PHYSIOLOGICAL STUDIES ON THE EFFECTS OF HORMONAL IMBALANCE ON THE CENTRAL NERVOUS SYSTEM

I. Effects of Hypophysectomy on Certain Biochemical Parameters in the Brain of Frog

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ABSTRACT

The acetylcholinesterase activity, RNA levels, total free amino acids and magnesium content in fore-, mid- and hind-brain were determined in the normal and hypophysectomised frogs (*Rana cyanophlyctus*). Hypophysectomy results in a marked decrease in the free $a - a$ and in Mg. RNA also decreases significantly, but not to the same degree as the $a - a$ and Mg. But AchE levels do not change significantly although there is a general decrease. It is suggested that the decrease in Mg is a reflection of the ionic imbalance resulting from hormonal imbalance. Hypophysectomy also results in reduced protein synthesis (and/or turnover) in the CNS as indicated by reduced RNA and $a - a$.

INTRODUCTION

THE pituitary gland has been shown to be a very important chemical co-ordinator of several metabolic and physiological functions in the vertebrates (Turner, 1966). It is now becoming increasingly clear that the other major co-ordinating and integrating system in the vertebrates, namely the Central Nervous System (CNS), is greatly influenced by the levels of certain chemicals and hormones in the blood and it is becoming evident that such effects on the CNS determine several functions which are dependent upon the CNS for expression. In view of the great importance of the pituitary, considerable amount of experimental work has been done and continues to be done using hypophysectomised vertebrates. Such hypophysectomised animals experience conspicuous alterations in their blood chemistry especially with regard to the levels of hormones circulating in the blood. Therefore it would be obvious that these changes in the blood level of hormones in such animals would have very important effects on the metabolism and activity of the CNS. However, little information has so far been gathered on such changes in the CNS in hypophysectomised animals.

There are some studies on the effects of certain hormones, especially of the thyroid hormone and some of the sex hormones, on the biochemistry and physiology of the CNS in the rat.

Thyroidectomy has been shown to result in a decrease in total cholinesterase activity in the brain of the rat (Hamburgh and Flexner, 1967). Likewise neonatal hypothyroidism results in a depression of the ATPase activity in cortical tissue in rat (Valcana and Timiras, 1969) and also decreases cortical RNA levels (Geel and Timiras, 1967). Hypothyroidism is known to alter the levels of glutamate decarboxylase (Balazs, Kovacs *et al.*, 1968) and GABA transaminase (Garcia Argiz *et al.*, 1967), while it also results in a decrease in protein synthesis. In Amphibia it has been shown that thyroid hormone has significant effect upon the differentiation of certain neuronal units (Kollros, 1968).

There is also some published evidence which shows that gonadal steroids are involved in the modification of the central nervous activity and function (Woodbury and Vernadakis, 1966; Vernadakis and Timiras, 1966). Specificity of hormonal action on discrete areas of the brain (Timiras, Vernadakis and Sherwood, 1968) and specific areas of the brain selectively concentrating estradiol (Woolley, Holinka and Timiras, 1969) have also been reported. Effects of growth hormone (GH) on the CNS resulting in an increase in brain DNA content have been reported (Zamenhof, Mosely and Schuller, 1966).

In the light of the relatively scant information available in this regard and in view of the common usage of frog and other amphibians in the laboratory for experimental purposes and in view of the great need for an understanding of the effects of hormonal imbalance on the physiological state of the CNS, the present study has been undertaken.

An attempt is made to measure changes in a few important metabolic parameters such as the RNA levels and the amino acid content. Further an important ion such as magnesium and a neuropharmacologically important constituent such as the acetylcholinesterase content have also been studied in the three major parts of the brain of frog.

MATERIAL AND METHODS

The commonly occurring frog, *Rana cyanophlyctus*, has been used in this study. The frogs were maintained in glass aquaria at laboratory temperature which varied around 25° C. The frogs were force-fed once every three days with frog muscle. In all cases the brain was dissected out from the ventral aspect and immediately used for analysis. The fore-brain, mid-brain and hind-brain were taken separately for purposes of analysis.

For hypophysectomy the animals were anaesthetized and were then operated. After hypophysectomy individual frogs were maintained in aquaria upto 7 days before they were sacrificed. The hypophysectomised animals were given injections of 1% NaCl solution daily and were also force-fed as mentioned above.

