EFFECT OF CORTICOSTEROIDS ON NEUROTRANSMITTER METABOLISM IN RAT CENTRAL NERVOUS SYSTEM

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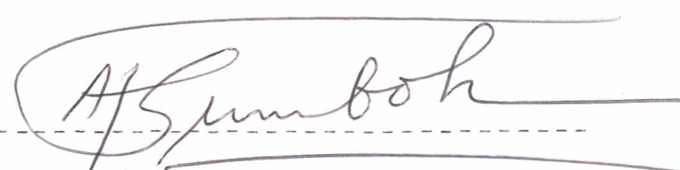

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A C K N O W L E D G E M E N T S

I would like to express my gratitude to the German Exchange Service (DAAD) and the University of Nairobi for the scholarships which enabled me to successfully carry out this work. In particular I wish to thank the seminar co-ordinator Dr. N. K. Olembo for her co-operation help and encouragement in organizing the seminars that gave me orientation in my work. The facilities, help and trust accorded me by the department of animal physiology which enabled me to run my experiments smoothly is highly appreciated.

I am grateful to the department personnel for their encouragement and interest in my work. I am indepted to all those who supervised my work partly or wholly. The help I got from Dr. R. M. Njogu is sincerely appreciated. I am thankful to Bob King of small animal house ILRAD for making available the animals used in this work. Special thanks also to the Pharmacist in charge of Kenyatta National Hospital for making available the drugs used.

I have been constantly stimulated by Dr. B. S. Bharaj who apart from directly supervising my work has been following my progress throughout. My gratitude to him runs deeper than is possible to express. Dr. Bharaj read all the drafts and the entire thesis.

His stimulating and scintillating advices, dedication, suggestions and criticisms are highly appreciated.

I am thankful to all those who worked in Lab B11 for their assistance, discussions, company, encouragements, and moral support during the entire period.

Lastly I would like to thank Mrs. Rose Birgen for typing this thesis with great keenness.

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
CRH	Corticotrophin releasing hormone
DA	Dopamine
5-HT	Serotonin
GABA	γ-Aminobutyric acid
GABA-T	γ-Aminobutyric acid transaminase
GAD	Glutamate decarboxylase
GAD-1	Glutamate decarboxylase 1
GAD-11	Glutamate decarboxylase 11
MAO	Monoamine oxidase
NAD	Nicotinamide - adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)

UNIT ABBREVIATIONS

gm	Gram
hr	Hour
M	Molar concentration
mg	Milligram
min	Minute
ml	Millilitre
mM	Millimolar
mmol	Millimole
nm	Nanometer
nmol	Nanomole
pm	Picomolar
sec	Second
µg	Microgram
µm	Micromolar
µmol	Micromole

SUMMARY

EFFECT OF CORTICOSTEROIDS ON NEUROTRANSMITTER METABOLISM IN RAT CENTRAL NERVOUS SYSTEM

Present data indicates that hormones especially corticosteroids, apart from being responsible for functional changes in the CNS, also bring about changes which alter the general metabolism of the brain, thus affecting different aspects of the neurotransmitter, plastic and energetic functions of the neurone. It is generally accepted that one of the most plastic morphofunctional structures of the CNS is the synapse, and most neurotropic compounds including steroid hormones affect neuronal activity and other associated structures by affecting synaptic transmission. Hormones can alter the levels of neurotransmitters in certain regions of the central and peripheral nervous system by either altering their data of synthesis or degradation, i.e. by affecting the activities of the enzymes responsible for their metabolism.

The study has examined the metabolism of several biogenic amines GABA, Norepinephrine (NE) Dopamine (DA) and Serotonin (5-HT) and how their metabolism is affected by different corticosteroid levels. In an attempt to elucidate the biochemical basis of behavioural effects of corticosteroids, we have studied the influence of two of these hormones, hydrocortisone

and dexamethasone on the relative concentrations of NE, DA, 5-HT and GABA and their metabolic enzymes in discrete regions of the brain with special emphasis being paid to the limbic structures especially the hypothalamus and hippocampus. We used a model of single and multiple administration of high doses of the hormones which causes hyper and hypocortisolism in blood plasma respectively. We have also studied how the metabolic enzymes vary under these hormonal conditions.

The results of our study show that alteration of corticosteroid status of rats, does cause changes in the concentrations of the putative neurotransmitters sufficient to cause gross behavioral changes. The data also indicates that changes seen in brain NE, DA, 5-HT and GABA are modulated through adrenocortical steroids and that these hormones play a role in the regulation of biogenic amine metabolism. It is suggested that corticosteroids could be used in therapy of affective disorders.

The work is original and helps to expand and shed more light on available data on the control of neurotransmitter metabolism by steroid hormones and possible control mechanisms.

CHAPTER 1

INTRODUCTION

1.1. GAMMA AMINOBUTYRIC ACID, PHYSIOLOGICAL ROLE, METABOLISM AND TRANSPORT IN THE CENTRAL NERVOUS SYSTEM.

γ-Aminobutyric acid (GABA) is currently considered a putative neurotransmitter because it meets the criteria of a neurotransmitter i.e. it is synthesized and accumulated by nerve endings and is released from them. The release of GABA is effected as a result of presynaptic excitation. Topographic distribution of GABA and its synthetic enzyme glutamate decarboxylase indicate their close relationship to nerve structures associated with inhibition. The inhibitory effect of GABA is reduced by its antagonists like picrotoxin, bicucullin and benzilpenicillin. The role of GABA as a neurotransmitter has been intensively studied (Roberts et al., 1976; Krogsgaard-Larsen et al., 1978; Sytinskii, 1977). For a compound performing neurotransmitter function, it is necessary that it has a mechanism for inactivation in the synaptic cleft. The absence of an enzymatic mechanism for GABA inactivation in the synaptic cleft has led to criticism of its role as a transmitter (Sytinskii, 1977). However, neurotransmitters can be inactivated as a result of active

transportation through carrier systems with high affinity mechanisms. Transport mechanisms specific for GABA have been found in the other cells of the organism as well, however, the kinetic properties of GABA uptake in peripheral nerves differ from those found in these cells. For brain cells a Km ~ 4.10^{-6} moles has been found (Sytinskii, 1972; Iversen, 1972). This value is considerably lower than the Km for other cells of the organism consequently in nervous tissue there exists an uptake mechanism which can transport GABA from a region of lower concentrations to that of higher concentrations i.e. a high affinity mechanism. The exact nature of the mechanism is however unknown. (Miller et al., 1978; Peck et al., 1976).

1.1.1. Distribution of GABA

According to biochemical, autoradiographic and electrophysiological data, GABA is widely distributed in the central nervous system of invertebrates. It has also been established that GABA is present not only in neurones of different regions of the brain, but also in glial elements. This suggests the possible participation of the latter in the metabolism of GABA (Hosli and Hosli, 1979). The highest concentration of GABA in invertebrates is located in the CNS, whereas its content in the spinal cord and peripheral nerves is considerably less. By examining tissue extracts

from the brains of different vertebrates i.e. Molluscs, lobsters, and crayfish, and comparing them with those of mammals, it was shown that the concentration of GABA in these animals is almost the same (Aoyama, 1958, Sytinskii, 1972).

Data on the distribution of GABA in the CNS shows great variance in its levels for different regions. Very high concentrations of GABA are characteristic for cells of the dorsal nucleus, purkinje cells, and cells of the granular layer of the cerebellum. Low concentrations or total absence of GABA is characteristic of most diencephalic and mesencephalic nuclei (Robinson and Wells, 1973). Concentrations of GABA higher than 100 nmoles were found in the globus pallidus and median eminence. Lower concentrations of GABA were found in the hypothalamus and septal nuclei. The highest concentrations of GABA were found in the caudal part and in the ventral regions at several levels of the striatum (Van der Heyden et al., 1979). In experiments carried out by Bertilsson (1977), it was found that the substantia nigra contained the highest GABA concentrations, followed by globus pallidus, N. accumbens, deep cerebella nuclei, N. caudatus and cerebellar cortex.

1.1.2. Synthesis and Degradation of GABA

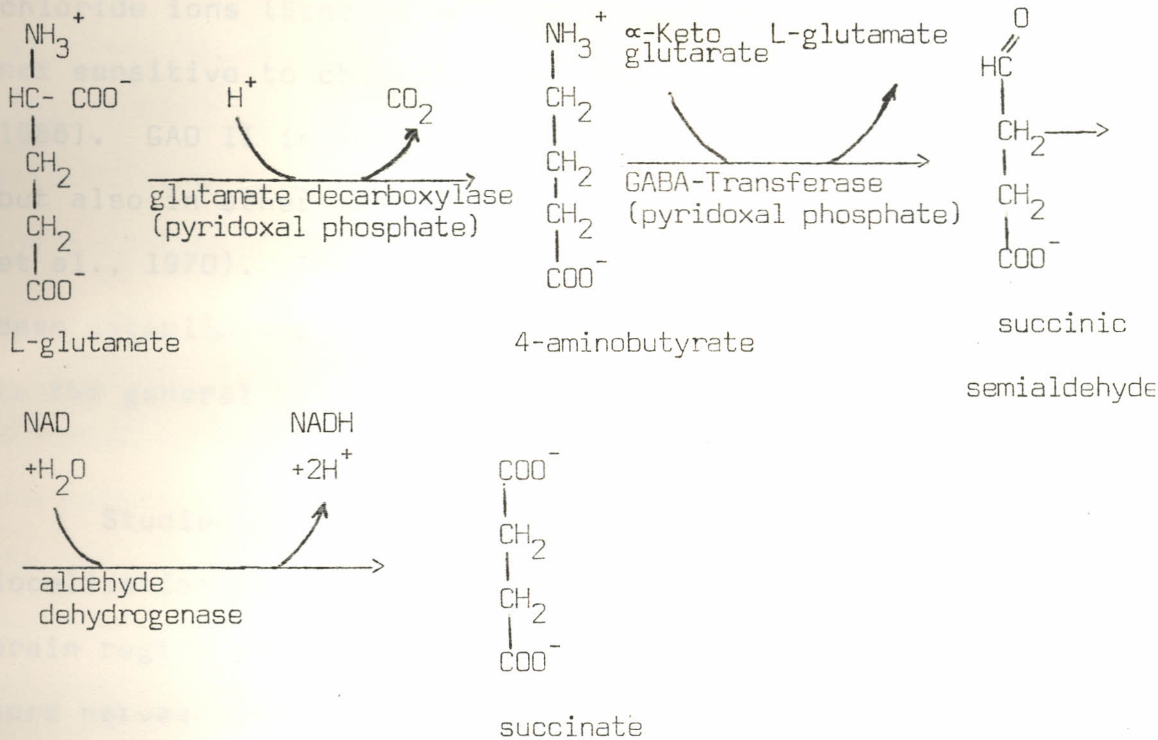


Fig 1. THE GABA Shunt

In nervous tissue GABA is synthesized as a result of the decarboxylation of L. glutamic acid by the enzyme glutamate decarboxylase in the presence of pyridoxal phosphate as a coenzyme (Susz et al., 1966). The first stage of GABA degradation involves its transamination with α-ketoglutaric acid to form succinic semialdehyde. The reaction again requires pyridoxal phosphate as a cofactor, the enzyme responsible is GABA-Transaminase. Synthesis and catabolism of GABA thus represent a bypass of the α-ketoglutarate dehydrogenase reaction in the citric acid cycle.

In mammals glutamate decarboxylase (GAD) exists in two forms: GAD I whose activity is inhibited by chloride ions (Susz et al., 1966) and GAD II which is not sensitive to chloride ions (Malinoff and Kravitz, 1968). GAD II is not only located in nervous cells, but also in other tissue of endodermal origin (Haber et al., 1970). The exact function of GAD II has not been established, however it is thought to play a role in the general biosynthesis of GABA.

Studies of GAD activity have shown its specific localization in the central nervous system. The mid-brain region shows the highest activity with the spinal cord nerves having little or no activity. In the spinal ganglia, GAD activity has not been demonstrated. The grey matter has GAD activity 4-5 times higher than the white matter, while high activities of GAD have also been established in the hippocampus and hypothalamus regions of the brain. In certain brain regions (cerebellum, thalamus and midbrain), GAD I activity is directly proportional to GABA content (Sytinskii, 1972). Glutamate decarboxylase is regarded as a cytoplasmic component of nerve endings. Different brain regions have been shown to have varying intercellular localization. In the cerebral cortex for example the activity of the enzyme was found only in the cytoplasm (Chan ke Pan, 1963). A study of the transamination of GABA has shown that the process proceeds not only in the brain but also in other tissues. However, the

highest activity of GABA-T was shown in nervous tissue. The activity of GABA-T in the brain was shown to be ten times higher than in the spinal cord (Padolfo, 1959; 1961; 1962). In the same experiments, high GABA-T activities were shown in the hypothalamus, Cerebellum and N. reticulata of rat brains.

Studies on the subcellular localization of GABA-T, showed mitochondria as having the highest activity of the enzyme, where it was ten times more active than in the non-mitochondrial fraction and six times more than the microsomal fraction (Buu and Van Gelder, 1974). The activity of GABA-T was also found in the fraction enriched with nerve endings, however GABA-T in mitochondria was five times higher than in the synaptosomes. It was speculated on the basis of this, that GABA absorbed in neuronal perikarya and glia (Cytoplasmic mitochondria) is rapidly degraded and does not accumulate in nerve endings (Buu and Van Gelder, 1974).

GABA is regarded as a physiological paradox since its high concentrations in mammalian brain (15-25 mg) do not correlate with the classical definition of transmitter compounds. Topographical distribution of GABA and its metabolic enzymes show, however, its localization in the nervous structures responsible mostly for inhibition processes.

1.1.3. GABA uptake and Transport

Nerve elements containing GABA and those synthesizing it have a transport system for GABA with a high affinity. Iversen and Neal (1968) speculated that the physiological effects of GABA in the synapses are terminated by the reuptake of GABA by a carrier mediated system of high specificity. This was later shown in whole brain slices and subcellular fractions. The transport of GABA and its binding, like most sodium -potassium dependent processes is sensitive to 2,4-dinitrophenol and ouabain. The results of GABA binding studies form the basis for suggesting that, GABA might be transported by a mechanism similar to that of sugars (Martin and Smith, 1973). According to this model of transport, GABA is translocated by a sodium-potassium dependend electrochemical potential. The Na^{+}, K^{+}-ATPase which acts as a pump in this process helps in maintaining the sodium and potassium gradients. Despite the fact that present data show GABA transport as transmembrane in nature, the actual mechanism of the neurotransmitter transport is far from being fully understood.

It has been established that, GABA quickly accumulates in the synaptosomes by a mechanism that is very sensitive to temperature. (Martin, 1972). By incubating synaptosomes at $0^{o}C$ for 10 min, only 3% of ^{3}H-GABA is accumulated as compared to the same

incubation at 27°C. These results prove that this is a membrane transport process. Studies of GABA reuptake as a function of time show, that after incubation for 10 min, uptake decreases and is practically complete after 15 min. Studies on the kinetics of GABA transport in the synaptosomes of rat brain have shown that the rate of reuptake is proportional to the protein concentration in the range 0.05 to 0.2 mg (Martin, 1973).

1.1.4. Effect of Ions on GABA Uptake

γ-Aminobutyric acid reuptake by synaptosomes can take place in a simple medium containing NaCl, KCl glucose and a buffer. (Martin, 1972). The absence of glucose from the medium does not significantly alter transport in the presence or absence of potassium, which shows that the synaptosomes have adequate energy reserves for maintaining transport without need for exogeneous substrates. In the same experiments, the effect of removing potassium from the incubation medium was significant. At the start of incubation, an accumulation of GABA was observed in the synaptosomes, later on this process stopped. Maximum reuptake was observed at concentrations of 3-6 nmoles. The complexity of the GABA-potassium interaction was demonstrated by the fact that maximum GABA reuptake was observed at potassium ion concentrations of 3-6 nmoles, however higher concentrations led to an inhibition of the

reuptake process in the synaptosomes. From these results it was evident, that potassium ions interract with GABA transport system in two ways since they stimulate transport at low concentrations and inhibit at higher concentrations.

The best established characteristic of GABA transport is the requirement for sodium which has been observed by several investigators (Iversen and Neal, 1965; Martin and Smith, 1972; Martin, 1973). The accumulation of GABA proceeds at a constant rate for at least eight minutes at all the sodium concentrations tested without any sign of a lag period or other anomaly. Thus, the data on GABA is suitable for analysis of transport by an enzyme kinetics approach. An interesting feature of the GABA transport system is the pronounced sigmoid shape of the sodium-dependent curves similar to those observed with allosteric enzymes. The fact that nearly identical sigmoid curves are obtained with both the sucrose and choline chloride replacements of sodium chloride indicates that the shape of the curves is not an artifact. By analysing the kinetic curves, it is possible to conclude that GABA transport systems exhibit allosteric properties, in which case, the GABA transport system would have more than one sodium site and these sites would interact in such a way that the binding of sodium at one site influences the binding of sodium at successively

occupied sites. (Martin and Smith, 1972; Martin, 1973).

the influence of other ions on GABA uptake has been surveyed at low sodium concentrations. Potassium and magnesium had little effect on uptake at low levels of sodium but the addition of calcium stimulated the uptake (Simon et al., 1974).

Further examination of the effects of ions show that calcium strongly stimulated GABA transport at low sodium concentrations but had only slight effects at high sodium concentrations (Martin, 1972; Wheeler, 1980). The addition of 20 mM calcium chloride clearly eliminated the sigmoid character of the curve. The fact that the double reciprocal plot for sodium is linear in the presence of 20 mM calcium is consistent with a mechanism in which only one sodium ion is required for GABA transport.

In view of the fact that allosteric systems typically function in a regulatory manner, the apparent allosteric effects observed for the GABA transport system suggests that the transport system may be subject to metabolic regulation and thereby serve to modulate synaptic transmission by controlling the time that GABA remains in the extracellular fluid (Martin and Smith, 1972).

In studies on GABA transport, a lot of emphasis has been laid on the process of GABA release from the synaptosomes. Thus, it has been shown that synaptosomes are capable of quickly releasing accumulated GABA. During this process, release was maximal over a duration of forty seconds following stimulation (Simon et al., 1974). The rate of ^{14}C GABA release from synaptosomes over a duration of twenty seconds before and after addition of calcium in the medium in the presence of depolarizing concentrations of potassium (56 mM) made it possible to demonstrate the dependence of the process on calcium which is an absolute stimulant. The release of GABA caused by calcium ions increases in the range upto 1 mM and is asymptotic in the proximity of the physiological concentrations of calcium. This observation is in agreement with available data on the asymptotic dependence of metabolite secretions on calcium in the central nervous system *in vivo* (William et al., 1973). The calcium dependent release is inhibited by magnesium and manganese ions which are known to be inhibitors of coupled stimulus-secretory processes. It should be noted that the stimulation of GABA release is specific for synaptosomes. Though myelin fractions and microsomes have the ability to accumulate GABA, none of these fractions has been shown to release measurable amounts of the transmitter following stimulation with calcium. Similarly, homogenates of glial cells of Hela tissue cultures or synaptic ganglia can also accumulate GABA.

(Williams et al., 1973). However, upon stimulation by calcium, less than 0.01% of the total radioactivity accumulated is released. By adding low concentrations of GABA in the incubation medium a sponteneous release of radioactive GABA from synaptosomes was observed in the same experiment. Moreover the said process required the presence of sodium ions although it was independent of its concentrations outside the cell.

The data cited above shows that, the secretory process of GABA is not a common phenomenon for all systems capable of transporting GABA, even if they are of glial or neuronal origin. Since the major contaminants of synaptosomal fractions don't secrete GABA, it can be concluded that synaptosomes are the centre of calcium dependent release. An analysis of the data describing GABA uptake by synaptosomes and its release has led to the conclusion that these two processes are modulated by one and the same carrier (Martin and Smith, 1972; Martin, 1973; William et al., 1973). The sodium insensitive spontaneous GABA release is possibly modulated through a different membrane mechanism. The release of radioactive GABA from synaptosomes in the presence of high concentrations of GABA in the medium (Simon et al., 1974) makes it possible to describe this process as the active transport of the neurotransmitter. This is further supported by the following data.

(i) The Km value of GABA uptake by synaptosomes through active transport is close to the half maximal extracellular concentrations of GABA required to stimulate its release.

(ii) Extracellular concentrations of sodium chloride exert the same effects on the uptake of GABA and on the GABA stimulated release of GABA from synaptosomes.

(iii) Both processes are completely inhibited at low temperatures while the rate of spontaneous release is less sensitive to low temperatures.

