Physiological studies on the effects of hormonal imbalance on the CNS:

2. Time course of the effects of extirpation of Pars distalis on the levels of cholesterol in the brain of the frog, *Rana cyanophlictis*

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MS received 6 February 1976

ABSTRACT

The levels of cholesterol in the fore, mid and hind brain regions were determined in normal and Pars distalis ablated *Rana cyanophlictis*, at various time intervals after the operation. The time course of the effects of removal of Pars distalis showed an initial overshoot (shock effect) and a recovery phase leading to a new stabilized stage. In general ablation of Pars distalis resulted in the elevation of the levels of cholesterol during the first week after the operation ($P > 0.05$, 0.001 and 0.001 for the fore, mid and hind brain on the first day after the operation). Regional differences in the magnitude of response were significant. A possible relationship between adrenal steroids and thyroid hormones and cholesterol synthesis is suggested.

1. INTRODUCTION

A NUMBER of hormones have been shown to affect the concentration of or turn over of cholesterol in various tissues and body fluids of vertebrates.¹⁻⁴ In the human and rat, ovariectomy produces an increase in the plasma cholesterol concentration¹ as does thyroidectomy.^{2,4} It has also been reported that injection of pituitary extracts into a hypophysectomized rat results in a marked decrease in the level of adrenal cholesterol.³

Reiss⁵ suggested that the growth hormone influences and regulates many phases of fat metabolism and Goodman and Knobli⁶ demonstrated that growth hormone plays an important role in fat mobilization in mammals. Gibson and Nalbandov^{7,8} studied lipid mobilization, lipolysis and lipogenesis in liver and adipose tissue of hypophysectomized cockerels and found an impairment of the lipid mobilizing function in hypophysectomized animals.

From the aforementioned studies, it is evident that hormones have specific influence on lipid metabolism in various tissues and body fluids in vertebrates.

However, no information is available on the effects of hormones on brain lipids. The present study was undertaken to clarify the relationship between thyroid stimulating hormones (TSH) and adrenocorticotrophic hormone (ACTH) and their effects on brain cholesterol.

The paper provides information about the time course of the effects of extirpation of Pars distalis on the levels of cholesterol in the fore, mid and hind brain regions of the frog, *Rana cyanophlictis*.

2. MATERIALS AND METHODS

Frogs, *Rana cyanophlictis* of medium size (22–26 grams) were used for the study. They were collected near Bangalore, India and were maintained in the laboratory in glass aquaria at $26 \pm 2^\circ \text{C}$. These animals were force-fed on frog leg muscle, only once in three days.

Animals were placed under mild ether anaesthesia and the Pars distalis was extirpated by the buccal approach.⁹ Control frogs were sham operated by drilling into the sphenoid bone, but without touching the brain. After the ablation of Pars distalis individual frogs were maintained in laboratory aquaria (at $26 \pm 2^\circ \text{C}$) up to three weeks before they were sacrificed. The operated animals were given (1 ml) daily injections of 1% aqueous NaCl and force-fed as mentioned above.¹⁰ The animals were sacrificed by decapitation and the brain was removed from the ventral side taking care to avoid damage. The adhering (to brain) blood vesicles were removed and kept in a petri dish kept on ice. The different regions of the brain were separated from one another with fine bent forceps and scalpel and immediately weighed (with single-pan electric balance). These regions were placed in amphibian Ringer solution and extracted with glacial acetic acid (BDH). The precipitated proteins were separated by centrifugation in clinical centri. fuge. Cholesterol content of the supernatant thus obtained was determined colorimetrically as described by Harper.¹¹ The color developed was read in SPEKOL colorimeter at 530 milli microns. The color-photo-electric concentration of cholesterol in the sample was read from a standard graph prepared by using cholesterol standards in glacial acetic acid (BDH).

3. RESULTS

From the data obtained (figures 1–3), it is clear that the level of cholesterol of the hind brain is more, compared to mid and fore brain levels and that of midbrain is more compared to the level in the fore brain of controls. Similar trend was also exhibited by the ablated animals.

It is clear from figures 1–3 that cholesterol increased up to the 5th day after operation in all three regions of the brain. On the first day after

Days after extirpation of Adenohypophysis

Figure 1. Time course of change in the level of cholesterol in the forebrain in frog, on extirpation of Pars distalis. ●—● = Normal. x—x = Ablated.

Figure 2. Time course of change in the level of cholesterol in the mid brain of frog on removal of Pars distalis. ●—● = Normal. x—x = Ablated.