The known weight of each part of the brain from the normal and the hypophysectomised individuals were analysed for the RNA, amino acids, magnesium and acetylcholine contents as follows:

RNA was determined by the orcinol colour reagent method (Schmidt-Schneider, 1946) where the tissue was homogenised with methanol and then treated with TCA, ethanol-ether, PCA and orcinol in step-wise reactions to remove traces of other components so as to leave only RNA. The optical density was read at 720 mμ.

Amino acids were determined by the photometric method (Folin and Danielson, 1933) from the colour developed by the reaction between the amino acids and naphthoquinone 4 sulphonic acid in alkaline solution, which is the basis of the method. The optical density was read at 490 mμ. Magnesium was determined by the Milton and Waters method, wherein magnesium was precipitated as magnesium ammonium phosphate and the latter was estimated by colorimetric phosphate determination as described in Milton and Waters.[19]

Acetylcholine esterase was determined by Hestrin's method (1949) which is based upon the disappearance of acetylcholine as determined by the ferric-acetyl droxamic acid complex where one unit is equivalent to the disappearance of one micromole of acetylcholine per minute at 25° C.

RESULTS

The data obtained is summarised in Table I. It is seen from this table that hypophysectomy results in very marked decrease in the levels of free

TABLE I

Comparison of the different constituents measured in the different parts of the brain of normal and hypophysectomised frogs

	Fore-brain		Mid-brain		Hind-brain	
	Normal	Hypo	Normal	Hypo	Normal	Hypo
Amino acids μgm/Mg	6·018± 1·6 (5)	1·68± 0·25 (6)	4·81± 1·04 (5)	2·52± 0·14 (6)	5·35± 1·7 (5)	2·92± 0· (6)
	t=5·81 P>0·005 V. Sign.		t=4·83 P>0·005 V. Sign.		t=2·73 P>0·025 <0·01 Sign.	
RNA μgm/Mg	78·18+13·6 (6)	46·15± 3·82 (5)	66·6 ± 7·5 (6)	46·3 ± 7·9 (5)	84·97±15·3 (6)	45·6 ± 3· (5)
	t=4·6 P>0·005 V. Sign.		t=3·9 P>0·005 V. Sign.		t=4·3 P>0·005 V. Sign.	
Magnesium μgm/Gm	47·0 ±15·3 (6)	12 ±10·6 (5)	54 ±26·99 (6)	15 ± 5·2 (5)	35 ±14·0 (6)	19 ± 6· (5)
	t=3·504 P>0·005 V. Sign.		t=5·72 P>0·005 V. Sign.		t=1·902 P>·05 <·025 Sign	
AChE Unit/Mg protein	6·98± 0·26 (6)	6·02± 0·52 (5)	5·2 ± 0·10 (6)	4·7 ± ·78 (5)	4·7 ± 0·15 (6)	4·06± 0· (5)
	t=2·73 P>·025 <·01 Sign.		t=1·6 P>·1 <·05 F. Sign.		t=2·914 P>·01 <·005 Sign.	

amino acids in all the three different parts of the brain, although the decrease is most conspicuous percentage-wise in the fore-brain. Likewise there is an equally marked decrease in the total magnesium content in all the three parts of the brain in hypophysectomised frogs. Here again the decrease is most conspicuous percentage-wise in the fore-brain while the mid-brain also exhibits a nearly similar drop. With regard to RNA levels, while a significant decrease is noticed in all the three parts of the brain after hypophysectomy the degree to which the change has occurred is not as great as in the other two parameters mentioned above. Further, the relative change in the different parts of the brain is nearly the same although the hind-brain seems to be the most affected unlike in the case of the amino acids and magnesium mentioned above.

On the other hand, it is quite noteworthy that the AchE content does not change very significantly after hypophysectomy although there is a general decrease in total AchE content in all the three parts of the brain. In fact here again the change seems to be most marked in the hind-brain rather than in the fore-brain.