1.2. CORTICOSTEROID INDUCED CHANGES IN GABA METABOLISM IN DISCRETE BRAIN REGIONS.

The inbalances in corticosteroid levels in the organisms affect the excitability of the brain. One of the mechanisms affecting the excitability can be through the effects of hormones on the neuronal system utilizing γ-amino butyric acid as a neurotransmitter viz the content and metabolism of GABA (Woodbury et al., 1957; Woodbury and Vernadakis, 1966; Dobrijanskaya, 1973; Komissarenko and Lisenko, 1974).

Adrenalectomy has been reported to result in

decreased levels of GABA in the brains of rabbits (Riudi and Ventura, 1961; Woodbury and Vernadakis, 1966; Lisenko, 1968). The levels of "Free" and "Bound" forms of GABA in the grey and white matter of the cortex, cerebellum and the spinal cord of rabbits and rats has been shown to decrease (Dovgalevich, 1969; Chirkosora and Pikulev, 1972). An increase in GABA content in the brains of rats has been reported (Woodbury et al., 1957; Komissarenko and Kononenko, 1980), and no changes in the levels of GABA after surgical removal of the adrenal glands in different regions of the brain have also been cited (Roberts, 1960; Sutherland and Riximarv, 1964; Kazarian, 1972; Hahn et al., 1977; Kendal et al.,1982).

The effects of hypocortisolism is expressed differently for different types of animals. Thus the removal of the adrenals has been shown to cause a decrease in the content of GABA in the cortex region of rat brain (Kononenko and Mishunina, 1980) but did not affect the levels of GABA in the cortex of rabbits (Mishunina and Masteshkina, 1977). Ten and seventeen days after adrenalectomy, the levels of GABA fell not only in the cortex, but also in the hippocampus. The content of GABA in the caudate-putamen, ten days after the operation, increased, and did not fall after 17 days. In these experiments adrenalectomy did not affect the levels of the amino acid transmitter in the hypothalamus (Kononenko and Mishunina, 1983). The studies on

the content of "Free" and "Bound" forms of GABA showed a decrease in the former in the hippocampus, caudate-putamen and cortex, and the latter in the hypothalamus and cortex respectively. In the caudate-putamen, the content of "Bound" GABA showed an increase (Kononenko and Mishunina, 1980). Pharmacological adrenalectomy caused an increase in the content of "Bound" GABA in the cortex and cerebellum (Chirkosova and Pikulev, 1972), but it decreased the "Free" form in these brain regions.

Studies on the enzymes involved in GABA metabolism showed that, the activities of glutamate decarboxylase in several brain regions was altered following adrenalectomy (Acs et al., 1980; Kendall et al., 1982). Glutamate decarboxylase and GABA-transaminase activities in the rat hippocampus did not change (Mayer et al., 1979). However, a number of investigators have shown a decrease in the activities of GAD and GABA-T in the brain (Padolfo and Moraine, 1964). The activities of GAD were shown to decrease following surgical adrenalectomy and to increase after pharmacological adrenalectomy (Chirkosova and Pikulev, 1972). The activities of GAD ten days after adrenalectomy, decreased in the hypothalamus, caudate-putamen, hypophysis but remained unaltered in the hippocampus and in the cortex. Ten days after surgical adrenalectomy, the activities of GAD increased in the hypothalamus and hippocampus but decreased in the hypophysis (Kononenko and Mishunina, 1983).

The distribution of GABA receptors in the brain after adrenalectomy did not change neither was the high affinity ^{3}H-GABA uptake system in the synaptosomes of different brain regions altered (Kendall et al., 1982; Miller et al., 1978). At the same time, in the hippocampus, the maximum velocity of GABA uptake (which acts as a measure of the number of functional sites for GABA transport) increased following adrenalectomy (Miller et al., 1978; Peck et al., 1976). This data suggests that in the absence of corticosteroids, the neuronal membranes of the hippocampus possess a high ability to transport GABA. An increase in the ability of hippocampal neuronal membranes to bind GABA occured very fast. This was observed even 24 hours after adrenalectomy and was consistent over a period of 7-14 days. Therapeutical implantation of corticosterone brought the maximal velocity of binding back to normal as compared to sham operated animals though the response time was much longer 6-10 days. Alterations in maximal velocity of GABA uptake by synaptosomes showed regional specificity uptake by synaptosomes derived from the cortex and cerebellum regions of the brain of adrenalectomised rats did not change (Miller et al., 1978). The fact that incubation of the hippocampal synaptosomes with corticosterone did not alter the maximal velocity of uptake which shows that the changes observed after adrenalectomy were as a result of the interaction of the corticosteroids

with receptors in the hippocampus. In other experiments, adrenalectomy increased the binding of GABA in the midbrain on the third day after the operation while in the caudate-putamen, the increase was observed between days 7-14. Administration of corticosterone to adrenalectomised rats prevented the changes in GABA binding to occur in these brain structures (Kendall et al., 1982).

The results on the effects of exogeneous corticosteroids on the metabolism of GABA vary widely. A number of authors note that hydrocortisone has no effects on GABA turnover in intact animals (Riudi and Ventura, 1961; Woodbury et al., 1957). However, after a single injection of hydrocortisone, the levels of GABA in the hypothalamus, cerebellum, cortex and brain stem of rat brain are reported to decrease (Hahn et al., 1970; Sadasivudu et al., 1977). Four hours after an injection of the hormone, the GABA content in the cortex region of rabbit brain increased but 24 hours later, its levels returned to normal (Masteshkina and Mishunina, 1975).

Studies on the effects of single hydrocortisone injection on GABA levels of the brain showed a decrease in GABA levels in the hippocampus and cortex regions of the rat brain, and an increase in its content in the medulla-pons and in the cerebellum. No such changes

were observed in the midbrain (Kononenko and Mishunina, 1982). Administration of hydrocortisone to newborn rats caused a decrease in GABA levels in the brain. This decrease in GABA was found to vary with the age of the rats (Vernadakis and Woodbury, 1971). Single administration of corticosterone caused a decrease in GABA content in the hypothalamus without affecting the relationship between "Free"/"Bound" GABA (Hahn et al., 1977). At the same time no changes were observed in the whole brain following corticosterone administration (Riudi and Ventura, 1961).

The effects of adrenal hormones therefore may be dependent on the duration of administration. A single injection of cortisone can decrease brain GABA content (Riudi and Ventura, 1961), while prolonged administration over a 16-20 days duration does not affect the levels of GABA (Yudaev and Gancharova, 1959). The initial hormonal status of the organism plays an important role in the effectiveness of hormonal action. Hydrocortisone injection (single and multiple) results in a decrease in the GABA content in the brain of adrenalectomised rats (Woodbury and Vernadakis, 1966), while in intact animals no such changes were observed (Woodbury et al., 1957). Corticosterone and cortisone caused an increase in the levels of GABA in the brains of adrenalectomised rats, while at the same time, corticosterone failed to cause any change in GABA levels

of intact animals. Cortisone however, decreased the levels of GABA in intact rats (Riudi and Ventura, 1961). Argiz (1967) also noted a difference in the effects of hydrocortisone dependent on the levels of the hormone in blood plasma at the time of administration.

Changes in the levels of GABA in the brain could partly be due to the effects of the exogeneous hormone on the uptake of GABA. *In vitro* studies show that hydrocortisone increased the incorporation of ^{3}H-GABA in brain slices (Banay et al., 1979). The release of GABA in this case was not affected. The content of GABA in the slices upon incubation with hydrocortisone increased and it is worth noting that the effects of the hormone were similar to the effects of aminooxy-acetic acid, an inhibitor of GABA-Transaminase (Banay et al., 1979). Hydrocortisone in these experiments increased the formation and decreased the catabolism of GABA. *In vitro*, neither hydrocortisone nor corti-costerone affected the activities of GAD or GABA-T in the brains of rats (Edwards and Rousse, 1980; Mayer et al., 1979). Studies on the activities of GABA meta-bolising enzymes in different brain regions, show that one hour after hydrocortisone administration, the acti-vities of GAD in the hippocampus and medulla-pons dec-rease. Four hours later in the hippocampus, an incr-ease was observed, while its activities decreased in the medulla-pons (Kononenko and Mishunina, 1982).

The activities of GAD in the hypothalamus, cortex, midbrain, cerebellum and caudate-putamen did not show any changes under these experimental conditions.

The above data indicates that adrenal hormones have an adverse effect on the metabolism of GABA in the brain, however the discrepancies in the results obtained due to the use of different corticosteroids, doses, times of experimentation, kinds and functional status of the animals make a follow up and interpretation of the effects of tbe hormones on brain metabolism of GABA difficult.

1.3. CORTICOSTEROIDS AND BIOGENIC AMINE (DOPAMINE, NOREPINEPHRINE, SEROTONIN) BALANCES AND METABOLISM IN THE CENTRAL NERVOUS SYSTEM.

The relationships between glucocorticoids and biogenic amines have received considerable attention in recent years especially with regard to biogenic amines' control of glucocorticoid production (De Vellis and Kukes, 1973; Lissark et al., 1973). The converse effects of glucocorticoids on biogenic amines' activity have not been well studied, although a number of clinical observations suggest that steroids can exert great effects on catecholamines and/or indoleamine systems. Patients receiving high doses of exogeneous adrenocorticotropic hormone (ACTH) or glucocorticoids

often exhibit psychiatric disturbances, including depressive, psychotic or excited states (Antony et al., 1985). Patients with conditions that are characterized in part by marked hypercortisolemia (e.g. Cushings Disease) often demonstrate psychiatric disturbances.

Adrenocorticotropic hormone (ACTH) is an adreno-hypophysial glandotrophic hormone, its main effects being stimulation of the adrenal cortex and glucocort-icoid secretion (Cortisol in man and corticosterone in certain other species e.g rat). ACTH is regulated by the hypothalamic corticotrophin releasing hormone (CRH) and by feed-back in which glucocorticoids especially cortisol or corticosterone act on ACTH secretion. The feed-back regulation secretion by the blood cortisol or corticosterone level mainly concerns the relatively slow adjustment of ACTH secretion to changed blood levels of the adrenal cortical hormones. Rapid ACTH secretory reactions (Stress) are relatively independent of the corticoid level; they can be only partly inhi-bited and only by large doses of corticosteroids (Schreiber, 1974).

ACTH secretion is regulated in two ways. The first is homoestatic regulation, the purpose of which is to keep the blood concentration of adrenal cortex hormones at a constant level. It takes effect mainly under conditions of diminished blood corticoid level, ACTH secretion being stimulated after adrenalectomy

and after chemical block or enzymatic disturbances of hormone biosynthesis in the cortex. The other type is reflex regulation, when ACTH secretion rapidly increases during exposure to the most diverse forms of stress (heat, cold, trauma, emotions, etc). In these situations, ACTH secretion rises to some extent independently of the blood corticoid level. Both types of regulation use a simple final common pathway, i.e. secretion of hypothalamic CRH into the adenohypophysial portal blood (White et al., 1980).

Diminished ACTH secretion after large doses of corticoids can also be accompanied by a decrease in the ACTH content of the adenohypophysis. Conversely the blood ACTH concentration and the amount of ACTH in the adenohypophysis both rise after adrenalectomy. It has also been reported that, the administration of corticosteroids inhibits the pituitary-adrenocortical system and that the regulation of this system is affected by corticosteroid feedback mechanisms (Szentagothai et al., 1968; Zimmerman and Critchov, 1969; Dafny et al., 1973). Exogeneous administration of corticosteroids are also reported to be followed by a decrease in cortisol levels and these levels of cortisol are prevented from rising during stress (Verniko-Danellis, 1964; Legori et al., 1965). This indicates that the blood corticoid level simultaneously influences both ACTH secretion and synthesis. It also shows that CRH likewise exerts both types of action.

The mechanisms of the feed-back action of the adrenal cortical hormones evidently involve effects of hypothalamic CRH secretions. Study of the possible site of action of corticoids (the adenohypophysis or the hypothalamus) show rather conclusively that the site of the inhibitory action of corticoids is the hypothalamus, and possibly other structures in the higher parts of the central nervous system as well (Schreiber, 1974).

Studies in man and other species have emphasized the effects of glucocorticoids on noradrenergic (NE) and serotonergic (5-HT) systems. These effects appear to vary greatly depending in part on dose and duration of corticosteroid treatment or the animal species and specific brain regions. The effects of corticosteroids on dopamine (DA) have been less emphasized, although a number of indirect observations indicate that steroids increase DA content. Some of these effects can be thought to reflect a possible corticosteroid effect on the pituitary (Kononenko, 1982).

A lot of data has enabled researchers to formulate a number of concepts on the role of brain monoaminergic systems and hormones in the functional regulation of not only the central nervous system, but also of other organs and systems. It is generally believed that, monoaminergic systems of the brainstem participate in the integration and primary co-ordination of

different signals originating from within and outside the organism (Dorner, 1978). One of such important signals is the hormonal signal. It is postulated that, hormones interact with neurotransmitters, especially biogenic amines and form a general co-ordinate neuro-hormonal system at CNS level, controlling important life processes such as metabolism and coordination of information.

It is known that excess or deficiency of corti-costeroids causes behavioural and psychological distur-bances in humans and experimental animals (Woodbury, 1958). Evidence that corticosteriods may affect beha-viour independently of the steroid's ability to modu-late the concentrations of ACTH also exists (De Wied, 1979). Bilateral adrenalectomy has been shown to suppress body growth and increase the activity of tyrosine hydroxylase (an allosteric enzyme in the synthesis of catecholamines) in rat striatum, in a time dependent manner. Fifteen days after adrenalec-tomy the concentrations of NE were found to be decreased significantly in the hypothalamus and striatum, as were those of DA in brainstem and striatum. Administration of corticosterone to these rats effectively reversed these effects on brain amine metabolism. It has been suggested that changes seen in brain NE and DA of adrenalectomised rats are specific to adrenocortical steroids and that these hormones play a role in the regulation of catecholamine biosynthesis (Rostogi

et al., 1978). It has also been shown that adrenalectomy decreases tryptophan hydroxylase activity and its substrate tryptophan in the brain stem (Rostogi et al., 1978). This was followed by a parallel change in the concentration of 5-HT in the brain stem and striatal regions of adrenalectomised rats. Replacement therapy with corticosterone produced time-dependent increases in total tryptophan, tryptophan hydroxylase and 5-HT, and decreased 5-hydroxyindolacetic acid levels. It was also noted that alterations in these neurochemical parameters were more conspicuous in adrenalectomised rats receiving corticosterone for 7 days as compared to those receiving it for 3 days. This data seems to suggest that adrenocortical hormones regulate brain 5-HT synthesis probably by enhancing both levels of tryptophan and the activity of tryptophan hydroxylase. It has been suggested previously that serotonergic nerve fibre containing structures like the cortex, hippocampus and amygdala constitute a functional unit that modulates the regulation of ACTH (Scapagnini et al., 1971). Hence there is some correlation between the daily rhythm of 5-HT content in the limbic system and that of circulating corticosteroids and thus of ACTH and CRH levels. Following corticosteroid administration, reports of increased (Azmitia and McEwen, 1969), unchanged (Shah et al., 1968) or even decreased brain 5-HT have been reported (Curzon and Green, 1970). One hour after bilateral adrenalectomy, the turnover

of 5-HT in the dorsal hippocampus was reported to have been significantly decreased as compared to that of sham operated animals. A low dose of corticosterone given immediately after adrenalectomy restored both 5-HT turnover rate and steroid receptor occupancy in dorsal hippocampus. Dexamethasone failed to produce any such changes (Versteeg et al., 1984).

The role of brain NE in the regulation of pituitary-adrenocortical function is controversial. It has been suggested (Ganong, 1972), that the central noradrenergic system inhibits the secretion of adrenocorticotropin, but it results in a drastic reduction in the hypothalamic NE content which results from intraventricular administration of 6-hydroxydopamine and it has no effects on the basal levels of corticosterone in rat plasma and adrenal tissue. The stress response is also not affected nor is the suppressive effects of dexamethasone on plasma and adrenal concentrations of corticosterone (Ganong, 1972).

Circadian ryhthms in brain levels of NE, DA and 5-HT have often been described (Simon et al., 1975; Anne et al., 1975). There has been evidence suggesting that, these circadian ryhthms could be modulated through the allosteric enzymes responsible for synthesis of these neurotransmitters, which have also been shown to vary circadially (Enna et al., 1975). Several investi-

gations on the functional role of biogenic amines in the brain and their interrelationships with hormones have enabled researchers to discover a number of common trends in neuroendocrine interaction. The role of biogenic amines in the realization of positive and negative feed-back mechanisms in the hypothalamus-pituitary-peripheral organs has been demonstrated (Rakitskaya and Shaliapina, 1976). The nature of interaction between individual corticosteroids and neuro-transmitters in the neurochemical mechanisms of neuro-glial interaction and also in behavioural reactions is of great value (De Vellis and Kukes, 1973; Lissark et al., 1973).

The data cited above shows the participation of monoaminergic systems of the brain in the realization of hormonal effects on the nervous system. This is not only of theoretical but also of practical importance. That is in fact why there is justification in attempts to use several drugs which act on serotonergic brain mechanisms with the sole aim of inhibiting hyperpro-duction of ACTH in Cushing Desease e.g cyproheptadine (Krieger, 1978).

It has been established that exogeneous adminis-tration of hydrocortisone has different effects on the brain content of norepinephrine and serotonin in rats (Kononenko et al., 1970; Davidienko., 1974). While

the levels of serotonin fell 30-60 min after hormonal administration, the concentrations of noradrenaline was not altered. Elevated serotonin levels caused by Impacide injections are normalised by hydrocortisone, while those of noradrenaline continue to increase consequently, despite the general metabolic pathways for catecholamine and phenylalkylamine, changes caused by hydrocortisone can vary for each one of them.

Further it was found that changes seen in the biogenic amine content can be caused by the effects of corticosteroids on the activities of the enzymes responsible for amine metabolism. It was thus shown that hydrocortisone administration alters monoamine oxidation of several neurotransmitters. (Kononenko., 1970; Valikina., 1972). After single injection of hydrocortisone 2-4 hours, intensive oxidative deamination of tyramine, tryptamine, and serotonin was observed in the brains of rats and rabbits. Further the process normalizes. These alterations in the activity of the major enzyme responsible for biogenic amine inactivation in nervous tissue is but one of the possible ways in which monoaminergic structures of the brain could respond to hydrocortisone. This would correspond to electrophysiological finding on the excitability of adrenergic structures of the nervous system following hydrocortisone administration (Gaidina, 1967). After adrenalectomy, there are changes in the subcellular distribution of monamines oxidase specific for particular

tissue. Total MAO activity of heart, brain and vas deferens was significantly increased in adrenalectomised rats, but there was no significant alteration of activity in the other tissue examined (Caesar et al., 1970). There was a greater than two fold increase in mitochondrial MAO activity in the heart and significant increases were detected in the mitochondrial fractions of the brain. This rise, however, does not occur in all brain regions and the intensity of the oxidative deamination of amines under corticosteroid deficiency varies from region to region (Youdim and Holzbaver, 1976).

The above results have shown two interesting facts. First, alterations in monoamine oxidase activity are not the same in all structures of the brain, and are more pronounced in the hypothalamus and cortex. Secondly hydrocortisone affects different amines differently. Thus while intensification of oxidative deamination of tyramine was observed in all brain regions, the deamination of serotonin increased only in the midbrain and cerebellum, while dopamine deamination was not altered (Valikina, 1972). Administration of ACTH has been shown to decrease oxidative deamination of tyramine in different regions, probably by directly acting on the brain (Valikina, 1972).

Hypocortisolism caused by administration of cloditane to dogs is accompanied by a decrease in the noradrenaline content of the hypothalamus, thallamus,

dorsal nucleus, medulla and the cerebellum. These alterations are more pronounced in the hypothalamus which has an important role in the regulation of corticosteroidogenesis. The lowering of noradrenaline content of the brain is by all means probably due to the hypocortisolism brought about by the drug (Komissarenko et al., 1977). This view was further supported by the fact that catecholamine levels in the affected animals returned to normal following corticosteroid therapy. No changes were observed in noradrenaline levels in those animals where chloditane did not suppress adrenal function.

1.4. AIMS OF THE STUDY

The present study, carried out in rats was aimed at:

(i) Finding out alterations in neurotransmitter metabolism in brain tissue under different corticosteroid levels in the organism.

(ii) Ascertaining the effects of plasma corticoid levels on the putative neurotransmitter (GABA, norepinephrine, dopamine and serotonin) distribution in the discrete brain structures with special emphasis on the hypothalamus and hippocampus regions.