Days after extirpation of Adenohypophysis

Figure 3. Time course of change in the level of cholesterol in the hind brain of frog, on ablation of Pars distalis. ●—● = Normal. x—x = Ablated.

operation the cholesterol level showed overshoot (percentage increase is 137.5, 111.1 and 174.4 mg/g wet. wt. for the fore, mid and hind brain regions respectively). A similar trend but different in magnitude was observed even 15 minutes after the operation, the percentage increase being 111, 66.5 and 62.5 mg/g wet wt. for fore, mid and hind brain regions respectively. This response appears to be the 'shock effect' exhibited by animals on exposure to stress. On the second day after the ablation a drop in the level of cholesterol in all the three regions of the brain was recorded but still remained at higher values as compared to controls. On the 3rd day after the extirpation of Pars distalis cholesterol level increased in the hind brain, the percentage increase being 112.5 over controls and formed

a plateau with the level on the fifth day after operation in the midbrain. However, in the fore brain the rise was not very significant compared to that on the second day (figure 1). It is thus evident that the nature of change is similar in all the three regions of the brain, but the magnitude of change is different.

The period between 3–5 days (after the operation) represented the recovery phase for the fore brain region whereas the recovery phase was 5–7 days (after the operation) for the midbrain. But in general, from the 3rd day (after the operation) onwards a decrease was noted in the levels of cholesterol in all the three regions of the brain till the 7th day and thereafter a tendency of stabilization at the new level was recorded. During the period for stabilization (from 7th–15th day) the fore and midbrain regions showed a new stabilized level which finally coincided with that of control level in the fore brain and closely approximated with the control level in the midbrain on the 15th day after operation. However, a decrease in the level of cholesterol was recorded in the hind brain compared to the control on the 15th day and an increase on the 20th day. In the fore brain the level remained almost stable between 15–20 days after the operation. In the midbrain both the control and the experimental groups exhibited overshoot in the level of cholesterol on the 20th day after the operation.

4. DISCUSSION

It is clear from the results that the response (increase in cholesterol levels to hormonal imbalance caused by extirpation of Pars distalis was of different magnitude in different regions of the brain on all the days experimented after operation (figures 1–3). For instance fore brain showed relatively higher response 15 minutes after the operation than the other regions.

When compared among the other regions of the brain the hind brain exhibited maximum response on the first day after operation. The midbrain showed maximum response on the 3rd day after operation. This response of the midbrain may be the direct consequence of the removal of pituitary from the midbrain. This is in accordance with what has earlier been reported by Suryaprabha and Rao¹⁰ that midbrain exhibited maximum response a week after hypophysectomy in the protein metabolism in the frog. However, it appears that the hormonal imbalance created by the removal of Pars distalis is the direct cause for the increase in cholesterol observed on removal of pituitary in all three regions of the frog brain. Another plausible explanation for the elevation in the levels of cholesterol on ablation of Pars distalis is the dependence of cholesterol metabolism on the influence of adrenocorticotrophic hormones since removal of adeno-hypophysis undoubtedly affects the functioning of the adrenals, viz., ACTH absence or its reduction.

Earlier studies also indicated a relationship between the adrenal steroids and cholesterol level.^{1,2,4,12} These authors reported that in adrenalectomized rats, the conversion of carbohydrate to fat increased markedly. This suggests that adrenal steroids inhibit fat synthesis. It is therefore possible that removal of the Pars distalis which approximates adrenocortical insufficiency or absence may be responsible for the elevated brain cholesterol level due to increased availability in the precursor substances.¹² Thus it is evident that adeno-hypophyseal hormones may exert their effects directly on the CNS or indirectly as a result of hormonal interaction. For instance, changes in thyroid functions are known to be accompanied by alteration in tissue cholesterol levels.^{1,3,4,13,14,15} Boyd¹ reported that the rate of synthesis of cholesterol from acetate is lower in hypothyroid rats and suggested that this decrease in synthesis is accompanied by the lower rate of cholesterol degradation or excretion in the absence of the circulating thyroid hormone. These results are in accord with Myant⁴ and suggest that the conversion of cholesterol to bile acids is accelerated by the presence of circulating thyroid hormone. In the light of the above facts it can be suggested that the elevated levels of cholesterol during the first week after the extirpation of Pars distalis may partly be due to changes in thyroid functions.

The reason for the decrease in the levels of brain cholesterol from (about) the 10th day up to 20th day after operation is not known. It is possible that cholesterol metabolism depends on genetic factors as well as differences in adrenal activity associated with different sensitivity towards emotional stimuli in the environment as suggested by Feuer.²

ACKNOWLEDGEMENT

The authors are grateful to the late Prof. K. Pampapathi Rao for encouragement and for offering laboratory facilities.

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