Comparing the different parts of the brain it could be seen that even in the normal animals, there are significant differences between the different parts of the brain in the level of occurrence of at least some of the constituents measured (Table II). Thus for example it may be seen that there are considerable differences betwen the mid-brain and the other two parts of the brain in the level of occurrence of RNA, the mid-brain having the lowest RNA content. Likewise, significant differences are noticed in the AchE content of different parts of the normal brain. However, here the hind-brain shows the lowest values while the forebrain the highest. On the other hand there do not appear to be any significant differences between the different parts of the normal brain in the occurrence of free amino acids and to a great extent in the level of magnesium. However, the magnesium content is lowest in the hind-brain and this is fairly significantly different from the magnesium content in the mid-brain where it is highest.

Instituting a similar comparison between the different parts of the brain in the hypophysectomised frogs (Table III) it could be seen that some of the differences noticed above in the normal animals are abolished. Thus for example it is noticed that the RNA content drops to a uniformly low level in all the three different parts of the brain after hypophysectomy. Likewise the AchE content also decreases to a nearly uniform level in the different parts of the brain, although still the difference between the fore-brain and hind-brain tends to be fairly significant. Although there is a remarkable decrease in the magnesium content after hypophysectomy the decrease is uniformly great in the three differnt parts of the brain and therefore the different parts of the brain show similarly low values, hence the differences between the different parts of the brain continue to be not significant. With regard to the amino acid content although there has been a considerable decrease after hypophysectomy such decrease was not uniform in the different parts of the brain and therefore the different parts of the brain in the hypophysectomised animals continue to be significantly different in the

TABLE II

The biochemical contents as compared between the different parts of the brain of the normal frog

	Fore-brain	Mid brain	Hind-brain	Mid-brain	Fore-brain	Hind-brain
Amino acids μgm/Mg	6·02 ± 1·63	4·81 ± 1·04	5·34 ± 1·7	4·81 ± 1·04	6·02 + 1·63	5·34 + 1·7
	$t=1\cdot25$ P > ·15 < ·1 Insign.		$t=0\cdot54$ P > ·35 < ·3 Insign.		$t=0\cdot59$ P > 0·3 < ·25 Insign.	
	Fore brain	Mid-brain	Hind brain	Mid-brain	Hind-brain	Fore-brain
RNA μgm/Mg	78·18 ± 13·6	66·6 ± 7·5	84·97 ± 15·3	66·6 ± 7·5	84·97 ± 15·3	78·2 ± 13·6
	$t=1\cdot67$ P > ·01 < 0·05 Sign.		$t=2\cdot414$ P > ·025 < ·01 Sign.		$t=0\cdot7605$ P > ·25 < ·2 Insign.	
	Mid-brain	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Hind-brain
Magnesium μgm/Gm	53·8 ± 26·9	47·0 ± 15·3	53·8 ± 26·9	35 ± 14	47 ± 15·3	35 ± 14
	$t=\cdot48$ P > ·35 < ·3 Insign.		$t=1\cdot4$ P > ·1 < ·05 F. Sign.		$t=1\cdot3$ P > ·15 < ·1 Insign.	
	Fore-brain	Mid-brain	Mid-brain	Hind brain	Fore-brain	Hind-brain
AChE Units/Mg protein	6·98 ± 0·26	5·2 ± ·10	5·2 ± ·10	4·7 ± ·14	6·98 ± ·26	4·7 ± ·4
	$t=1\cdot44$ P > ·1 < ·05 F. Sign.		$t=\cdot65$ P > ·3 < ·25 Insign.		$t=1\cdot73$ P > ·1 < ·05 F. Sign.	

amino acid content. However the hind-brain and mid-brain do not exhibit a significant difference.

DISCUSSION

The foregoing results show that there is a marked decrease in the magnesium content of the different parts of the brain as a result of hypophysectomy. This may partly be due to the systemic ionic imbalance known

TABLE III

The biochemical contents as compared between the different parts of the brain of the hypophysectomised frog