(iii) Assessing the correlation between the metabolism

of GABA, NE, DA and 5-HT in the limbic structures with plasma cortisol levels as a possible indicator of adrenocorticotropic response to neuroendocrine reactions.

(iv) Finding out the possible level at which the endocrine system interacts with the central nervous system to bring about a feed-back response exhibited by the hypothalamus- pituitary - adrenal gland, system.

CHAPTER 2

MATERIALS AND METHODS

2.1. ANIMALS

Male sprague-Dawley rats 3-4 months old and weighing 250-300g were obtained from the International Laboratories for Research on Animal Diseases (I.L.R.A.D). The animals were housed, given rat pellets and water *ad libitum*. Hydrocortisone hemisuccinate sodium salt "Ritcher" or dexamethasone sodium phosphate "Ikpharm" (a courtesy of Kenyatta National Hospital Nairobi) were injected intramuscularly in the doses of 50mg/Kg and 4mg/Kg body weight respectively. Control animals were injected with an appropriate amount of 0.85% saline. All the injections were carried out at 0800 hrs local time.

2.2. DISSECTION PROCEDURE

The animals were quickly killed by decapitation into an ice bucket. The brains were carefully removed onto a pre-frozen tile, blotted and chilled. Dissections were performed on an ice-cooled tile essentially by the method described by Glowinski and Iversen, (1966). Seven regions were separated, and these will be described in the text by the following simplified names:

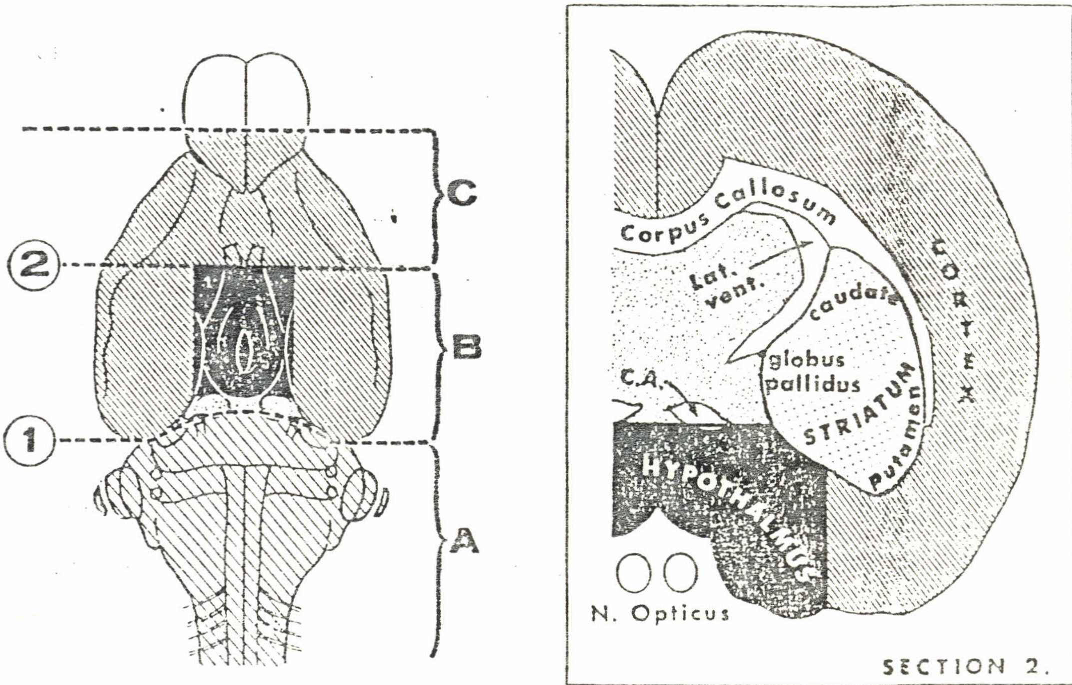
(1) hypothalamus, (2) hippocampus, (3) cerebellum (4) medulla and pons (5) midbrain (6) caudate-putamen (7) cortex.

Five of these regions include more than one distinct anatomical structure: The "Medulla pons" corresponds to the medulla oblangata and pons; The "Midbrain" corresponds to midbrain, subthalamus (the latter structures being part of the diencephalon). The "Caudate-putamen" contains the putamen nucleus and caudate nucleus (striatum) and the globus pallidus nucleus (i.e., the basal ganglia of the telencephalon without the amygdala). The "Cortex" corresponds to the telencephalon without the "Striatum"; It includes white and grey matter of the cerebral cortex. (see Fig 2 for the diagramatic representati of the dissection). All these and subsequent events including homogenization were carried out in the cold room at 4°C.

2.3. PREPARATION OF TISSUE

a) Short term experiments

Sprague - Dawley rats 3-4 months old weighing 250-300g were injected intramuscularly with either hydrocortisone 50mg/Kg body weight or dexamethasone 4mg/Kg body weight respectively. The injections were in all cases carried out at 0800 hrs local time. The animals were decapitated 4 hours later at 1200 noon and used for the assays.



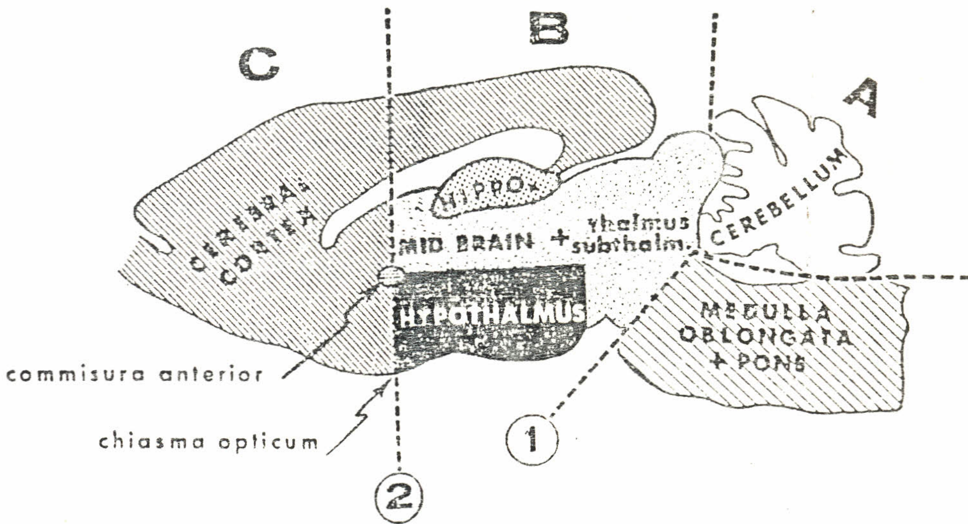


Fig. 2 - Diagramatic representation of dissection procedure for rat brain (Glowinski and Iversen, 1966). Dotted lines indicate positions of initial sections.

b) Long term experiments.

The animals were injected intramuscularly with hydrocortisone 50mg/kg body weight at 0800 hours. The injections were carried out continuously every day for seven consecutive days. The animals were killed 24 hours after the last hydrocortisone injection between 0800 and 0900 hours and used for the assays. Control animals injected with an appropriate amount of 0.85% NaCl solution in both long and short term experiments were ran side by side.

2.3.1. Preparation of Synaptosomes and Free Mitochondria

The preparation of synaptosomes and free mitochondria was carried out using the homogenization and fractionation procedures described by Cotman, (1973) as follows.

The hypothalamus and hippocampus tissue from 5-6 rats was pooled, and homogenized in 20% (w/v) homogenization medium [0.32M sucrose, 1mM EDTA (sodium salt), 10mM-Tris/HCl, pH 7.4], with 18 up and down strokes, diluted to about 10% (w/v) with isolation medium and fractioned as outlined in the scheme. The 13% or 17% Ficoll/sucrose medium contained: 13% or 17% (w/v) Ficoll, 0.32M sucrose, 1mM EDTA (sodium salt) and 10mM Tris/HCl, pH 7.4. The 8.5% Ficoll medium contained:

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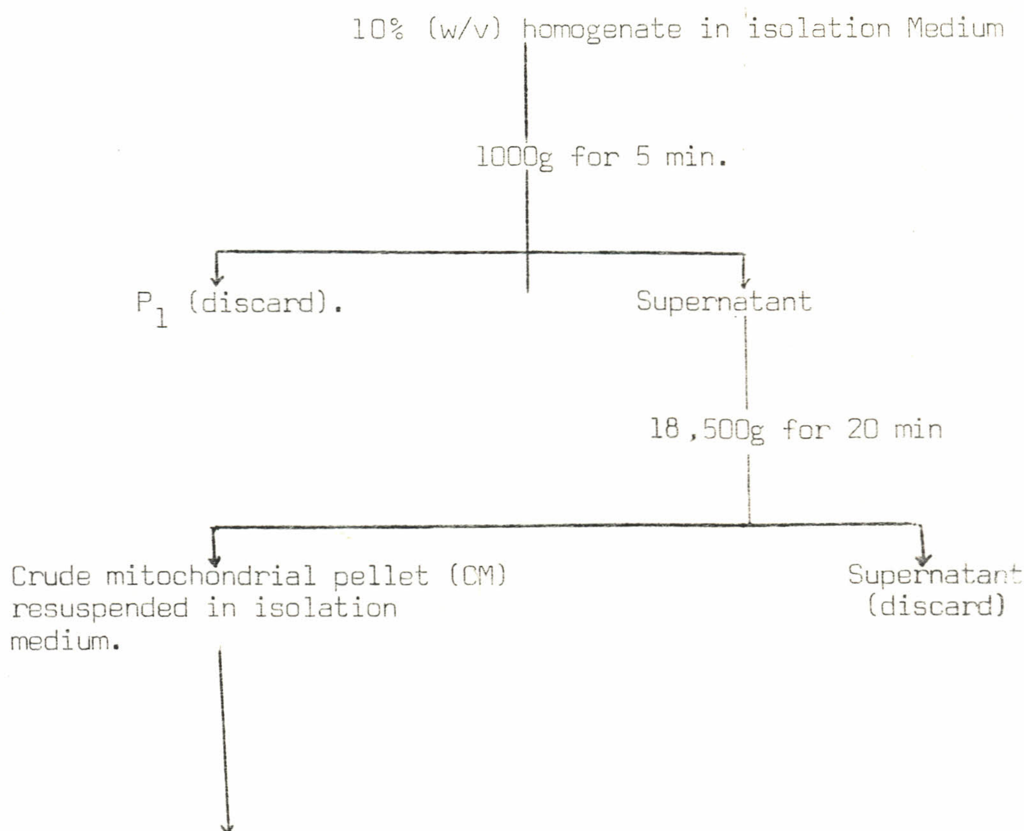
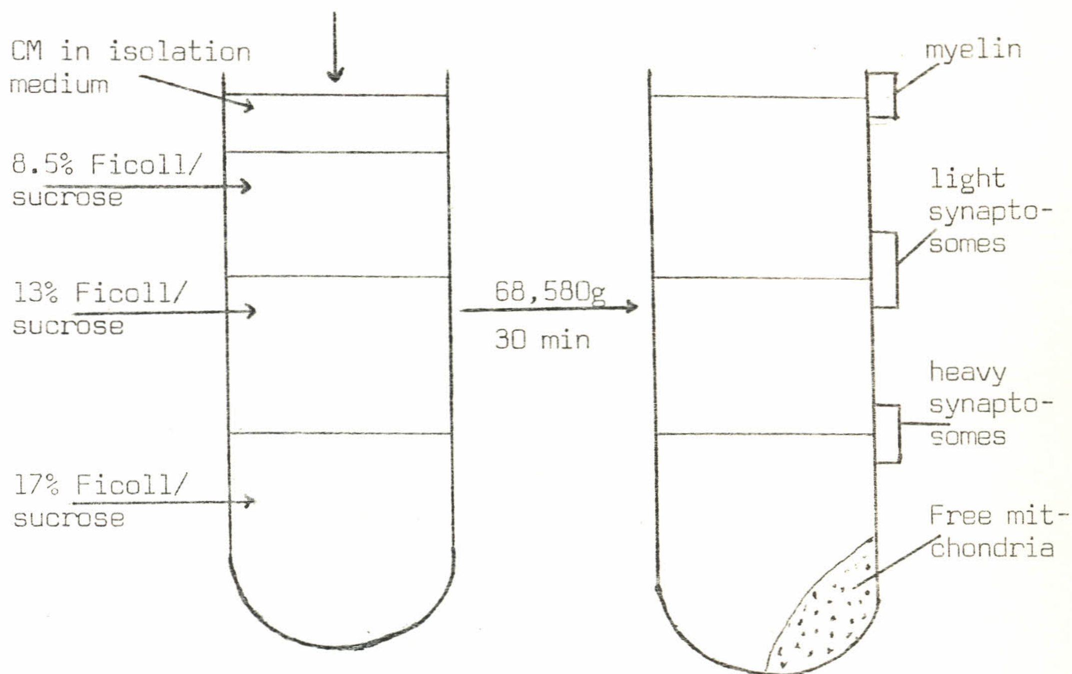
8.5% (w/w) Ficoll, 0.12M-mannitol, 30mM sucrose, 20mM-EDTA (sodium salt) and 5mM-Tris/HCl pH 7.4. The centrifugation was carried out in an MSE 50 ultracentrifuge using a 3 x 23 ml swing out rotor. Under these conditions light and heavy synaptosomes were pooled together as one fraction. After dilution with isolation medium, the synaptosomes were recentrifuged at 18,500g for 20 min. The synaptosomal and mitochondrial pellets were resuspended to about 1-2mg protein in isolation medium or buffer and used for the experiments. The distribution of glutamate decarboxylase and GABA-Transaminase enzymes among the fractions was used as a marker for the purity of the separations.

ISOLATION SCHEME





2.4. PROTEIN MEASUREMENTS

The protein concentrations of the samples were determined by the Biuret method (Gornall et al., 1949) with some modifications or alternatively by the method as described by Hartree, (1972). To remove the phospholipid layer from brain tissues, the following procedure was followed: 50-100µl of the sample was washed with 1.5mls of an acetone: ethanol (1:1) mixture and centrifuged at 4000 rpm for 10 min. The pellet was then rewashed with 1.5mls of ether and centrifuged at 4000 rpm for 10 min. The ether was discarded and the pellet assayed for protein content.

a) Biuret Method

The protein-acetone powder pellet obtained from the washing above was dissolved in 50µl of

7% NaOH in a waterbath at 60°C. To 1ml of diluted sample was added 4mls of the biuret reagent (1.50g. $CuSO_4$. $5H_2O$ and 6.0g Na^+-K^+-tartarate in 500ml of water. 300ml of 10% NaOH was added and diluted to 1 litre with water) and vortexed. The mixture was immersed in boiling water for 1 min cooled to room temperature and the optical density read at 540nm on a zeiss pm Q 11 spectrophotometer. Standards containing 1-10mg protein/ml were run in a similar manner.

b) Hartree Method

Protein samples were dissolved in 50µl of 7% NaOH and diluted to 1ml with water and treated with 0.9mls of solution A. (2gm potassium sodium tartarate and 100gm Na_2CO_2 dissolved in 500mls IN NaOH and diluted with water to 1 litre). A blank and a standard are set up in the same way. The tubes were placed in a water bath at 50°C for 10 min, cooled to room temperature and treated with 0.1mls B (2gm potassium sodium tartarate and 1gm $CuSO_4$. $5H_2O$ dissolved in 90mls water and 10mls IN NaOH). The solutions were left at room temperature for at least 10 min then 3mls of solution C (1 vol Folin-Ciocalteu reagent diluted with 15 vol water) was forced in rapidly to ensure mixing within 1 sec. The

tubes were again heated at 50°C for 10 min and cooled to room temperature. Absorbancies were read at 650nm in a Zeiss pm Q 11 spectrophotometer against bovine serum albumin as standard.

2.5. DETERMINATION OF TOTAL 11-OXYCORTICOSTEROID CONTENT IN PLASMA

Determination of 11-oxycorticosteroids was carried out by the method according to De Moor et al., (1960) as follows:-

to 0.1ml of heparinized trunk blood plasma from experimental animals was added 3.0mls H_2O and 12mls hexane in graduated tubes. The tubes were vortexed vigourously and the upper layer of the supernatant extract was discarded by aspiration using a drawn-out polyethylene tube connected to a water aspirator leaving behind a volume of 3ml. 4.5mls of water was added followed by 15mls of methylchloride and vortexed for 20 seconds. Immiscible liquids were separated by brief centrifugation at 3000 rpm for 5 min. The upper organic layer was aspirated leaving 12mls. To this was added 1.0mls of 0.1N NaOH, vortexed for 15 sec and the upper layer aspirated leaving behind 10mls. A cold mixture of concentrated sulphuric acid: Absolute ethanol (3:1) was then added and vortexed briefly. The upper layer of this mixture was then aspirated off

leaving 10mls. A standard containing cortisol was run in a similar manner. Fluorescence was recorded 90 min later at 470nm excitation and 524nm emission wavelengths respectively on an Amico Bowmen spectrophotofluorimeter. Results were expressed in nmoles 11-oxycorticosteroids/ml ± SEM.

2.6. DETERMINATION OF "FREE" AND "BOUND GABA

Extraction of "Free and "Bound" GABA was carried out as described by Lovell and Elliot, (1963) as follows: Brain hypothalamus and hippocampus tissue following dissection (see 2.2) was weighed and homogenized quickly at 4°C in five volumes of physiological saline (1:5 w/v) using a glass homogenizer. After centrifugation at 18,000g for 20 min, the supernatant containing "Free" GABA was separated from the residue which contained "Bound" GABA. To the residue was then added the original volume of 0.85% saline and the resulting suspension heated in a boiling water bath for 15 min and centrifuged. The pellet was discarded. To the first and second supernatants were added three volumes of absolute ethanol. These two alcoholic solutions were centrifuged after standing for 12-18 hours at 4°C. The supernatants were then evaporated in a Water bath. To the residues was added 0.1ml H_2O and the whole volume was pippeted onto a chromatographic paper. Determination of GABA was carried out following

descending paper chromatography using the upper layer of the solvent system: butanol-glacial acetic acid-water (4:1:5. v/v). The chromatography was run three times 20-24 hours each, drying the paper every time before the next run. Development of the chromatogram and subsequent determination of GABA were done by the methods of Giri, (1953) and Bode, (1975) respectively. The chromatograms were sprayed with a solution of ninhydrin in acetone (0.5g ninhydrin, 95mls acetone, 0.9ml glacial acetic acid and water to 100mls). They were then dried by heating in an oven at 65°C for 30 min. The spots carrying GABA were cut out and eluted with 5ml of 0.005% copper sulphate solution in 76% ethanol (w/v). After 1-1½ hours the optical density was read at 500nm in a Zeiss PM Q 11 spectrophotometer against a standard concentration of GABA. The results were expressed as µmoles of GABA per gm wet tissue.

2.7. DETERMINATION OF NOREPINEPHRINE, DOPAMINE AND SEROTONIN IN SAME BRAIN REGION

The assay was carried out essentially by the method of Jacobowitz et al., (1978) as follows. 5ml of butanol was pipetted into a homogenizing tube and placed in ice cold water. Each tissue sample was rapidly weighed and added to the butanol and an appropriate amount of 0.01N HCl added such that the tissue water, plus 0.01 HCl equaled 0.75ml (assuming tissue

weight is 70% water) in 5ml butanol. The tissue samples were homogenized in a glass homogenizing tube with a motor driven teflon pestle until the tissue was uniformly distributed throughout the butanol. The homogenate was poured in a 12ml conical centrifuge tube and centrifuged for 10 min to sediment the tissue debris leaving a clear supernatant.

A 2ml aliquote of the butanol supernatant was put into a 12ml conical centrifuge tube containing 1.5ml of 0.1M phosphate buffer (pH 6.5) in ice water. For analysis of 5-HT an additional 2ml of butanol supernatant was put in a 15ml glass stoppered centrifuge tube containing 5ml heptane and 0.5ml of 0.1N HCl in ice water. Both the mixtures were vortexed for 20 sec. NE and DA were extracted into the phosphate buffer and 5-HT was extracted into the 0.1N HCl. The tubes were then centrifuged (3000 rpm) to separate the organic and aqueous layers and, using a vaccum with a water trap. The top organic layer including any tissue discs was aspirated, leaving as much of the aqueous layer as possible. A 1ml aliquot of the phosphate extract was then placed into 16 x 150mm test tube. A 0.3ml aliquot of the 0.1N HCl was put into another similar tube for the 5-HT assay.

The NE and DA in the phosphate buffer extract were oxidised into fluorophores by addition of 0.25ml

versene 4g disodium ethylenediamine tetra-acetic acid (EDTA) in 95mls of distilled water (pH 6.0-6.5) and vortexed briefly. Then at exactly 2 min intervals the following reagents were added in the order indicated. (1) 0.2ml iodine (4.8g potassium iodide plus 0.25ml iodine to 100ml with distilled water), vortexed briefly (2) 0.25ml alkaline sodium sulphite (2.5g Na_2SO_3 in 100mls 4N sodium hydroxide), vortexed briefly and (3) 0.3ml 5N acetic acid, vortexed briefly. The test tubes were placed in boiling water for 5 min, then in ice water for 1 min. The NE fluorescence was read immediately in an Amico-Bowmen spectrophotofluorometer excitation 385/emission 485. The DA fluorescence was read at least 20 min later at 320/385. The fluorescence reading was converted into µg NE or DA per g of tissue as described below.

The highly fluorescent condensation product of orthophthaldialdehyde and the 5-HT in the 0.1N HCl extract was prepared by adding 0.2ml of OPT solution (50mg of orthophthaldialdehyde in 100ml of absolute methanol) followed immediately by 1.5ml of concentrated (10N) HCl. The test tubes were vortexed briefly and then placed in boiling water for 10 min and then cooled to room temperature in tap water. The 5-HT fluorescence was read at 360/470 and converted into µg 5-HT per gm tissue as described below.

Standards were prepared and calculations performed as follows: Separate standard stock solutions of NE, DA and 5-HT were mixed as 400μg of base per ml of 0.01N HCl. All subsequent solutions were kept in ice water. 1ml of NE stock (400μg NE) and 1ml DA stock (400μg DA) were placed in 10ml volumetric flask and 0.01 N HCl added to 10mls (40μg/ml NE and 40μg/ml DA) and mixed. 1 ml of this solution was transferred into a 25ml volumentric flask and 0.01 N HCl added to volume (1.6μg/ml). 2mls of this solution was added to a test tube containing 2mls of 0.01N HCl and vortexed. This is standard A stock (0.8μg/ml). 1ml of standard A stock was added to 3mls 0.01N HCl in a test tube and vortexed. This is standard B stock (0.2μg/ml). 1ml of standard B stock was taken and added to 3mls of 0.01N HCl in a test tube and vortexed. This is standard C stock (0.05μg/ml). 0.5ml of A stock and 0.25mls of 0.01N HCl was added to 5mls butanol in a 12ml conical centrifuge and vortexed for 20 sec. This is A standard (0.4μ). 0.5mls of B stock and 0.25mls of 0.01N HCl was added to 5mls butanol and vortexed. This is C standard (0.025μg).

Two untreated rats of a size sex and strain similar to those used in the experiment were decapitated and the various brain regions dissected out. The brain parts were then weighed, homogenized and the homogenates centrifuged as described above. A 4ml aliquote of the clear butanol supernatant from

the A, B and C standards and from these tissue samples were placed into separate centrifuge tubes containing 3ml of the phosphate buffer, vortexed, centrifuged and the top organic phase aspirated off including any tissue disc leaving at least 2ml of the bottom phosphate extract. Duplicate 1ml aliquot were transferred into two test tubes labelled to identify initial and duplicate aliquot. One aliquot was oxidized into a fluorophore as above, the second aliquot was processed to provide a blank reading by adding, at exactly 2 min intervals, first the alkaline sulphite, then the iodine and finally the acetic acid. By reversing the order in which the iodine and the alkaline sulphite are added, the oxidation of NE and DA into fluorophores was prevented. The tubes were boiled cooled and read as above.

The blank fluorescence reading of the standards was subtracted from their corresponding oxidized fluorescence reading. The blank readings for a particular brain area averaged. This mean was subtracted from the mean blank value from each oxidized reading of similar area from the brains of experimental and control rats. The corrected fluorescence reading of each standard was divided by the amount of added NE or DA (ie corrected A÷0.4 = X_1, corrected B÷0.1 = X_2 corrected C÷0.025 = X_3) and the average slope calculated. $(X_1 + X_2 + X_3) \div 3$ = average slope . The corrected

tissue fluorescence reading was then divided by the average slope to determine µg NE or µg DA per sample and this was then divided by the weight in gm of the particular tissue sample to determine the amount of NE or DA per gram of tissue.

The 5-HT standards were prepared in a similar manner (1ml of 400µg/ml stock up to 10ml, 1ml of this up to 25ml, 2ml of this plus 2ml 0.01 N HCl is standard X stock; 1ml X stock plus 3ml of 0.01 N HCl is standard Y stock; 1ml X stock plus 3ml 0.01 N NCl is standard Z stock; 0.5ml of each (X, Y, and Z stocks) + 0.25ml 0.01N HCl each into separate centrifuge tubes containing 5ml butanol). Two reagent blanks were made by adding 0.75mls of 0.01 N HCl to 5ml butanol. These 5-HT standards and reagent blanks were processed as butanol homogenates as in the assay. They were then reacted with OPT reagent and read as above. The reagent blank fluorescence readings were averaged and this mean blank valve subtracted from the fluorescence readings of all the standards and all of the tissues. The corrected standard readings were divided by the amount of 5-HT in each corrected reading and the average slope to determine total 5-HT in the sample and then by the weight of the sample in grams to give 5-HT per gram wet tissue.

2.8. DETERMINATION OF ^{3}H-GABA REUPTAKE IN THE SYNAPTOSOMES

Determination of High-affinity ^{3}H-GABA uptake by synaptosomes was carried out by the method described by Miller et al., (1978). The initial velocity of ^{3}H-GABA uptake was determined by incubating aliquotes of synaptosomes for 2 min at 37°C in Krebs - phosphate buffer containing 100mmoles glucose, (124mM NaCl, 5mM KCl, 20mM NaH_2PO_4, 1.2mM KH_2PO_4, 1.3mM $MgSO_4$ and 0.75mM $CaCl_2$. pH 7.4), with various (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 10µmoles) GABA with labelled ^{3}H-GABA as a tracer, in a final incubation volume of 1ml. The reaction was stopped by the addition of 5 volumes of ice cold buffer. The samples were quickly filtered through millipore membrane filters with pore size ~ 0.45µm. The synaptosomes on the filters were re-washed twice with buffer, dried in air and radioactivity counted by liquid scintillation in a MARK-3 scintillation counter. The V_{max} and K_m for transport were found by ploting Eadie-Hofstee, Hanes plots of $\frac{G}{Pm}$ Vs G and Pm Vs Pm/ G and lineweaver-burk plots of 1/Pm Vs 1/ G which yielded straight lines.

2.9. DETERMINATION OF GLUTAMATE DECARBOXYLASE ACTIVITY (E.C. 4.1.1.15 GAD).

The determination of glutamate decarboxylase

activity was carried out by the fluorimetric method of Lowe et al., (1958) by the modification of Graham and Aprison, (1969).

To 100µl of tissue in a centrifuge tube (in an ice bath) were added 100µl substrate buffer pH 6.4, (1ml of 100mM glutamic acid, 40µl of 50mM pyridoxal phosphate and 40µl of 10% Triton X-100) and incubated for 30 min at 37°C. The reaction was stopped by adding 100µl of 15% TCA. To the blank tube, TCA was added prior to addition of substrate to precipitate the protein. 300µl ninhydrin (14mM Ninhydrin in 500mM sodium-carbonate buffer pH 9.4) was then added, vortexed, and centrifuged at 5000 rpm for 10 min. The supernatant was then placed in a clean test tube to which another 300µl ninhydrin was added. The tubes were incubated in a water bath at 60°C for 30 min, cooled and then 3ml of tartaric reagent (1.6g Na_2CO_3, 329mg Tartaric acid and 300mg $CuSO_4 . 5H_2O$ in 1000ml distilled water) added.

2-4µmoles/ml of standard GABA was carried through the assay. The fluorescence was read at 380nm excitation and 455nm emission on an Amico Bowmen spectrophotofluorometer. GABA produced was expressed in nmoles/min/mg protein.

2.10. DETERMINATION OF GLUTAMIC-γ-AMINOBUTYRIC TRANSAMINASE (E.C. 2.6.1.19)(GABA-T)

The assay was carried out using the incubation medium described by Vasilyev and Yeremin, (1968). The succinic semialdehyde produced from γ-aminobutyrate during incubation of the enzyme was measured fluorimetrically by the method of Salvador and Abers, (1959). In which the reagent 3, 5-Diaminobenzoic acid, condenses with α-methylenic aldehydes to form quinaldine derivatives as follows.

The incubation mixture including enzyme homogenate contained 83mM Tris-HCl buffer, pH 8.6, 67mM α-ketoglutarate, 67mM γ-Aminobutyric acid. 1% v/v Triton X-100 in a final volume of 500µlitres. The mixture was incubated for 30 min at 37°C and the reaction terminated by adding 100µlitres of 15% TCA. The tubes were centrifuged for 10 min at 5000 rpm. To 500µl of the supernatant was added 100µlitres of 0.25M Diaminobenzoic acid and placed in water at 60°C for 60 min. The fluorescence was read on an Amico-Bowman spectrophotofluorimeter at an excitation and emission wavelength of 405nm and 505nm respectively. The results were expressed as nmoles succinic semialdehyde formed per min per mg protein.

2.11. ASSAY FOR MONOAMINE OXIDASE ACTIVITY (E.C.1.4.3.4. MAO)

The assay was carried out essentially by the method described by Harada and Nagatsu, (1973).

MAO activity was determined by measuring the ammonia formed from the deamination of the amines. The fluorescence of NAD in alkaline solution which was described by Undenfriend, (1962), was applied for the assay and the fluorometric assay of ammonia described by Roche-Ramd,(1967), was modified for this MAO assay.

The standard procedure for fluorometric determination of ammonia based on coupling with GLDH assay system was carried out as follows. Standard ammonia solution 100μlitres, containing 0-100μlitres of ammonium sulphate was mixed with 400μlitres of GLDH assay mixture which was made up with; NADH, 0.5mg; 0.15M Tris-Malate buffer (pH 8.6), 1.7ml; 0.08M α-ketoglutarate, 100μlitres, and GLDH 5μlitres (50μg). The GLDH assay mixture was freshly mixed in that order mentioned above just before each assay. The reaction mixtures containing the standard ammonia solution in different concentrations and the GLDH assay mixture were shaken and left at room temperature for 60 min. Excess NADH in the reaction mixture was destroyed by the addition of 500μl of 1N HCl. The acidified

mixture was agitated with 1.6ml of a mixture composed with 9.0ml of 8N NaOH and 1.0ml of 0.1% H_2O_2 for transformation of NAD into a highly fluorescent compound. The alkaline mixture was kept for 5 min in a boiling water bath. The fluorescence intensity of each sample was measured at excitation and fluorescence wavelengths of 360nm and 460nm, respectively, in an Amico-Bowman spectrofluorometer.

The incubation mixture for MAO assay was made up in a final volume of 500µl, 200µl of 0.5M sodium phosphate buffer, pH 7.4; 200µl of 10mM substrate; 100µl of tissue homogenate. The tubes were incubated 30 min with gentle shaking at 37°C. The reaction was stopped by adding 100µl of 15% TCA. The tubes were centrifuged at 4000 rpm for 10 min. The liberated ammonia was determined by the GLDH system as described above. The monoamine oxidase was given as nmoles of ammonia liberated per min per mg protein.

2.12. STATISTICAL ANALYSIS

The data given in the text represents the mean of five to seven independent determinations in dublicate. The data were evaluated for statistical significance by the students 't' test after testing for homogeneity of variance. Statistical differences were assessed at the 95% level of confidence using a

two-tailed test between groups. The differences were considered significant for values of $p < 0.05$. Results are represented as means ± SEM.

CHAPTER 3

RESULTS

TABLE 1. The 11-oxycorticosteroid levels of rat blood plasma following cortisol administration

4 HOURS AFTER INJECTION			
Saline treated	Cortisol treated	P	n
325.1±14.7	208.0±7.7	< 0.05	6

7 DAYS CONTINUOUS TREATMENT			
Saline treated	Cortisol treated	P	n
254.1±7.5	434.4±12.8	< 0.05	7

Results expressed as nmoles cortisol/ml plasma ± SEM.

Table No. 1 shows the effects of hydrocortisone injection on the 11-oxycorticosteroid content of rat blood plasma. The levels of 11-oxycorticosteroids in the cortisol treated animals 4 hours after injection were decreased significantly by 36% as compared to control animals receiving normal saline. Following

7 days continuous injection of cortisol, the 11-oxy-corticosteroid levels in rat blood plasma increased by 71% when compared with saline treated animals.

TABLE 2. The effects of single administration of cortisol on GABA content in the hypothalamus and hippocampus of rat brain.

4 HOURS AFTER INJECTION			
"TOTAL" GABA CONTENT			
Brain region	Saline treated (N=6)	Cortisol treated N=5	P
Hypothalamus	3.95±0.06	4.88±0.05	< 0.05
Hippocampus	3.03±0.03	4.00±0.08	< 0.05

"FREE" GABA CONTENT			
Hypothalamus	1.97±0.03	2.43±0.03	< 0.05
Hippocampus	2.05±0.04	2.98±0.06	< 0.05

"BOUND" GABA CONTENT			
Hypothalamus	1.88±0.04	2.44±0.04	< 0.05
Hippocampus	0.98±0.02	1.02±0.02	> 0.1

The values are expressed as µmoles GABA/gm wet tissue ± SEM.

Table 2 shows the effects of hydrocortisone o the "Free", "Bound" and Total GABA content of the

hypothalamus and hippocampus regions of rat brain, 4 hours after injection. An increase in the total content of GABA was observed in both the brain regions. In the hypothalamus approximately equal increases in both the "Free" and "Bound" forms of GABA were noted. In the hippocampus only the "Free" form increased significantly. The "Bound" GABA was not altered significantly.

TABLE 3. Effects of multiple cortisol administration on GABA content in the hypothalamus and hippocampus of rat brain.

7 DAYS CONTINUOUS TREATMENT

Brain region	Saline treated	Cortisol treated	P	n
TOTAL GABA CONTENT				
Hypothalamus	4.11±0.09	6.67±0.18	< 0.05	6
Hippocampus	4.07±0.14	4.54±0.05	> 0.1	6
"FREE" GABA CONTENT				
Hypothalamus	2.69±0.07	4.03±0.14	< 0.05	6
Hippocampus	2.28±0.06	2.97±0.05	< 0.05	6
"BOUND" GABA CONTENT				
Hypothalamus	1.41±0.05	2.64±0.12	< 0.05	6
Hippocampus	1.80±0.09	1.58±0.01	> 0.1	6

The values are expressed as µmoles GABA/gm wet tissue ± SEM.

Table 3 shows the effects of prolonged cortisol injections on the three forms of GABA "Free", "Bound"

and Total in the hypothalamus and hippocampus regions of the rat brain. Prolonged injections of hydrocortisone increased the total GABA content of the hypothalamus by 62%. It did not have any effect on the total GABA content of the hippocampus region of the rat brain. The levels of "Free" GABA increased in both the regions by 50% and 30% respectively. The "Bound" form increased in.the hypothalamus by 87%. No such increases were observed in the hippocampus.

TABLE 4. The effects of hydrocortisone on glutamate decarboxylase enzyme and its distribution in discrete regions of rat brain.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	9.27±0.58	5.23±0.27	< 0.05	6
Hippocampus	4.34±0.87	5.19±0.70	> 0.1	6
Cerebellum	2.84±0.10	2.96±0.35	> 0.1	6
Pons-Medulla	2.06±0.38	2.22±0.35	> 0.1	6
Mid-brain	5.44±1.01	6.17±1.07	> 0.1	6
Caudate-Putamen	3.58±0.10	3.21±0.20	> 0.1	6
Cortex	4.82±0.14	3.78±0.28	< 0.05	6

The values are expressed as nmoles of GABA formed/min/mg protein ± SEM.

Table 4 shows the effects of a single hydrocortisone injection on the activity and distribution of glutamate decarboxylase in discrete regions of the rat brain. The activity of GAD was highest in the hypothalamus and lowest in the medulla and pons region, and also the cerebellum. The activity of the enzyme in the hippocampus was 53% lower than in the hypothalamus. Hydrocortisone injection caused significant decreases in GAD activity in the hypothalamus by 44% and the

cortex by 22% respectively. No significant changes were observed in the other brain regions. In cortisol treated animals, the GAD enzymatic activities of the hypothalamus and hippocampus regions were almost the same.

Table 5. Effects of dexamethasone on GAD activity in discrete regions of rat brain.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	dexamethasone treated	P	n
Hypothalamus	9.27±0.58	5.56±0.45	< 0.05	6
Hippocampus	4.34±0.87	2.72±0.53	01≤P≤0.05	6
Cerebellum	2.84±0.10	2.34±0.27	> 0.1	6
Medulla and Pons	2.06±0.38	2.00±0.18	> 0.1	6
Mid-Brain	5.44±1.01	5.43±0.54	> 0.1	6
Caudate-putamen	3.58±0.10	2.47±0.20	< 0.05	6
Cortex	4.82±0.14	3.59±0.43	< 0.05	6

The values are expressed as nmoles of GABA formed/min/mg protein ± SEM.

Table 5 shows the effects of dexametharone injection on glutamate decarboxylase activity in discrete brain regions. Dexamethasone injection caused a decrease in GAD activity in the hypothalamus, Caudate-putamen and Cortex regions of the brain respectively. The relative activities of the enzyme in the hippocampus and hypothalamus regions did not change after dexamethasone treatment, ie. 47% for saline treated animals and 49% for dexamethasone treated

animals. The highest decrease in activity was in the cortex where the activity of the dexamethasone treated animals was 74% less than in the control animals. The other decreases in activity were 60%, 63% and 69% for the hypothalamus, hippocampus and caudate-putamen respectively. Though statistically significant results were not recorded for most of the brain regions, the enzymatic activities of the dexamethasone treated animals remained well below those of the control animals.

TABLE 6. Effects of hydrocortisone on GAD activity in the synaptosomes of the hypothalamus and hippocampus.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	20.22±0.79	24.15±0.24	> 0.1	6
Hippocampus	14.73±0.58	15.53±0.40	> 0.1	6
7 DAYS CONTINUOUS TREATMENT				
Hypothalamus	15.93±0.66	17.08±1.00	> 0.1	6
Hippocampus	11.68±0.52	9.28±0.50	> 0.1	6

The values are expressed as nmoles GABA formed/min/mg protein ± SEM.

Table 6 shows the effects of hydrocortisone injection on the activities of glutamate decarboxylase in synaptosomes isolated from the rat hippocampus and hypothalamus regions. The activities of GAD was higher in synaptosomes isolated from the rat hypothalamus than in those from the hippocampus by 36.5%. There were no alterations in enzymatic activity following hydrocortisone injections, both single and prolonged.

TABLE 7. Effects of cortisol on GAD activity in the mitochondrial fraction of the hypothalamus and hippocampus.

4 HOURS AFTER TREATMENT				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	8.83±0.26	11.62±0.26	< 0.05	6
Hippocampus	8.80±0.33	8.55±0.38	> 0.1	6

7 DAYS CONTINUOUS TREATMENT				
Hypothalamus	8.98±0.47	10.17±0.42	> 0.1	7
Hippocampus	7.32±0.23	6.47±0.22	> 0.1	7

Values are expressed as nmoles GABA formed/min/mg protein ± SEM.

Table 7 shows the effects of single (after 4 hours) and prolonged (7 days) hydrocortisone injection on glutamate decarboxylase activity in the mitochondria isolated from the hypothalamus and hippocampus regions of the rat brain. The activities of GAD in the mitochondria isolated from both the hypothalamus and hippocampus remained the same. The activity of GAD in the hypothalamus mitochondria 4 hours after cortisol injection increased by 31.6% , while a 13%

increase was observed for the mitochondria of the same region following prolonged injection. No significant changes were observed in the hippocampus mitochondria.

TABLE 8. Effects of hydrocortisone on the regional distribution of GABA - Transaminase activity in the brain.

4 HOURS AFTER TREATMENT				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	30.23±3.05	26.99±2.35	> 0.1	6
Hippocampus	53.74±5.81	43.69±5.19	> 0.1	6
Cerebellum	30.23±2.37	40.31±6.77	> 0.1	6
Medulla and Pons	61.30±1.77	38.38±3.98	< 0.05	6
Mid-Brain	73.90±13.54	75.02±6.60	> 0.1	6
Caudate-Putamen	59.35±7.15	34.72±2.90	< 0.05	6
Cortex	20.58±2.72	not detectable	< 0.05	6

The values are expressed as nmoles of succinic semialdehyde formed/min/mg protein ± SEM.