	Mid-brain	Fore-brain	Hind-brain	Mid-brain	Hind-brain	Fore-brain
Amino acids μgm/Mg	2·52± 0·14	1·7 ± 0·25	2·92± 0·27	2·52± 0·14	2·92± 0·27	1·7 ± 0·25
	t=2·08 P>·05 <·025 Sign.		t=0·96 P>·2 <·15 Insign.		t=2·4 P>·025 <·01 Sign.	
	Mid brain	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Hind-brain
RNA μgm/Mg	46·3 ± 7·9	46·2 ± 3·8	46·3 ± 7·9	45·9 ± 3·7	46·2 ± 3·8	45·9 ± 3·7
	t=0·51 P>·35 <·3 Insign.		t=0·57 P>·35 <·3 Insign.		t=0·62 P>·3 <·25 Insign.	
	Mid-brain	Fore-brain	Hind-brain	Mid-brain	Hind-brain	Fore-brain
Magnesium μgm/Gm	15 ± 5·2	12 ±10·6	19 ± 6·07	15 ± 5·2	19 ± 6·1	12 ±10·6
	t=0·53 P>·35 <·3 Insign.		t=0·94 P>·2 <·15 Insign.		t=0·64 P>·3 <·25 Insign.	
	Fore-brain	Mid-brain	Mid-brain	Hind-brain	Fore-brain	Hind-brain
AChE Units/Mg protein	5·02± ·52	4·67± ·78	4·67± ·78	4·06± ·47	5·02± 52	4·06± ·47
	t=·75 P>·25 <·2 Insign.		t=1·3 P>·15 <·1 Insign.		t=2·07 P>·025 <·01 Sign.	

to occur in many vertebrates after hypophysectomy as a result of the imbalance caused in the mechanisms of water balance. The fact that the decrease noticed is greatest in the mid-brain is likely to be due to the fact that the hypophysis is removed from this region, while for normal control values the hypophysis is included in these estimations.

Likewise the very marked reduction in the Amino acid content in all parts of the brain after hypophysectomy may primarily be due to general reduction in the protein metabolism. Part of the causating mechanisms may be systemic. However, the magnitude of the change noticed is such that it cannot be entirely due to systemic causes. It appears therefore that the hormonal imbalance caused by the removal of the hypophysis may be the direct causating factor. This appears to be likely especially in view of the fact that the different parts of the CNS reacted in different degrees; thus for example, the decrease is most marked in the fore-brain. It has earlier been shown (Gomez *et al.*, 1967) that early post-natal deprivation of thyroid hormone results in a decreased aerobic conversion of labelled glucose into amino acids (particularly aspartic) in the cerebral cortex of rat.

It is very noteworthy that the RNA content shows a remarkable decrease in all parts of the brain as a result of hypophysectomy. This may be a reflection of the diminished activity of the synthetic machinery. It is known that imbalances in thyroid function cause significant changes in protein synthesis. It is also known that increased levels of growth hormone (GH) result in neuronal hyperplasia and also cause improvement in certain kinds of behaviour such as learning capacity in rats (Block and Essman, 1965). Therefore the fact that there is a remarkable decrease in RNA in the brain of frog as a result of hypophysectomy may indicate that the drop in hormonal function has directly effected adversely the RNA and protein synthetic machinery in the CNS. This may in fact be augmented by the considerable decrease in magnesium levels, since it is known that the incorporation of amino acids into cerebral ribonucleoproteins is markedly dependent on the levels of Mg^{++}, K^{+} and Na^{+} in the medium (Roberts and Zomzely, 1966).

However, the acetylcholine esterase content in the brain does not exhibit an equally marked alteration as a result of hypophysectomy although there is a certain decrease in its level. It is quite possible that this decrease is only a reflection of the decrease in the hormone dependent synthetic processes and may not be due to specific action of any hormone resulting from the imbalance caused due to hypophysectomy.

SUMMARY

1. The free amino acid content, magnesium, RNA and acetylcholine esterase levels in the fore-, mid- and hind-brain of normal and hypophysectomised frogs, *Rana cyanophlyctus* were estimated.

2. Within a week after hypophysectomy the free amino acids and magnesium show a marked decrease, which is most conspicuous in the

fore-brain. RNA also shows a significant decrease in all the three parts of the brain although the decrease is not as marked as in $a - a$ and Mg.

3. Although acetylcholine esterase shows some decrease, this is neither conspicuous nor very significant.

4. It is suggested that decrease in Mg is the result of the general systemic ionic imbalance caused by the alterations in water balance caused by hypophysectomy.

5. The decrease in RNA and amino-acid levels perhaps reflects the reduction in protein synthesis. This may be a direct result of hypophysectomy and partly due to the imbalance in thyroid function resulting from hypophysectomy.

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