Table 8 shows the distribution of GABA-Transaminase activity in discrete brain region following hydrocortisone treatment.

The activities of GABA-T in the hippocampus region is 78% higher than in the hypothalamus. The highest enzymatic activity was however, recorded in the mid-brain region. The lowest activity of the enzyme was in the cortex. Hydrocortisone injection

caused significant decreases in GABA-T activities in the medulla and pons (37%) and caudate putamen (41%). In the cortex region, no GABA-T activity could be detected after hydrocortisone injection.

TABLE 9. Dexamethasone induced changes in the distribution of GABA-T in discrete brain regions.

4 HOURS AFTER TREATMENT				
Brain region	Saline treated	dexamethasone treated	P	n
Hypothalamus	30.23±3.05	47.50±7.06	< 0.05	6
Hippocampus	53.74±5.81	43.68±2.74	> 0.1	6
Cerebellum	30.23±2.37	32.48±7.70	> 0.1	6
Medulla and Pons	61.30±1.77	36.95±6.19	< 0.05	6
Mid-Brain	73.90±13.54	57.11±5.40	> 0.1	6
Caudate-Putamen	59.35±7.15	57.87±5.10	> 0.1	6
Cortex	20.58±2.72	not detectable	< 0.05	6

The values are expressed as nmoles succinic semialdehyde formed/min/mg protein ± SEM.

Table 9 shows the effects of dexamethasone injection on GABA-Transaminase activity in discrete brain regions. Dexamethasone had a mixed effect on the activities of GABA-T in different brain regions. An increase in the transamination of GABA was noted in the hypothalamus where the activity of GABA-T increased by 58% as compared to control animals. The activity of the enzyme decreased in the rest of the brain regions reaching statistical significance in the medulla and Pons where a 40% decrease activity was recorded.

The activity of the enzyme in the cortex after dexamethasone injection could not be detected.

TABLE 10. Effects of hydrocortisone on GAGA-T activity in the synaptosomes isolated from the hypothalamus and hippocampus.

Brain region	Saline treated	Cortisol treated	P	n
4 HOURS AFTER INJECTION				
Hypothalamus	114.62±4.73	74.66±3.67	< 0.05	6
Hippocampus	106.25±7.86	127.24±13.92	> 0.1	6
7 DAYS CONTINUOUS INJECTION				
Hypothalamus	69.44±2.95	42.43±1.80	< 0.05	6
Hippocampus	59.44±3.84	38.92±2.94	01<P<0.05	6

The values are expressed as nmoles succinic semialdehyde formed/min/mg protein ± SEM.

Table 10 shows the effects of cortisol injections on GABA-Transaminase activity in synaptosomes isolated from the hypothalamus and hippocampus brain regions following single (after 4 hours) and prolonged (7 days).

The activity of GABA-Transaminase in synaptosomes isolated from the rat hypothalamus was slightly higher (10-15%) than in synaptosomes isolated from the hippothalamus. The activity of GABA-T decreased in the synaptosomes isolated from the hypothalamus by 65%.

No change was observed in the synaptosomes isolated from the hippocampus. Prolonged hydrocortisone injections produced decreases in GABA-Transaminase activity in both the synaptosomes isolated from the hypothalamus (61%) and hippocampus (65%) respectively.

TABLE 11. Effects of cortisol on GABA-T activity in the mitochondria of the hypothalamus and hippocampus.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	Cortisol	P	n
Hypothalamus	338.0±24.9	345.1±18.8	> 0.1	6
Hippocampus	238.4±18.0	115.3±12.2	< 0.05	6

7 DAYS CONTINUOUS INJECTION				
Hypothalamus	270.5±13.9	269.1±12.2	> 0.1	6
Hippocampus	121.4±5.3	132.3±6.9	> 0.1	6

The values are expressed as nmoles of succinic semialdehyde produced/min/mg protein.

Table 11 shows the effects of hydrocortisone injection on the activities of GABA-Transaminase in hypothalamus and hippocampus mitochondria, following single (after 4 hours) and prolonged (7 days) injections.

The activities of GABA-T in the hypothalamus mitochondria was higher than the hippocampus mitochondria. There was also a difference in GABA-T activity for the same region between rats receiving single and

prolonged injections. Hydrocortisone injection caused a significant decrease in GABA-Transaminase activity in hippocampal mitochondria by 48%. No such changes were observed in other brain fractions.

TABLE 12. The effects of hydrocortisone on the K_m and V_{max} of ^{3}H-GABA uptake in synaptosomes derived from the hypothalamus and hippocampus 4 hours after injection.

HYPOTHALAMUS SYNAPTOSOMES		
	K_m	V_{max}
Saline treated	2.84±0.47	0.58±0.06
Cortisol treated	2.59±0.36	0.61±0.10
P	> 0.1	> 0.1
n	6	6

HIPPOCAMPUS SYNAPTOSOMES		
Saline treated	2.27±0.29	0.57±0.06
Cortisol treated	2.12±0.25	0.56±0.04
P	> 0.1	> 0.1
n	6	6

The K_m and V_{max} values are expressed as moles x 10^{-6} and nmoles/min/mg protein respectively ± SEM.

Table 12 shows the effects of hydrocortisone on the K_m and V_{max} values of the ^{3}H-GABA uptake by synaptosomes isolated from the hypothalamus and hippocampus brain regions 4 hours after cortisol injection. No changes were observed in either the K_m or the V_{max} of the two synaptosomal populations from the two regions.

TABLE 13. The effects of hydrocortisone on the K_m and V_{max} of ^{3}H-GABA uptake in synaptosome derived from the hypothalamus and hippocampus following 7 days of cortisol injection.

HYPOTHALAMUS SYNAPTOSOMES		
	K_m	V_{max}
Saline treated	2.32±0.28	0.62±0.07
Cortisol treated	2.09±0.26	0.58±0.06
P	> 0.1	> 0.1
n	6	6

HIPPOCAMPUS SYNAPTOSOMES		
Saline treated	1.72±0.23	0.51±0.05
Cortisol treated	3.85±0.34	1.19±0.18
P	< 0.05	< 0.05
n	6	6

The K_m and V_{max} values are expressed as moles x 10^{-6} and nmoles/min/mg protein respectively ± SEM.

Table 13 shows the effects of hydrocortisone on the K_m and V_{max} of the ^{3}H-GABA uptake by synaptosomes isolated from the hypothalamus and hippocampus brain regions following 7 days of continuous injection. A significant increase in the K_m and V_{max} values for

^{3}H-GABA uptake was observed in synaptosomes isolated from the hippocampus in rats receiving prolonged cortisol injections. The increase in K_m was 124% while an increase of 133% was observed for the V_{max} in these fractions. No changes were observed in synaptosomes isolated from the hypothalamus.

TABLE 14. Effects of hydrocortisone on the regional distribution of norepinephrine in the rat brain.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	1.48±0.16	1.40±0.11	> 0.1	6
Hippocampus	0.40±0.04	0.44±0.11	> 0.1	6
Cerebellum	0.23±0.18	0.23±0.01	> 0.1	6
Medulla and Pons	0.75±0.09	0.69±0.04	> 0.1	6
Mid-Brain	0.93±0.06	0.57±0.05	< 0.05	6
Caudate-putamen	0.61±0.06	0.44±0.01	< 0.05	6
Cortex	0.17±0.01	0.10±0.01	< 0.05	6

Results are expressed as µg norepinephrine/gm wet tissue ± SEM.

Table 14 shows the effects of hydrocortisone injection on the regional distribution of NE in brain regions. Norepinephrine content was highest in the hypothalamus and lowest in the cortex regions of the brain respectively. The concentration of norepinephrine in the hippocampus was between 70 - 73% lower than in the hypothalamus. Cortisol injection decreased the NE content of the midbrain, caudate-putamen and the cortex by 37%, 27% and 41% respectively. No significant changes could be noted in the other brain regions.

TABLE 15. Effects of dexamethasone on the regional distribution of NE in the rat brain.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	dexamethasone treated	P	n
Hypothalamus	1.66±0.08	2.50±0.42	$<$ 0.05	6
Hippocampus	0.50±0.04	0.52±0.05	$>$ 0.1	6
Cerebellum	0.45±0.01	0.42±0.01	$>$ 0.1	6
Medulla and Pons	1.07±0.08	1.24±0.19	$>$ 0.1	6
Mid-Brain	0.96±0.09	1.24±0.11	$>$ 0.1	6
Caudate-Putamen	0.80±0.06	0.84±0.12	$>$ 0.1	6
Cortex	0.16±0.01	0.20±0.02	$<$ 0.05	6

Results are expressed as µg NE/gm wet tissue ± SEM.

Table 15 shows the effects of dexamethasone injection on the NE content of discrete brain regions.

Dexamethasone injection increased the content of NE in the hypothalamus by 51% and in the cortex by 25% respectively.

TABLE 16. Effects of hydrocortisone on the regional distribution of dopamine in the brain.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	0.30±0.02	0.22±0.01	< 0.05	6
Hippocampus	0.13±0.01	0.08±0.02	> 0.1	6
Cerebellum	0.04±0.01	0.03±0.01	> 0.1	6
Medulla and Pons	0.08±0.01	0.03±0.01	< 0.05	6
Mid-Brain	0.18±0.01	0.09±0.01	< 0.05	6
Caudate-Putamen	0.17±0.02	0.10±0.02	> 0.1	6
Cortex	0.12±0.01	0.02±0.01	< 0.05	6

Results are µg dopamine/gm wet tissue ± SEM.

Table 16 shows the effects of hydrocortisone injection on the distribution of dopamine in discrete brain regions.

Dopamine content was highest in the hypothalamus and lowest in the cerebellum. The hippocampus content of DA was (57%) lower than that of the hypothalamus. Hydrocortisone injection decreased the content of DA in the hypothalamus (27%) medulla and pons (62%), mid-brain (50%) and cortex (83%) respectively. No statistically significant decreases were observed in th_ other brain regions. The levels of dopamine remained

low in cortisol treated animals as compared to saline treated ones.

TABLE 17. Effects of dexamethasone on the regional distribution of dopamine in rat brain.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	dexamethasone treated	P	n
Hypothalamus	0.30±0.02	0.28±0.04	> 0.1	6
Hippocampus	0.13±0.02	0.12±0.03	> 0.1	6
Cerebellum	0.07±0.01	0.06±0.01	> 0.1	6
Medulla and Pons	0.09±0.01	0.15±0.02	> 0.1	6
Mid-Brain	0.16±0.01	0.16±0.01	> 0.1	6
Caudate-Putamen	0.20±0.04	0.14±0.01	> 0.1	6
Cortex	0.11±0.01	0.10±0.01	> 0.1	6

Results are expressed as μg dopamine/gm wet tissue ± SEM.

Table 17 shows the effects of dexamethasone injection on the content of dopamine in discrete brain regions.

Dexamethasone injection did not alter the content of dopamine in the brain regions studied.

TABLE 18. Effects of hydrocortisone on the regional distribution of 5-HT in the brain.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	1.50±0.09	1.22±0.13	> 0.1	
Hippocampus	0.85±0.06	0.69±0.04	> 0.1	
Cerebellum	0.30±0.02	0.34±0.02	> 0.1	
Medulla and Pons	1.05±0.08	0.33±0.04	< 0.05	
Mid-Brain	1.95±0.22	1.19±0.07	< 0.05	
Caudate-Putamen	1.31±0.12	0.77±0.06	< 0.05	
Cortex	0.22±0.01	0.11±0.04	< 0.05	

Results are expressed as µg serotonin/gm wet tissue ± SEM.

Table 18 shows the effects of hydrocortisone on the distribution of 5-HT in discrete brain regions. Serotonin concentration was highest in the mid-brain and hypothalamus and lowest in the cortex. The hypothalamus serotonin content was (76%) higher than in the hippocampus. Hydrocortisone injection decreased the concentrations of serotonin in the medulla and pons (69%), mid-brain (39%), caudate-putamen (41%) and cortex (50%), respectively. Similar decreases in 5-HT content were observed in the other brain regions though they were not statistically significant.

TABLE 19. Effects of dexamethasone on the regional distribution of 5-HT in the brain.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	dexamethasone treated	P	n
Hypothalamus	1.23±0.15	1.54±0.09	> 0.1	6
Hippocampus	0.98±0.01	1.91±0.14	< 0.05	6
Cerebellum	0.14±0.01	0.50±0.01	< 0.05	6
Medulla and Pons	0.76±0.07	1.20±0.07	< 0.05	6
Mid-Brain	1.34±0.16	1.37±0.01	> 0.1	6
Caudate-Putamen	1.09±0.07	1.27±0.13	> 0.1	6
Cortex	0.25±0.04	0.35±0.03	> 0.1	6

Results are expressed as µg serotonin/gm wet tissue ± SEM.

Table 19 shows the effects of dexamethasone on the distribution of 5-HT in discrete brain regions. Dexamethasone injection increased the 5-HT content of most brain regions. Statistically significant increases were observed in the hippocampus (94%) cerebellum (257%), and medulla and pons (58%) respectively.

TABLE 20. Effects of hydrocortisone on Monoamine oxidase activity towards dopamine in specific brain regions

4 HOURS AFTER INJECTION				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	1.33±0.11	0.48±0.01	< 0.05	6
Hippocampus	0.80±0.16	0.39±0.16	> 0.1	6
Cerebellum	0.56±0.05	0.23±0.10	< 0.05	6
Medulla and Pons	0.48±0.03	0.36±0.10	> 0.1	6
Mid-Brain	0.72±0.17	0.50±0.13	> 0.1	6
Caudate-Putamen	0.86±0.16	Not detectable	< 0.05	6
Cortex	0.58±0.10	Not detectable	< 0.05	6

Results are expressed as nmoles Ammonia formed/min/mg protein ± SEM.

Table 20 shows the effects of hydrocortisone injection on monoamine oxidase activity with dopamine as substrate, in discrete brain regions.

Monoamine oxidase activity was highest in the hypothalamus and lowest in the medulla and pons region of the rat brain. The activity of MAO in the hippocampus was 40% lower than in the hypothalamus. Hydrocortisone injection decreased the activities of the

enzyme in most brain regions. Statistically significant decreases were recorded in the hypothalamus (64%) cerebellum (59%). In the caudate-putamen, and cortex regions, no MAO activity could be detected following hydrocortisone administration.

TABLE 21. Effects of dexamethasone on monoamine oxidase activity towards dopamine in specific brain regions.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	dexamethasone treated	P	n
Hypothalamus	1.33±0.11	1.78±0.25	> 0.1	6
Hippocampus	0.80±0.16	1.12±0.31	> 0.1	6
Cerebellum	0.56±0.05	0.48±0.15	> 0.1	6
Medulla and Pons	0.48±0.03	0.39±0.12	> 0.1	6
Mid-Brain	0.72±0.17	0.28±0.06	< 0.05	6
Caudate-Putamen	0.86±0.16	0.55±0.14	> 0.1	6
Cortex	0.58±0.10	0.31±0.09	> 0.1	6

The values are expressed as nmoles ammonia produced/min/mg protein ± SEM.

Table 21 shows the effects of dexamethasone injection on monoamine oxidase with dopamine as substrate.

Injection of dexamethasone to intact rats caused a significant decrease in only the mid-brain region of the rat brain. No significant changes were observed in the other brain regions studied.

TABLE 22. Effects of hydrocortisone on monoamine oxidase activity towards 5-HT in brain regions.

4 HOURS AFTER INJECTION				
Brain regions	Saline treated	Cortisol treated	P	n
Hypothalamus	2.86±0.01	3.25±0.51	> 0.1	6
Hippocampus	3.03±0.28	3.25±0.31	> 0.1	6
Cerebellum	1.17±0.18	1.39±0.20	> 0.1	6
Medulla and Pons	1.19±0.09	1.16±0.07	> 0.1	6
Mid-Brain	2.16±0.18	2.84±0.27	> 0.1	6
Caudate-Putamen	2.15±0.13	2.56±0.15	> 0.1	6
Cortex	1.19±0.23	0.90±0.06	> 0.1	6

Results are expressed as nmoles ammonia produced/ min/mg protein ± SEM.

Table 22 shows the effects of hydrocortisone injection on monoamine oxidase activity with serotonin as substrate.

Monoamine oxidase showed highest activity in the hippocampus and lowest in the cerebellum and cortex regions when 5-HT was used as substrate. The actiyity of the enzyme in the hippocampus was slightly higher by 6% than in the hypothalamus. Hydrocortisone injection failed to cause any changes in the enzymatic activity in any of the brain regions studied.

TABLE 23. Effects of dexamethasone on monoamine oxidase activity towards 5-HT in brain regions.

4 HOURS AFTER TREATMENT				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	2.86±0.01	3.55±0.32	< 0.05	6
Hippocampus	3.03±0.28	2.26±0.29	> 0.1	6
Cerebellum	1.17±0.18	1.17±0.17	> 0.1	6
Medulla and Pons	1.19±0.09	0.93±0.07	> 0.1	6
Mid-Brain	2.16±0.18	2.30±0.18	> 0.1	6
Caudate-Putamen	2.15±0.13	2.15±0.09	> 0.1	6
Cortex	1.19±0.22	0.88±0.12	> 0.1	6

Results are expressed as nmoles ammonia produced/ min/mg protein ± SEM.

Table 23 shows the effects of dexamethasone injection on monoamine oxidase activity towards serotonin as substrate.

Injection of dexamethasone increased the activity of MAO in only the hypothalamus region of the brain. In this brain region the ammount of ammonia produced from serotonin was 24% higher for animals receiving cortisol when compared to the saline treated animals. The other brain regions were not affected.

TABLE 24. Effects of hydrocortisone on monoamine oxidase activity towards tryptamine in discrete brain regions.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	Cortisol treated	P	n
Hypothalamus	1.84±0.44	3.45±0.40	< 0.05	6
Hippocampus	0.97±0.19	0.78±0.05	> 0.1	6
Cerebellum	0.51±0.17	0.68±0.06	> 0.1	6
Medulla and Pons	0.37±0.10	0.35±0.04	> 0.1	6
Mid-Brain	0.62±0.14	1.29±0.04	< 0.05	6
Caudate-Putamen	1.34±0.16	0.64±0.08	< 0.05	6
Cortex	0.67±0.21	0.30±0.10	> 0.1	6

Results are expressed as nmoles of ammonia produced/min/mg protein.

Table 24 shows the effects of hydrocortisone on monoamine oxidase activity towards tryptamine following hydrocortisone injection. Hydrocortisone injection increased the MAO activity towards tryptamine in the hypothalamus (87.5%) and the mid-brain (108%), but decreased, the activity in the caudate-putamen (52%). No changes were observed in the other regions.

TABLE 25. Effects of dexamethasone on monoamine oxidase activity towards tryptamine in discrete brain regions.

4 HOURS AFTER INJECTION				
Brain region	Saline treated	dexamethasone treated	P	n
Hypothalamus	1.84±0.44	2.83±0.75	> 0.1	6
Hippocampus	0.94±0.19	2.00±0.29	< 0.05	6
Cerebellum	0.51±0.17	2.12±0.43	< 0.05	6
Medulla and Pons	0.37±0.10	1.07±0.20	< 0.05	6
Mid-Brain	0.62±0.14	2.03±0.29	< 0.05	6
Caudate-Putamen	1.34±0.16	1.70±0.31	> 0.1	6
Cortex	0.67±0.21	1.64±0.21	< 0.05	6

Results are expressed as nmoles of ammonia produced/min/mg protein.

Table 25 shows the effects of dexamethasone injection on monoamine oxidase activity with tryptamine as substrate. Dexamethasone had a profound effect on MAO activity increasing the transamination of tryptamine in the hippocampus (54%) cerebellum (316%), medulla and pons (189%), mid-brain (227%) and the cortex (145%) respectively.

CHAPTER 4

DISCUSSION

4.1. 11-OXYCORTICOSTEROID CONTENT OF BLOOD PLASMA FOLLOWING HYDROCORTISONE INJECTION

The main aim of the study was to determine the alterations in neurotransmitter metabolism in brain tissue under the influence of different corticosteroid levels in the organism. The fall in 11-oxycorticosteroid levels observed in table 1 four hours after injection of hydrocortisone could be attributed to several reasons:-

That administration of corticosteroids inhibits the pituitary-adrenocortical system and that regulation is affected by corticosteroid feedback mechanism (Critchlow, 1973; Dafny et al., 1973). Exogeneous administration of corticosteroids has been known to decrease cortisol levels and the exogeneous corticoids prevent the glucocorticoid levels from rising during stress (Verniko-Danellis, 1964; Legori et al., 1965). In humans, it was shown that fifteen minutes after hydrocortisone injection, 95% of the hormone dissappeared exponentially within an hour (Padolfo and Maraine, 1964). The exogeneous administration of glu-

cocorticoids brings about a short term inhibition to the secretion of endogeneous corticosteroids by a negative feedback mechanism (Lissark and Endreci, 1967). There is reason, therefore to believe that it may be these two basic factors which bring about a decrease in the concentrations of 11-oxycorticosteroids in the plasma of rats studied.

Prolonged elevation of corticosteroids was achieved by several injections of cortisol. 24 hours after the last injection of hydrocortisone following seven days of continuous injection, the concentration of 11-oxycorticosteroids in the blood plasma remained high. It should be taken into account that, the major corticosteroid in rat adrenal glands is corticosterone and cortisone, while hydrocortisone which is predominant in humans, is found in small quantities (Lissark and Endreci, 1967). It can be assumed therefore, that the increase in 11-oxycorticosteroids in blood plasma seen in these rats receiving prolonged cortisol injections was solely due to the exogeneous hormone, since endogeneous corticosteroid production in such cases is greatly inhibited. (Yudaev and Afinogenova, 1960).

Dexamethasone has a more potent depressive action on the central control of ACTH secretion than other corticosteroids (Sapronov, 1973). In animals treated

with dexamethasone (300μg/Kg i.p), two to three hours after dexamethasone injection, the blood plasma concentration of corticosteroids decreased about two fold as compared to intact animals. This shows the inhibition of the hypothalamo-pituitary-adrenal system by both dexamethasone and cortisol 4 hours after injection (Dafny et al., 1973; Lissark and Endreci, 1967).

4.2. EFFECTS OF CORTICOSTEROIDS ON THE METABOLISM OF GAMMA γ-AMINOBUTYRIC ACID IN THE BRAIN:-

A study of γ-Aminobutyric acid content in the hypothalamus and hippocampus regions of the brain is of major importance as a tool towards getting adequate information as to how corticosteroids affect GABA metabolism in the limbic system. The present study reports the "Free", "Bound" and "Total" GABA content of these two regions of the limbic system and how the three forms of GABA respond to hydrocortisone administration (tables 2 and 3). It is apparent that, the increases in GABA content of the hypothalamus region following hydrocortisone administration were due to approximately equal increaments in "Free" as well as "Bound" GABA. This was not the case with the hippocampus where a significant increase was observed in the "Free" form. Consequently, short term as well as prolonged elevations of cortisol in the organisms' blood causes an elevation in the content of GABA

("Total", "Free" as well as "Bound" forms) in the hypothalamus. The effects of the hormone was however more pronounced during prolonged injections (table 3). In the hippocampus, approximately equal increases were observed in only the "Free" form in both cases of cortisol administration. In earlier studies, single injections of hydrocortisone did not alter the hypothalamus GABA content (Kononenko and Mishunina, 1982) or decreased its levels altogether (Hahn et al., 1977). Varrying changes in the GABA content of the hippocampus under corticosteroid influence have been reported. Kononenko and Mishunina, (1982) reported that one hour after hydrocortisone injection, the levels of the amino acid transmitter had decreased while four hours latter showed an increase. The latter finding seems in agreement with the findings in the present study.

Considering the important role played by glutamate decarboxylase in the metabolism of GABA and the functional status of the central nervous system, the regional distribution of GAD enzymatic activity in discrete brain regions and the effects of hydrocortisone and dexamethasone on its distribution were studied (table 4 and 5). The effects of hydrocortisone on the activities of this GABA synthesizing enzyme in synaptosomes and mitochondrial fractions isolated from the rat hypothalamus and hippocampus were also studied. Both hydrocortisone and dexamethasone injections

brought about a decrease in GAD activity in the hypothalamus and cortex regions of the brain. Single as well as prolonged injections of hydrocortisone however, did not significantly alter the activities of GAD in the subcellular fractions of the brain regions studied. There were no significant changes in GAD activity in the synaptosomal fractions isolated from the hypothalamus and hippocampus regions of the brain (table 6). The absence of changes in the specific activity of GAD in synaptosomal fractions of the hypothalamus and hippocampus as opposed to the decrease in the enzymatic activity observed in tissue homogenates might be due to the effects of the hormones on the protein content and distribution in the fractions isolated from these brain regions. It has been shown that, hydrocortisone affects the content and distribution of proteins in subcellular fractions isolated from the brain (Kalinskaya, 1979).

Single injections of cortisol increased GAD activity in mitochondrial fractions of both the hypothalamus and hippocampus regions (table 7). Prolonged injections of the hormone failed to alter the enzymatic activity in mitochondria isolated from the hippocampus (table 7). These results indicate an activation in the biosynthesis of GABA in the synaptosomes and mitochondria isolated from the hypothalamus after single cortisol injection. The biosynthesis of GABA

in the brain tissue seems to be inhibited as seen from the effects of the hormone on GAD activity in the crude homogenates from the hypothalamus and hippocampus regions of the brain. These findings do not agree with results reported earlier which show an increase in GAD activity in the rat hypothalamus 4 hours after hydrocortisone injection (Kononenko and Mishunina, 1982). The decrease in GAD activity in whole hypothalamus and hippocampus tissue as opposed to the increase in its activity in the synaptosomes isolated from these regions of the brain can be explained if hydrocortisone causes a redistribution of the GAD enzymatic protein in subcellular fractions. This would be in agreement with the findings reported by Kalinskaya (1979) that hydrocortisone affects the content and distribution of proteins in subcellular fractions isolated from the brain.

Alterations in the GABA content of the brain regions following corticosteroid administration to rats could be brought about not only by the effects of the steroid hormone on the GABA synthesis, but also on its degradation. The effects of hydrocortisone and dexamethasone on the regional distribution of the GABA degrading enzyme GABA-transaminase was also studied. The activity of the enzyme in subcellular fractions of the hypothalamus and hippocampus regions of the ra brain and the effects of hydrocortisone on this acti-

vity was examined too. The subcellular localization of GABA-T in the hypothalamus and hippocampus regions of the brain of control animals is in agreement with earlier reports as to the enzyme being located mainly in the mitochondria (Sytinski, 1977; Buu and Van Gelder, 1974). The study has thus shown the regional specificity in the distribution of GABA-transaminase. Firstly, the activity of the enzyme in the mitochondria isolated from the hypothalamus was higher than in those isolated from the hippocampus. Secondly, the activity of GABA-T in the mitochondria was 3-4 times higher than in the synaptosomal fractions while this difference in the hippocampal fractions was about 200% (tables 10 and 11). The difference in GABA-T activity of control animals receiving single and prolonged injections of saline also exhibited significant differences. These differences could be due to several possible reasons namely:-

(i) Due to differences in injection stress between the groups of animals.

(ii) The enzymatic activity of GABA-T possibly shows circadian periodicity. This has been shown with tyrosine hydroxylase (Cahill et al., 1981).

These results, thus show that hydrocortisone injections decrease the intensity of GABA transamination. Moreover, while the effects of single cortisol injection

was shown to affect the enzyme activity in the mitochondria isolated from the hippocampus and synaptosomes isolated from the hypothalamus, prolonged excess of cortisol did not affect the activity of mitochondrial GABA-T but decreased the activity of the synaptosomal enzyme in both the hypothalamus and hippocampus (tables 10 and 11). These experiments thus show that cortisol is an inhibitor of GABA-transaminase at both synaptosomal and mitochondrial levels in the hypothalamus and hippocampus regions of the brain. This might probably explain why an accumulation of GABA was found in the two brain regions studied (tables 2 and 3). The nature and mechanism of inhibition however remains unknown.

In view of the lack of an enzymatic mechanism for deactivation of GABA in the synaptic cleft, a probable physiological role of the high affinity GABA re-uptake (Km ~ 4.10^{6}M) was proposed and has recently been intensively investigated. It is thought that, the re-uptake of GABA is the main mechanism behind the utilization of GABA as a neurotransmitter in the synaptic cleft (Martin, 1973; Miller et al., 1978; Robinson and Wells, 1973; Simon et al., 1974). The effects of corticosteroids on the re-uptake of GABA by synaptosomes is therefore of major interest since an alteration in the re-uptake process under different hormonal levels in the body organism could turn out to be one of the loops in the mechanism of action of corticosteroids on the functional status of the neuronal activity

(Miller et al., 1978).

To evaluate the kinetic parameters of ^{3}H-GABA uptake by synaptosomes, it was established in preliminary experiments the conditions suitable for initial velocity of ^{3}H-GABA uptake. The uptake velocity of the amino acid transmitter in the presence of 10μmoles of ^{3}H-GABA at 37°C was linear for 5 minutes. The uptake of ^{3}H-GABA under these conditions was proportional to the quantity of protein in the sample in the range of 50-400μg.

The uptake of ^{3}H-GABA by synaptosomes from various brain regions of control animals varied between 0.33 to 0.95 nmoles/min/mg protein and for the hypothalamus it ranged between 0.58-0.62, while for the hippocampus it ranged from 0.51-0.57 nmoles/min/mg protein. The Km values of GABA uptake was close for the regions studied. It was however slightly higher for the hypothalamus. The V_{max} and K_m values characterising the high affinity GABA uptake found in our study are in agreement with those found by other investigators (Hitsemann and John, 1978; Simon et al., 1974). The results of the study demonstrate the existance of a high affinity energy dependent, saturable ^{3}H-GABA transport system in a preparation of rat hypothalamus and hippocampus nerve ending particles. There are several possibilities concerning the necessity and significance of such a system. One possibility is

that the transport was occuring into resealed glial elements which contaminate the nerve ending preparations (Hitsemann and John, 1973). However the marked even regional distribution of the transport system observed in the study would tend to argue against such a conclusion. Secondly it has been shown that the high affinity GABA uptake is associated with a homogeneous synaptosomal population of neuronal origin (Miller et al., 1978). There is also the possibility that ^{3}H-GABA transport system is associated with a specific population of nerve ending particles derived from GABA neurons. Another possibility is that a large amount of GABA is normally synthesized in GABA nerve terminals via glutamate decarboxylase and this pool can be stored and released. If such a situation existed, a high affinity transport system may have developed in order to deactivate the released GABA (Hitsemann and John, 1978).

The intesity of high affinity ^{3}H-GABA uptake by the hypothalamic synaptosomes during short durational as well as during prolonged injections was not altered. (tables 12 and 13). In the hippocampus, during prolonged injections of cortisol, a sharp increase in the maximum velocity and K_m of ^{3}H-GABA uptake was observed which on one side indicates an increase in the number of binding sites and on the other side a decrease in the velocity of the carrier and therefore a decrease

in the net transport of the amino acid neurotransmitter. Miller et al., (1978) have shown that adrenalectomy specifically increases the synaptosomal transport of GABA in the hippocampus of rats. It can be proposed from the results of the study that prolonged administration of hydrocortisone caused a suppresion of GABA re-uptake in the hippocampal synaptosomes, which led to an accumulation of GABA in the synaptic cleft. Excess of GABA in the synaptic cleft would act not only on the postsynaptic receptors of the effector cell, but also on the presynaptic GABA - receptors, which would trigger off a feed-back mechanism, this in turn could cause the release of the neurotransmitter and probably block further accumulation of the transmitter in the synaptic cleft (Bandarienko, 1979). The tiggering off of the feed-back mechanism through the regulation of the presynaptic GABA - receptor activity could lead to an increase in the functional sites for binding and transport of GABA. There is evidence about the presence of GABA receptors (Brennan, 1982).

The presynaptic receptors can serve as the centre of action for different modulators, transported in the brain by the blood currrent, amongst these modulators are hormones (Starke, 1977). It is also known that the action of glucocorticoids on GABA uptake can be modulated through their interaction with receptors

for steroid hormones in hippocampus nervous cells (McEwen, 1979; Miller et al., 1978). A decrease in synaptosomal uptake of catecholamines caused by the action of glucocorticoids has already been established (Shaliapina, 1980). Inhibition of GABA uptake in the hippocampus following prolonged hydrocortisone injection would undoubtedly lead to an accumulation of GABA in the synaptic cleft. Coupled with a malfunction of the uptake mechanism observed in the study, this would result in intensified post-synaptic inhibition of impulses caused by GABA. The latter agrees well with the observed increase in "Free" GABA observed in the hippocampus. As established earlier, an alteration in the content of "Free" GABA in the hippocampus is connected to changes in the excitability of this brain region (Kononenko and Mishunina, 1982).

By studying the total GABA content of the hypothalamus and hippocampus regions of the brain, the concentrations of the "Free" and "Bound" forms, the activities of the enzymes responsible for GABA metabolism glutamate decarboxylase and GABA - transaminase, and the ^{3}H-GABA uptake by synaptosomes, the present study has set up a simple animal model that can be used in the study of endocrine interactions with the central nervous system. The present study has shown that single and prolonged hydrocortisone administration leads to an increase in the total GABA content of the hypothalamus and also the "Free" and "Bound" forms of

the neurotransmitter. The effects of the hormone after prolonged injections is even more pronounced (tables 2 and 3).

The increase in GABA observed in the hypothalamus 4 hours after cortisol administration was probably due to an increase in the synthesis as well as the inhibition of the catabolism of GABA (tables 6, 7, 10 and 11). This is evidenced by an increase in GAD activity in both the synaptosomes and mitochondria derived from the hypothalamus and a corresponding decrease in GABA-T activity in the synaptosomes of this brain region. A decrease in GAD activity in whole hypothalamus tissue observed following hydrocortisone injection should be taken to mean that subcellular GAD activity increases at the expense of the cytosolic enzyme, since hydrocortisone has been shown to affect the distribution of proteins in subcellular fractions in the brain (Kalinskaya, 1979). Note that during the 7 day administration of cortisol, only a decrease in the degradation of GABA was observed. A decrease in GABA-T activity was also seen only in the synaptosome derived from the hypothalamus.

4.3. BALANCES AMONG CENTRAL BIOGENIC AMINES IN DISCRETE REGIONS AND THE EFFECTS OF CORTICOSTEROIDS ON THEIR DISTRIBUTION.

In the peripheral autonomic nervous system there exists a balance between the effects of its two opposing components, sympathetic (adrenergic) and parasympathetic (cholinergic) on the organ systems. In the event of induced alterations of function of some of these systems, a homeostasis mechanism operates to restore the activity to normal levels. Within the central nervous system similar reciprocal balances between opposing actions in the functional system may also exist (Fuxe et al., 1973). However, such balance within the CNS become complicated by the presence of a number of putative neurotransmitters. In addition to norepinephrine and acetylcholine, as in peripheral nervous system, there are also other transmitters, such as dopamine, serotonin and γ-aminobutyric acid which participate in modulation of various functions including behaviour. Attempts to explain the role which biogenic amines play in behaviour must answer one main empirical question. Whether the monoamine in question is excitatory or inhibitory to behaviour. Several studies carried out on self-stimulation according to Pradhan, (1975) have showed that:-

(i) Norepinephrine is excitatory

(ii) Serotonin is inhibitory

(iii) The influence of norepinephrine and serotonin on stimulation is reciprocal.

(iv) Dopamine is essential to motor-function and in this way enters into stimulation behaviour.

A relative distribution study of three putative neurotransmitters studied, norepinephrine, dopamine and serotonin in discrete brain regions showed that serotonin was most abundant followed by norepinephrine while dopamine was least abundant in all the brain regions studied (tables 14 - 19). These results show that there is probably a higher density of NE fibres in the brain as compared to 5-HT and DA fibres (Saavedra et al., 1974; Versteeg et al., 1976). It was also apparent that NE was highest in the hypothalamus and midbrain and lowest in the cortex and cerebellum, this suggests the presence of most NE fibres being concentrated in the former brain regions. The results obtained in the present study show that hydrocortisone administration decreased brain amine levels in several brain regions. These findings are similar to the effects of adrenalectomy on brain amine levels reported by Rastogi et al., (1978). The neurotransmitters most affected by cortisol were dopamine and serotonin. Since neurotransmitter levels in brain regions are normally associated with the brain excitability a fall

in neurotransmitter levels as seen in the study would most probably be accompanied by a fall in neuronal response to stimuli. Dopamine and not norepinephrine was most affected by cortisol injection, and since dopamine is essential to motor-function and in this way enters into stimulation behaviour, it shows that the excitation behaviour affected by hydrocortisone is through the motor-function (Pradhan, 1975). This would imply that the peripheral nervous system is involved in the possible excitation mechanism caused by hydrocortisone. In the hypothalamus and hippocampus, brain regions of the limbic system which play a major role in neuroendocrine regulation, only dopamine levels were affected (tables 14 - 19), suggesting that neuroendocrine modulation by hydrocortisone may be affected through DA in these regions. Serotonin levels also fell in the hypothalamus and hippocampus but could not reach statistical significance. The fall in 5-HT levels under cortisol influence in most brain regions studied with the exception of the cerebellum is in agreement with what other authors have reported (Curzon et al., 1968, Scapagnini et al., 1969). The results of the above experiments show that hydrocortisone administration has a similar effect on neurotransmitter levels as that observed in adrenalectomised rats (Rostogi et al., 1978). This is not surprising when one considers that cortisol injection like adrenalectomy, caused the levels of 11-oxycorticosteroids to fall (table 1).

The major route of tryptophan degradation is via the liver enzyme tryptophan pyrrolase. The activity of this enzyme has been shown to be increased by hydrocortisone administration (Knox, 1951). It has been suggested that increased activity of this enzyme would result in a greater rate of metabolism of tryptophan down this pathway with a resultant decrease of tryptophan available to the brain (Curzon, 1965). These observations are in good agreement with the findings in this study since a decrease in brain tryptophan would most probably result in a decrease in brain 5-HT as was observed in this study. (table 18). The decrease in 5-HT levels observed above are of major pharmacological interest since it has been suggested that depressive illness may be caused by the induction of liver tryptophan pyrrolase by plasma corticosteroids leading to decreased synthesis and turnover of 5-HT in the brain (Knox, 1951., Curzon and Green, 1971).

Curzon and Green (1968) showed that increasing the activity of tryptophan pyrrolase by hydrocortisone injection caused moderately decreased brain 5-HT and that inhilation of the induction of pyrrolase blocked the amine changes. The brain amine change following hydrocortisone has thus subsequently been confirmed in this study (table, 18). Similar changes have been seen following betamethasone, corticosterone and cortisol administration (Yuwiler et al., 1971.,

Scapagnini, 1969). A likely reason for the brain changes is that increased pyrrolase activity has a more direct effect on the availability of tryptophan for transport into the brain. Following hydrocortisone injection, brain tryptophan is decreased (Knott and Curzon, 1975). There is also a decrease in free plasma and total tryptophan and the tryptophan content of muscle and liver (Curzon, 1965). These data suggest therefore that stimulations of acute stress as caused by hydrocortisone injection in this study leads to a decrease in brain 5-HT content, an odd situation since stress also appears to lead to increased 5-HT release from nerve endings. (Curzon and Green, 1971). This suggests the presence of other factors regulating brain 5-HT concentrations (Curzon and Green, 1968). It may also be of significance that after injection of hydrocortisone, the mean fall in the concentrations of 5-HT in the hypothalamus and hippocampus was less than in other areas. This might point to a particular insensitivity of 5-HT metabolism in these regions to adrenocortical/ or pyrrolase changes which could be relevant to suggested possible special response of the hypothalamus and hippocampus to stress (Curzon, 1971).

Dexamethasone a synthetic corticosteroid had a mixed effect on the neurotransmitter levels. It caused a fall in 5-HT levels in the hypothalamus and DA levels in the caudate-putamen, while causing a rise in NE, DA and 5-HT levels in most brain regions (tables 15, 17, 19). This latter effect of dexamethasone on the levels of the biogenic amines is opposite to that one caused by hydrocortisone. The mechanisms for the changes in CA turnover found after treatment with exogeneous hormones or after experimentally induced changes in hormone levels are unknown. The following two principal possibilities are the most likely ones:-

(i) The hormones acts directly on the central catecholamine neuron systems via a direct effect on the catecholamine cell bodies and/or nerve terminals facilitating or counteracting the nervous impulse induced release of catecholamine in the brain (Fuxe et al., 1973).

(ii) The hormones could act indirectly via other neuron system, which in turn could change the nervous impulse flow in the central catecholamine neurons. It may be pointed out that in this case the hormones may act either directly on central neurons or via

peripheral afferent pathways (Fuxe et al., 1973).

It can be argued that the action of the adreno-cortical steroids on biogenic amine turnover observed in the present study could be due to an increase in ACTH secretion, since rapid and dramatic falls of corticosteroids levels are known to enhance ACTH secretion with a latency of about 2 hours (Smelik, 1963). Such an action could very well contribute to the changes in biogenic amine turnover observed but cannot be the only effect of the corticosteroids in view of the relatively varying effects obtained with exogeneous cortisol and dexamethasone on the monoamines, seen in the present study. Furthermore the study has shown that cortisol and dexamethasone both cause a fall in 11-oxycorticosteroid levels and thus a rise in ACTH production, but have varying effects on neurotransmitter levels in different brain regions. These results thus suggest an action of the corticoids independent of effects on ACTH secretion probably by directly acting on the hypothalamus (Fuxe et al., 1973). On the basis of the data from the present studies, it seems possible that in hypocortisolism as observed in the present study, the changes in monoamine turnover were partly mediated via the increase in ACTH secretion and partly via a loss of circulatory levels of glucocorticoids. This view is supported by the fact that the same cor-

ticosteroid (dexamethasone) while decreasing the 11-oxycorticosteroid levels (and thus increasing ACTH production) could cause two different effects on the same neurotransmitter in different brain regions. Since it has been shown earlier that corticosteroids can alter biogenic amine levels (Rastogi et al., 1978), it seems logical to think that some of the changes in monoamine turnover found after hormonal administration should partly be due to increases in ACTH secretion.

It is interesting to evaluate whether the effects on biogenic amine turnover by adrenocortical steroids and ACTH can be related to other well known effects of these hormones such as changes in brain excitability (Woodbury, 1954; Woodbury and Vernadakis, 1966) and/or changes in behaviour (De Wied, 1969). To elucidate this question, it is important to note that glucocorticoids such as hydrocortisone and dexamethasone have similar actions on the central catecholamine neurons i.e they counteract the catecholamine turnover found in hypocortisolism (Fuxe et al., 1973). In view of the above it seems possible that behavioural effects found in relation to conditional responses after changes of pituitary-adrenal activity are partly mediated via changes in activity of monoamine neurons. Weiss et al., (1970) have suggested that the pituitary-adrenal axis mainly moderates fear-motivated response. ACTH increases fear and glucocorticoids decrease fear

in this way increasing and decreasing fear-motivated responses, respectively. The present results are in good agreement with this view, since fear could probably be of a higher intensity and better retained with increased arousal, which probably is present in states with low activity of 5-HT neuron systems coupled with high NE levels observed during cortisol treatment. The reverse is true with high activity of 5-HT neuron systems observed in dexamethasone treatment. It should be added that high doses of glucocorticoids is regarded as a relatively stressful condition in general. These results thus suggest that glucocorticoids can act to reduce stress and anxiety and can therefore normalise overstimulated behaviour (Woodbury, 1958) by increasing activity in 5-HT neurons.

Evidence that NE neurons could be involved in the inhibition of ACTH secretion by reducing activity in the corticotrophin releasing factor containing neurons (Fuxe et al., 1973). The evidence is mainly based on pharmacological experiments. It has been demonstrated that catecholamine depletors and receptor blocking agents such as reserpine and chloropromazine and tyrosine-hydroxylase inhibitors increase ACTH secretion. Catecholamine releasing agents such as amphetamine or catecholamine precursors such ad Dopa inhibit stress - induced hypersecretion of corticosterone secretion (Van Loon et al., 1969; Ganong, 1970;

Scapagnini et al., 1970). The inhibitory systems here seem to be noradrenergic and not dopaminergic. In view of the above the NE changes in the hypothalamus and the limbic system in relation to changes in the pituitary - adrenal axis activity should be interpreted along the lines that the noradrenergic system is inhibited with regard to release of CRF and subsequently of ACTH (Ganong, 1971). The inhibitory feedback action of circulating corticosteroids on ACTH secretion can therefore be mediated via the NE neurons, since the corticosteroids decrease NE neuronal activity (Fuxe et al., 1970). Such an action would aim at increasing ACTH secretion as was found in our study and could possibly explain the great descrepancies in variations in this feedback mechanism, since obviously corticoids also inhibit inhibitory and not only excitatory systems in relation to ACTH secretion.

4.4. EFFECTS OF CORTICOSTEROIDS ON MONOAMINE OXIDASE ACTIVITY IN BRAIN REGIONS.

It has been reported that changes in the concentrations of biogenic amines can be due to the effects of corticosteroids on the activities of some enzymes involved in their metabolism. It has been reported that hydrocortisone causes a change in monoamine metabolism by affecting the activities of monoamine oxidase (Kononenko et al., 1970; Valikina, 1972). Alte-

rations in biogenic amine content in brain regions observed in the present study following hydrocortisone and dexamethasone administration to rats can be attributed to the effects of the steroid hormone on the neurotransmitter degradation by monoamine oxidase. In the present study, the activity of monoamine oxidase was studied using different specific substrates. The effects of hydrocortisone and dexamethasone on the MAO activities was also studied (tables 20 - 25).
When specific brain regions were analysed for MAO activity following corticosteroid administration, different regions showed different specificities towards individual amines. The enzyme showed the highest activity towards 5-HT in all the brain regions studied. For all the three substrates used (serotonin, dopamine and tryptamine), MAO activity was highest in the hypothalamus and hippocampus brain regions. The distribution of the enzyme activity was in close agreement with the distribution of the neurotransmitters, i.e the highest specific activity was found in the regions with the highest amine content. These findings are in agreement with those found by Valikina, (1972).

The amine hypothesis of depression states that at least some cases of endogeneous depression are caused by a depletion of certain monoamines in the brain (Shildkraut, 1965). The hypothesis is supported by the findings that all drugs which elevate mood in-

crease the brain concentrations of free monoamines, such as noradrenaline, whereas drugs which depress mood decrease these concentrations (Schanberg et al., 1967). In the present study, the effects of corticosteroids on the activity of MAO showed that hydrocortisone may decrease the oxidative deamination of dopamine in several brain regions including the hypothalamus. No enzymatic activity could be detected in the caudate-putamen and cortex following cortisol treatment. It is well known that monoamine oxidase plays an important role in regulating intraneuronal levels of dopamine, norepinephrine and serotonin in central aminergic neurons. The inhibition of MAO therefore seen in the present study caused by hydrocortisone suggests that more dopamine would be available following release. This would contribute towards the excitability of the neurons by affecting the motor-neuronal function (Fuxe et al., 1973). Monoamine oxidase inhibitors are valuable antidepressants, frequently producing dramatic improvements in patients who have failed to respond to treatment (Randrup et al., 1979). They inhibit a number of enzyme systems, and it is often assumed that the inhibition of MAO is the most likely way in which they effect mental depression. It is also known that increased ACTH production corresponds to an excited hypothalamus (Vernikos-Danellis, 1964; Legori et al., 1965; Critchlow, 1973).

Dexamethasone had a mixed effect on the oxidative deamination of dopamine. The activity of the enzyme increased in the hypothalamus and hippocampus but decreased in the rest of the brain regions studied. The significance of these results are unclear but could probably be attributed to the different functional roles played by the hypothalamus and hippocampus in the control of hormonal secretions. Stimulation of the hippocampus is normally followed by a suppression of the secretion of adrenocorticotrophic hormone (Porter, 1953; Endroczi and Lissak, 1959). Stimulation of the hypothalamus, on the contrary, results in a marked rise in ACTH (Vernikos-Danellis, 1964, Critchlow, 1973). Dexamethasone also increased the deamination of 5-HT in the hypothalamus. The increase in 5-HT deamination would mean that less extraneuronal serotonin would be available after release, while a decrease in dopamine deamination following cortisol treatment would avail more extraneuronal dopamine (Schanberg et al., 1967).

The results of the study thus indicate that corticosteroids do affect monoamine oxidase activity in discrete brain regions, thus affecting the amount of amines available after release. This would undoubtedly affect interneuronal levels of monoamines, thus affecting the excitability of the brain (Schanberg et al., 1967). The effects however vary from brain region

region and the corticosteroid used. This could be due to the structural diversity of the brain regions and the different roles they play in maintaining body homeostasis. It may be evident therefore that the effects brought about by corticosteroids to biogenic amine metabolism could be complex and probably involve other factors not connected with ACTH production.

4.5. CONCLUSION

Finally from the present studies, it can be concluded that:

(1) The administration of exogeneous corticosteroids can inhibit the pituitary-adrenocortical system and regulation can be affected by corticosteroid feedback mechanisms through several neurotransmitters in the central nervous system.

(2) The increase in GABA content of the hypothalamus and hippocampus regions following corticosteroid administration is due to equal increase in the "Bound" and "Free" GABA. This is brought about by an activation in the biosynthesis of GABA at the mitochondrial and synaptosomal levels and a decrease in the intensity of GABA transamination in these subcellular organelles.

(3) In the rat hypothalamus and hippocampus brain regions, there exists a high affinity energy dependent saturable ^{3}H-GABA re-uptake system. This transport system is very stable to flactuations in corticosteroid levels. The efficiency of transport in the hippocampus is however impaired during prolonged hormonal administration.

(4) Corticosteroid administration alters biogenic amine levels in several brain regions by affecting monoamine oxidase activity in discrete brain regions, thus affecting the amount of amine available after release. This undoubtedly affects interneuronal levels of amines and thus the excitability of the brain. The amines affected most by corticosteroids are dopamine and serotonin.

(5) Corticosteroid injections result in a greater rate of metabolism of tryptophan down the liver pyrrolase pathway due to increased tryptophan pyrrolase activity with a resultant decrease of free tryptophan available to the brain. Increasing the activity of tryptophan pyrrolase by corticoid injections causes moderately decreased brain 5-HT and the inhilation of the induction of pyrrolase blocks the amine changes.

(6) The inhibitory feedback action of circulating corticosteroids on adrenocorticotropin secretion can be mediated via putative neurotransmitter neurons, by inhibiting inhibitory systems in relation to ACTH secretion.

(7) Behavioural effects found in relation to conditional responses after changes of pituitary-adrenal activity may be partly mediated via changes in the activity of biogenic amine neurons.

(8) Alterations of corticosteroid status of rats, does cause changes in concentrations of the putative neurotransmitters sufficient to cause gross behavioural changes. The changes seen in brain NE, DA 5-HT and GABA are modulated through adrenocortical steroids and these hormones play a role in the regulation of biogenic amine metabolism. Corticosteroids may be therefore used in therapy of affective disorders, under certain conditions.

REFERENCES

Acs, Z., Palkovits, M., Stark, E. (1980): Changes of glutamic acid decarboxylase activity after dexamethasone in selected areas of the rat brain. Neurosci. Letters, 19: 97 - 101.

Argiz, C. A. G., Pasquini, J. M., Kaplun, B. A., Gomez C. J. (1967): γ-Aminobutyric acid in the brains of adrenalectomised rats receiving hydrocortisone. Brain Res. 6: 635.

Aoyama, T. (1958): Distribution of GABA in the Crayfish and Lobsters. Biochemistry Japan, 30: 452.

Azmitia, E. C., McEwen, B. S. (1969): Corticosterone regulation of tryptophan hydroxylase in mid-brain of the rat. Science 166: 1274 - 1276.

Antony, J. R., Philip. J. L., Schatzberg. A. F. Marijean, M. M. (1985): The effects of a single acute dose of dexamethasone on monoamine and metabolite levels in rat brain. Life sciences 36: 2491 - 2501.

Azmitia, E. C. Jr., McEwen, B. S. (1974): Adrenal-cortical influence on rat brain tryptophan hydroxylase activity. Brain Res. 78: 291 - 302.

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Balikina, E. M. (1972): Monoamine oxidase activity of some organs in the rat following prolonged hydrocortisone administration. In: Fiziologia, Biokhimia i patologia Endocrinoi systemi. Kiev: Zdarovia 2nd ed 9 - 11.

Banay, Schwartz M., Zanchin G., Gurman, T. De., Lajtha, A. (1979): The effects of corticosteroids on amino acid content of brain tissue preparations. Psychoneuroendocrinology. 4: 207 - 217.

Bandarienko, T. T. (1979): Presynaptic receptors and their role in the regulation of release of neuropharmacological substances. Pharmacology and Toxicology. 42: 559 - 567.

Bertilsson, Leif., Mao, Chi-Chiang and Erminio Costa. (1977): Application of principles of steady state kinetics to the estimation of γ-amino-butyric acid turnover rate in nuclei of rat brain. J. Pharmacol and exp. Ther. 200: 277 - 284.

Bode, F. (1975): Eine vereinfachung und Verbesserung der methode zur quantitativen Bestimmung von Aminosauren und peptiden mittels der Ninhydrin kypferkomplexes. Biochem. Ztschr. 326: 453 - 456.

Brennan, M. J. W. (1982): GABA autoreceptors are not coupled to benzodiazepine receptors in rat cerebral cortex. J. Neurochem. 38: 264 - 266.

Buu, N. T., Van Gelder, N. M. (1974): Differences in biochemical properties of GABA, aminotransferase from synaptosome - enriched and cytoplasmic mitochondria enriched subcellular fractions of mouse brain. Can. J. Physiol. and Pharmacol. 52: 674 - 680.

Caesar, P. M., Collins, G. G. S., Sandler, M. (1970): Catecholamine metabolism and monoamine oxidase activity in adrenalectomised rats. Biochem. Pharmacol. 19: 921 - 926.

Cahill, A. L., Ehret, F. C. (1981): Circadian variations in the activities of tyrosine hydroxylase, tyrosine aminotransferase and tryptophan hydroxylase: Relationship to catecholamine metabolism. J. Neurochem 37: 1109 - 1115.

Chan Ke Pan (1963): Intercellular localization of glutamate decarboxylase in the rat cortex. Acta Physiologica 26: 275 - 281.

Chirkasova, L. S., Pikulev, A. T. (1972): The action of glucocorticoids on glutamic acid metabolism. Endokrinologie. 60: 273 - 278.

Cotman, C. W. (1974): Isolation of synaptosomal and synaptic plasma membrane fractions. In: Methods in Enzymology V.XXXI. Ed. by Colowick, S. P. and Kaplan, N. O.: 445 - 452. Academic Press. N.Y. San Fransisco, London.

Critchlow, V. (1978). Pituitary adrenal function following ablation of medial basal hypothalamus. Proc Soc Exp Biol Med. 142: 749 - 754.

Curtis, D. R. (1978): Amino acid neurotransmitters. Proc. Austral. Physiol. and Pharmacol Soc. 9: 99 - 103.

Curzon, G. (1965). The Biochemistry of depression In; Biochemical aspects of Neurological Disorders. 2nd Series Eds. J. N. Cummings and M. Kremer. 1 Blackwell. Oxford.

Curzon, G., and A. R. Green. Effects of hydrocortisone on rat brain 5-hydroxytryptamine. Life Sci. 7: 657 (1968).

Curzon, G., Green, A. R. (1970): Rapid method for

the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Brit. J. Pharamacol. 39: 653 - 655.

Curzon, G. and Green, A. R. (1971). Regional and subcellular changes in the concentrations of 5-HT and 5-HIAA in the rat brain caused by hydrocortisone, Dl-α-methyltryptophan, L-kynurenine and immobilization. Brit. J. Pharmacol. 43: 39 - 52.

Dafny, N., Phillips, M. I., Taylor. A. N. Gilman, S. (1973): Dose effects of cortisol on single unit activity in hypothalamus, reticular formation and hippocampus of freely behaving rats correlated with plasma steroid levels. Brain Res., 59: 257 - 272.

Davgalevich N. N. (1969): Alterations in free and bound aminoacids linked to the TCA cycle and their acetylated products in the CNS following X-ray exposure. PhD thesis Minsk.

Davidienko, L. M. (1974): The effects of hydrocortisone on the content of catecholamines in the brains of newborn rats. Fiziol. Zhur. 20: 71 - 76.

De Vellis, J., Kukes, G. (1973): Regulation of glial cell functions by hormones and ions. Texas Rep. Biol. Med. 31: 271 - 295.

De Wied D. (1977): Pituitary adrenal system hormones and behaviour. Acta Endocr., Copenh 85: 9 - 18.

Dobrijanskaya, A. K. (1973): Psychological and Neuro-physiological disturbances in endocrine diseases. Medicine "Moscow": 190 - 210.

Dorner, G. (1978: Hormones, brain development and fundamental processes of life. In:- Hormones and brain development. Amsterdam: Elsevier/ North -Holland Biochem. Press: 13 - 25.

Edwards, P. M., Rousse G. G. (1980): Effects of adrenalectomy and corticosteroids on three enzymes involved in amino acid metabolism in the brain of adult and newborn rats. J. steroid Biochem. 13: 567 - 569.

Enna, S. J., Snyder, S. H. (1975). Properties of γ-aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fractions. Brain Res. 100: 81 - 87.

Endroczi, E. and Lissark, K. (1959): The role of the

mesencephalon and archicortex in the activation and inhibition of the pituitary - adrenocortical system. Acta Physiol. Acad. Sci. hung 15: 25 - 31.

Endroczi, E., Lissak K. (1963): Effect of hypothalamic and brain stem structure stimulation on pituitary - adrenocortical function. Acta. Physiol. Acad. Sci. Hung. 24: 67 - 77.

Fonnum, F. (1968): The distribution of glutamate decarboxylase and aspartate transaminase in subcellular fractions of rat and guinea - pig brain. Biochem. J. 106: 401 - 412.

Fuxe, K, Ungersted, U. Hokfelt (1970): Central monoaminergic systems and hypothalamic function. In: The Hypothalamus. Ed. by L. Martini, M. Motta and F. Fraschini. Academic Press, N. Y, London.

Fuxe, K., Hokfelt, T. Jonsson, G., Lidbrink, P. (1973): Brain-endocrine interaction: Are some effects of ACTH and adrenocortical hormones on neuroendocrine regulation and behaviour mediated via central catecholamine neurons? In: Hormones and brain function: 409 - 425. Plenum Press N.Y.

Gaidina, G. A. (1967): Excitatory state of adrenergic structures of the central nervous system following ACTH and hydrocortisone administration. Probl. Endocr. 13: 71 - 77.

Ganong, W. F. (1970): Control of adrenocorticotropin and melanocyte - stimulating hormone secretion. In: The Hypothalamus. Ed. by L. Martini, M. Motta and F. Fraschini. Academic Press N.Y, London.

Ganong, W. F. (1971): Evidence that adrenergic systems in the brainstem inhibit ACTH secretion. In: Median Eminence. Karger, Basel, Munchen, Paris, New York.

Ganong, W. F. (1972): Role of catecholamines and Acetylcholinesterase in regulation of endocrine function. Life Sciences. 15: 1401 - 1414.

Giri, K. V: (1953): Quantitative amino acid determination on chromatograms through the formation of complexes between copper and ninhydrin. Indian Inst. Sci. 35: 145 - 174.

Glabov, R. N., Krizhanovski, G. N. (1978). Functional biochemistry of synapses. Medicine M.: 328 - 335.

Glowinski, J., Iversen, L. L. (1966): Regional studies of catecholamines in the rat brain. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-Dopa in various regions of the brain. J. Neurochem. 13: 665 - 669.

Gornall, A. G., Bardawill, C. S., David, M. M. (1949): Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751 -766.

Graham. I., Aprison, M., (1969): Distribution of sóme enzymes associated with the metabolism of glutamine in rat spinal cord. J. Neurochem., 16: 559 - 566.

Haber, B. Kuriyama, K., Roberts, E. (1970): An anion stimulated L-glutamic acid decarboxylase in non-neural tissues: occurence and subcellular localization in mouse kidney and developing chick embryo brain. Biochem. Pharmacol. 19: 1119 -1136.

Hahn, Z., Telegdy, G., Lissak, K. (1977): The rat of the pituitary-adrenal system in changes induced by noxious stimuli in hypothalamic gamma-aminobutyric acid content in the rat. Acta Physiol. Acad. Sci. hung. 40: 325 - 330.

Harada, M., Nagatsu, T. (1973): A sensitive fluorometric assay for monoamine oxidase, activity Anal. Biochem. 56: 283 - 288.

Hartree, E. (1972): Determination of protein. a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48: 423 - 427.

Henke, H. Pannum, F. (1976): Topographical and subcellular distribution of choline acetyltransferase and glutamate decarboxylase in pigeon epic rectum. J. Neurochem. 27: 387 - 391.

Hitsemann, B., John, A. (1978): A comparison of GABA and β-alanine transport and GABA binding in the rat. J. Neuro-Chem. 30: 471 - 477.

Hosli, L., Hosli, C. (1979): Autoradiographic studies on the cellular localization of GABA and β-alanine uptake by neurons and glial tissue in culture. Adv. Exp. Med. Biol. 128: 205 - 218.

Iversen, L. L., Bloom, F. E. (1972): Studies on the uptake of ^{3}H-GABA and ^{3}H-glycine in slices of homogenates of rat brain and spinal cord by electron microscopic autoradiography. Brain Res., 41: 131 - 143.

Iversen, L., L., Johnston, G. A. R. (1971): GABA uptake in rat central nervous system: Comparison of uptake in slices and homogenates and the effects of some inhibitors. J. Neurochem, 18: 1939 - 1950.

Iversen, L. L., Neal, M. J. (1968): The uptake of ^{3}H-GABA by slices of rat cerebral cortex. J. Neurochem. 15: 1141 - 1149.

Jacobowitz, D. M. and Richardson, J. S. (1978): Method for the rapid determination of NE, DA and 5-HT in the same brain region. Pharm Biochem & Behaviour 8: 515 - 519.

Kalinskaya, L. N. (1979): Proteolitic activity of different regions and subcellular fractions of rabbit brains following changes in corticosteroid levels in the organism. Ph.D thesis. Kiev, University.

Kazarian, B. A. (1972): The connection between gamma-aminobutyric acid and tyrosine in the livers of intact and adrenalectomised rats. Vaprosi biokhimia mozga Yerevan. 7: 101 - 110.

Kendall, D. A., McEwen, B. S., Enna, S. J. (1982): The influence of ACTH and corticosterone on

^{3}H-GABA receptor binding in rat brain
Brain Res., 236: 365 - 374.

Kitamura, M. J. 1960. Distribution of the enzymes of the glutamic acid and GABA Cyeles in rat brain. J. Med. Soc. 66: 238.

Kizer, J. S., Palkovits, M., Zivin, J. Brownstein, M., Saavedra, J. M., Kopin, I. J. (1974): The effect of endocrinological manipulations on tyrosine hydroxylase and dopamine-β-hydroxylase activities in individual hypothalamic nuclei of the adult male rat. Endocrinology 95: 799-812.

Klimenko, G. A., Sergeev, O. N. (1963): Method of calculating the quantity of hydrocortisone, secreted by adrenal glands in response to stimulation of their function by hypophysial ACTH. In: Theories on new apparatus and methods. 2nd ed: 52 - 57.

Komissarenko, V. P., Lisénko, V. C. (1974): The effects of adrenalectomy on the metabolism of Nitrogeneous substances in the brain. In: X-Congress of all union of physiological society. Moscow. 1: 399 - 401.

Komissarenko, V. P., Kononenko, V. Y. (1980): The effects of hormones on the metabolism of the central nervous system. Vest. Acad. Med. Nauk U.S.S.R. 7: 37 - 43.

Komissarenko, V. P., Kononenko, V. Y., Davidienko, L. P. (1977): The effects of inhibitors of the adrenal cortex function on the metabolism of catecholamines in some brain regions. Fiziol. Zhur. 23: 291 - 296.

Kononenko, V. Y., Mishunina, T. M. (1983): Content of gamma-aminobutyric acid and glutamatedecarboxylase activity in the brains of intact and adrenalectomised rats following injection of cyproheptadine. Fiziol. Zhur. 54: 31 - 35.

Kononenko, V. Y., Mishunina, T. M. (1982): Effects of hydrocortisone on the content of gamma-aminobutyric acid and glutamate decarboxylase activity in some brain regions of rats. UKr. Biochem. J. 54: 31 - 35.

Kononenko, V. Y., Zriakov, O. M., Kononenko, T. K. (1970): Effects of hydrocortisone on serotonin metabolism and monoamine oxidase activity in the brains of rabbits. Physiology Journal. 16: 600 - 608.

Knizley, H. (1972): The hippocampus and septal area as primary target sites for corticosterone. J. Neurochem. 19: 2737 - 2745.

Knott, P. J. and Curzon, G. (1975): The effects of nicotinamine on brain tryptophan metabolism in the rat. Abstract 5th Int. Meeting of Int. Soc. Neurochem. Barcelona. Spain.

Krieger, D. T. (1978): The central nervous system and Cushing's disease. Med. Clin. N. Amer. 62: 261 - 268.

Krogsgaard-Larsen, P., Scheel-Kruger, J, Kofod, H., (1979): Gaba-Neurotransmitters. The Alfred Benzon foundation symposium - 12. Academic Press N. Y.

Krnjevic, K., (1974): Chemical nature of synaptic transmission in vertebrates. Physiol. Rev. 54: 418 - 540.

Legori, M., Motta, M., Zanisi, M., Martini, L., (1965): Pituitary-adrenal activity following hydrocortisone and ACTH administration. Acta Endocr. (Kbh), Suppl 100: 155 - 161.

Levi, W. B., Redburn, D. A., Cotman, C. W. (1973): Stimulus-coupled secretion of γ-aminobutyric acid from rat brain synaptosomes. Science 18: 676 - 686.

Lisenko, V. C. (1968): Some parameters of nitrogen metabolism in the brains of adrenalectomised rabbits. In: Hormones and the brain. Kiev: 62 - 66.

Lissark, K. (1973): Hormones and brain function. In. Proceedings of the second congress of the international society of psychoneuroendocrinology Budapest. Hungary.

Lissark, K. Endreci, E. (1967): Neuroendocrine regulation of adaptative behaviour. Budapest Acad. Sc. : 219 - 227.

Lovell, R. A., Elliot, K. A. G. (1963): The γ-aminobutyric acid and factor I content of brain. J. Neurochem. 10: 479 - 488.

Lowe, I. P., Robins, E., Eyerman, G. S. (1958): The fluorimetric measurement of glutamic decarboxylase and its distribution in brain. J. Neurochem. 3: 8 - 18.

Martin, D. L. (1973): Kinetics of the sodium dependent transport of GABA by synaptosomes. J. Neurochem. 21: 345 - 356.

Martin, D. L., Smith, A. A. (1972): Ions and the transport of GABA by synaptosomes. J. Neurochem. 19: 841 - 855.

Mastashkina, A. Y., Mishunina, T. M. (1975): Metabolism of some amino acids in the brain tissue and blood plasma of rabbits under the influence of hydrocortisone. UKr. J. Biochem. 47: 379 - 384.

Mayer, J. S., Luirie, U. N. (1979): Glucocorticoids and hippocampal enzyme activity. Brain Res. 166: 172 - 175.

McEwen, B. S. (1973): Adrenal steroid binding to presumptive receptors in the limbic brain of the rat. In: Neuroendocrinologie de l'axe corticotrope. Ed by Paul Dell. 79 - 98 INSERM PARIS.

McEwen, B. S. (1976): Steroid hormone receptors in developing and mature brain tissue. Soc. Neuroscience symposia. 1: 50 - 66.

Miller, A., Chaptal, C., McEwen, B. (1978): Modulation of high affinity GABA uptake into hippocampal synaptosomes by glucocorticoids. Psychoneuroendocrinology. 3: 155 - 164.

Mishunina, T. M: Masteshkina, A. Y. (1977): Amino acid metabolism in the tissue of rabbits following adrenalectomy and subsequent hydrocortisone and ACTH administration. UKr J. Biochem. 49: 12 - 18.

Molinoff, P. B., Kravitz, E. A. (1968): The metabolism of gamma-aminobutyric acid (GABA) in the lobster nervous system glutamic decarboxylase. J. Neurochem. 15: 391 - 409.

Moor, P. de, Steeno, O. (1960): Fluorimetric determination of the plasma 11-hydroxycorticosteroids in man. Acta Endocrinol. 33: 297 - 307.

Nana, A., Mircioiu, C., Newmann, E. 1965. γ-Aminobutyric system in the rat brain. Fiziol. norm. sipatol. 11: 251.

Padolfo, L., Moraine, S. (1964): Influenza della surrenectomia sulla attivita della GABA transaminasi e della glutamino decarbossilasi di corteccia cerebrale di ratto. Giorn. Biochim. 13: 1790 - 1797.

Peck, E. J.,Shaeffer, J. M., Miller, A. L., Clark, J. M. (1976): Analysis of receptive sites in synaptosomal fractions. In: J. A. Ferrendelli, B. S. McEwen and S. H. Synder (Eds). Neuroscience symposia, Bethesda, 1: 1 - 27.

Porter, R. W. (1953): The central nervous system and stress induced eosinopenia. Recent Progr. Hormon Res. 10: 1 - 27 - 31.

Pradham, S. N. (1975): Balances among central neurotransmitters in Self-Stimulation Behaviour. In Neurotransmitter Balances regulating behaviour. Edited and published by E.F. Domino and J. M. Davis, Ann Arbor.

Rakitskaya, V. V., Shaliapina, V. G. (1976): Distribution of catecholamines in the rat hypothalamus following alterations in hormonal balance. In: Pituitary-adrenal system in the brain. Len. Nauka: 87 - 106.

Randrup, A., Munkvad, I., Fog, R. Braestrup, C., Molander, L. (1979): The case for involvement of dopamine in depression and mania. In: Neuro-psychopharmacology Ed. by Saletu, B., Berner, P. and Hollister, L. Perbamon Press, N. Y, Oxford: 139 - 150.

Rastogi, R. B., Singhal, R. L. (1978): Evidence for the role of adrenocortical hormones in the regulation of noradrenaline and dopamine metabolism in certain brain areas. Brit. Jour. Pharmacol, 62: 131 - 136.

Riudi, G., Ventura, M. (1961): Influence of adrenalectomy of adrenal cortex hormones and of cold on the γ-aminobutyric acid and glutamic acid content of rat brain. Ital. J. Biochem. 10: 135 - 146.

Roberts, E. (1960): Some aspects of the biochemistry and Physiology of γ-aminobutyric acid in the central nervous system. Amer. J. Arthopsychial. 30: 15 -22.

Roberts, E. Chase, T. N., Tower, D. B. (1976): Gaba in Nervous System Function. Raven Press, N.Y.

Roberts, E., Kuriyama, K. (1968): Biochemical-physi-

ological correlations in studies of the γ-amino-butyric acid system. Brain Res. 8: 1 - 35.

Robinson, N., Wells, F. (1973): Distribution and localization of sites of gamma-aminobutyric acid metabolism in the adult rat brain. J. Anal. Biochem. 114: 365 - 378.

Roche-Ramel, G. (1967): An enzymatic and fluorophotometric method for estimating urea concentrations in nonoliter specimens. Anal. Biochem. 21: 372 - 381.

Ryzhenkov, V. E. (1973): Brain monoaminergic mechanisms and hypothalamic-Pituitary-adrenal activity. In: Hormones and brain function. Plenum Press. N.Y: 285 - 286.

Ryzhenkov, V. E. (1970): Meeting of Prebalt-Leningrad-Finland Pharmacological Societies, Tartu; 24 - 27.

Saavedra, J. M., Brownstein, M., Polkovits, M. (1974): Serotonin distribution in the limbic system of the rat. Brain Res. 79: 437 - 441.

Sadasivudu, B., Rao, T. D., Murthy, C. R. K. (1977): Metabolic effects of hydrocortisone in mouse brain. Neurochem. Res. 2: 521 - 532.

Salvador, R. A., Alberts, R. W. (1959): The distribution of glutamic γ-aminobutyric transaminase in the nervous system of the Rhesus monkey. J. Biochem. 234: 922 - 925.

Sapronov, N. S. (1973): Influence of some neurotropic drugs on the hypothalamo-pituitary-adrenal system after dexamethasone blockage. In: Hormones and brain function. : 287 - 292. Plenum Press N.Y.

Sapronov, N. S. (1975): Excitability of the hypothalamus and the brain limbic structures of the rabbits under the influence of glucocorticoids and ACTH. Pharmacology and Toxicology. 38: 672 - 675.

Scapagini, V. P., Preziosi and De Schaepdryrer (1969). Influence of restraint, stress corticosterone and bethamethasone on brain amine levels. Pharmacol. Res. Commun. 1: 63 - 69.

Scapagnini, U., Preziosi and A. De Schaep. Dryver (1969). Influence of restraint, stress corticosterone and betamethasone on brain amine levels. Pharmacol, Res. Commun. 1: 63 - 66.

Scapagnini, U., Moberg, G. G., Van Loon, G. R., DeGroof, J., Ganong, W. F.: (1971): Relation of brain 5-HT content to the diurnal variation in plasma corticosterone in the rat. Neuroendocrinology 7: 90 - 96.

Scapagnini, U., Van Loon, G. R., Moberg, G. P. Ganong, W. F. (1970): Effects of α-methyl-p-tyrosine on the circadian variation of plasma corticosterone in rats. Europ. J. Pharmacol. 11: 266 - 268.

Seligman, B., Miller, L. P., Brochman, D. E., Martin, D. L. (1978): Studies on the regulation of GABA synthesis, the interaction of adenine nucleotides and glutamate with brain glutamate decarboxylase. J. Neurochem. 30: 371 - 376.

Shah, N. S., Stevens, S., Himwich, H. E. (1968): Effect of chronic administration of cortisone on the tryptophan induced changes in amine levels in the rat brain. Arch. Int. Pharmacodyn. Ther. 171: 285 - 295.

Shaliapina, V. G. (1980): Regulation of catecholamine metabolism by corticosteroids in the brain. In. VII All union. Conference of Biochemistry of the nervous system. Minsk, Nauka: Texnika: 100 - 101.

Shanberg, S. M., Schildkraul, J. J., Kopin, I. J. (1967): The effects of psychoactive drugs on norepinephrine-3H metabolism in brain. Biochemical Pharmacology, 16: 393 - 399.

Shildkraut, J. J., (1965): The catecholamine hypothesis of affective disorders: A review of supporting evidence. Am. J. Psychial. 122: 509 - 522.

Shreiber, V. (1974): Adenohypophysial hormones: Regulation of their secretion and mechanisms of their action. In. MTP international Review of Science 8: 61 - 100 Butterworths.

Simon, M. L. and George, R. (1975): Diurnal variations in plasma corticosterone and growth hormone as correlated with regional variations in norepinephrine, dopamine and serotonin content of rat brain. Neuroendocrinology 17: 125 - 138.

Simon, J. R., Martin, D. L., Kroll, M. (1974): Sodium-dependent efflux and exchange of GABA in synaptosomes. J. Neurochem. 23: 981 - 991.

Smelik, P. G. (1963): Failure to inhibit corticotropin secretion by experimentally induced increases in corticoid levels. Acta endocr. (Kbh) 44: 36 - 46.

Snisar, I. A., Oreknov, V. C. (1982): The content of GABA metabolites in the brains of rabbits and cats. Ukr J. Biochem. 54: 565 - 568.

Starke, K. (1977): Presynaptic receptor systems in catecholaminergic transmission. Biochem. Pharamacol. 26: 259 - 268.

Susz, J. P., Haber, B., Roberts, E. (1966): Purification and some properties of mouse brain L-glutamic decarboxylase. Biochemistry, 5: 2870 - 2876.

Sutherland, V. C., Riximaru, M. (1964): The regional effects of adrenalectomy and ethanol of cerebral aminoacids in the rat. Int. J. Neuropharmac. 3: 135 - 139.

Sytinskii, I, A; Thinh, N. T. (1964): Distribution of gamma-aminobutyric acid in the monkey brain during picrotoxin induced seizures. J. Neurochem. 11: 551 - 556.

Sytinskii, I. A. (1972): In: Gamma-aminobutyric acid in the central nervous system function. Leningrad. Nauka : 1 - 107.

Sytinskii, I. A., (1977): Gamma-aminobutyric acid. inhibitory neurotransmitter. Leningrad, Nauka.

Szentagothai, J., Flerko, B., Mess, B., Halasz, B., (1968): Hypothalamic control of the anterior pituitary. Akadamia Kiado Budapest 14: 103 - 113 (1974).

Tapia, R., Sandoval, M., Contraras, P. (1975): Evidence for a role of glutamate decarboxylase activity as a regulatory mechanism of cerebral excitability. J. Neurochem. 24: 1283 - 1285.

Udenfriend, S. (1962): In: Fluorescence assay in biology and medicine, : 245 - 250. Academic Press New York.

Valikina, E. M. (1972): Monoamine oxidase activity of some rat organs following prolonged administration of hydrocortisone. In. Physiology, Biochemistry and Pathology of the endocrine system. Kiev: Health, 2nd Ed: 9 - 11.

Van der Heyelen., De Kloet, J. A. M., J. Korf., D. H. G. Versteeg. (1979): GABA content of discrete brain nuclei and spinal cord of the rat. J. Neurochem. 33: 857 - 861.

Van Loon, C. R., Hilger, L., Cohen, R., Ganong, W. F. (1969): Evidence for a hypothalamic adrenergic system that inhibits ACTH secretion in the dog. Fed. Proc. 28: 438.

Vasilyev, V. Y., Yeremin, V. P. (1968): Separation and some properties of aminobutyrate-glutamate transaminase. Biochemistry (U.S.S.R) 33: 1143 - 1149.

Vernadakis, A., Woodbury, D. M. (1971): Effects of cortisol on maturation of the central nervous system. In: D. M. Ford eds., Influence of hormones on the nervous system. Proc. Int. Soc. Psychoneuroendocrinology. Brooklyn, 1: 141 - 152.

Vernikos-Danellis, (1964): Estimation of corticotropin releasing activity of rat hypothalamus and neurohypophysis before and after stress. Endocrinology, 75: 514 - 520.

Vernikos - Danellis, J. (1965): Effects of rat median eminence extracts on pituitary ACTH content in normal and adrenalectomised rats. Endocrinology, 76: 240 - 247.

Versteeg, D. H. G., Van der Gugten, J., de Jong, W. and Polkovits, M. (1976): Regional concentrations of noradrenaline and dopamine in rat brain. Brain Res. 113: 563 - 574.

Versteeg, D. H. G. I van Zoest and E. R. De Kloet. (1984): Acute changes in dopamine metabolism

in the medio - basal hypothalamus following adrenalectomy. Experimentia 40: 112 - 114.

Wied, D. DE. (1969): Effects of peptide hormones on behavior. In. Frontiers in Neuroendocrinology. Ed. by W. F. Ganong and L. Martini. Oxford University Press, N. Y. : 97 - 140.

Weiss, J. M., McEwen, B. S., Silva, M. T. Kalkut, M. (1970): Pituitary - adrenal alterations and fear responding. Amer. J. Physiol. 218: 864 - 868.

Wheeler, D. D. (1980): A model for GABA and glutamic acid transport by cortical synaptosomes. Pharmacology. 21: 141 - 152.

White, A., Handler, P., Smith, E. L. (1980): Principles of Biochemistry 4th Edition. McGraw-Hill. New York. Toronto. London.

William, B. L., Dianne, A. R., Carl, W. C. (1973): Stimulus-coupled secretion of γ-aminobutyric acid from synaptosomes. Science. 181: 696 - 698.

Wood, J. D. Pesesker, S. S. (1974): Development of an expression which related the excitable state

of brain to the level of GAD activity and GABA content with particular reference to the action of hydrazine and its derivates. J. Neurochem. 23: 707 - 712.

Woodbury, D. M. (1954): Effect of hormones on brain excitability and electrolytes. Recent Progr. Hormone Res. 10: 65 - 107.

Woodbury, D. M. (1958): Relation between the adrenal cortex and the central nervous system. Pharmacol. Rev. 10: 275 - 357.

Woodbury, D. M., Timiras, P. S. Vernadakis, A. (1957): Influence of adrenocortical steroid on brain function and metabolism. In: Hormones, brain function and behaviour. Acad. Press. N. Y. :27 - 54.

Woodbury, D., Vernadakis, A. (1966): Effects of steroids on the central nervous system. In: methods in hormone research. N.Y. 5: 1 - 57.

Youdim, M. B. H., Halzbauer, M. (1976): Physiological and Pathological changes in Tissue Monoamine oxidase activity. J. Neural. Transm. 38: 193 - 229.

Yudaev, N. A., Afinogenova, S. A. (1960):
Changes in adrenal function of rabbits following administration of cortisol, ACTH and sodium salisilate. Prob. Endocrinol. 6: 19 - 25.

Yudaev, N. A., Gancharova, V. N. (1959): The effects of cortisol and glutamic acid on the Ammionia content of the brain.
Probl Endocrinol, 5: 3 - 9.

Yuwiler, W. L., Wetterberg, E., Geller (1971):
Relationship between alternate routes of tryptophan metabolism following administration of tryptophan peroxidase inducers or stressors
J. Neurochem. 18: 543 - 599.

Zimmerman, E., Critchlow, V., (1969):
Suppression of pituitary-adrenal function with physiological plasma levels of corticosterone.
Neuroendocrinology 5: 183 - 192.

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