EFFECT OF ELECTRICAL STIMULATION OF THE MEDIAL MID-BRAIN RETICU FORMATION AND ADMINISTRATION OF ACTH ON THE PLASMA ELECTROLYTES*.

By

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Our previous study(12) suggested that stimulation of medial parts of the mid- reticular formation produces increased adrenocorticotropic activity. The experimental available on the relative influence of ACTH on cortisol and aldosterone secretion tend to that ACTH increases the aldosterone secretion to a very slight extent but produces a signi rise in cortisol (1, 3, 4, 5, 10). Further, this rise of aldosterone in response to the stimulati ACTH returns to the normal level within a very short time. As electrical stimulation o medial mid-brain reticular formation increases the ACTH release (12), it is felt that pro the mid-brain reticular formation has something to do with the corticosteroid level a turn, with the ionic level of the blood. The present work is therefore, designed to whether the responses following the injection of exogenous ACTH and stimulation o mid-brain reticular formation differ from each other.

MATERIALS AND METHODS

Male guineapigs weighing 450-500 *gms.* were selected for the experiments. N butal 40*mg./Kg.* of body weight was used intraperitoneally for anaesthesia. Microelect of 10-15μ diameter were used. They were inserted into the brainstem reticular form by means of a micromanipulator of a stereotaxic instrument. For collection of blood sa a polythene canula was inserted into the jugular vein under anaesthesia as described b sher (13). After this operative procedure for seven days, all the animals were habituate blood collection by withdrawing and returning blood to the animals, with the help o ringe. The animals were given food *ad libidum* and maintained in a temperature and controlled room.

Two weeks after the implantation of electrodes, rectangular stimulating pulses milliseconds duration and 4-5 volts intensity were delivered for 5 minutes in conscious a and one to two *ml* of blood was collected through the indwelling catheter over a per 3-4 minutes at intervals of 2,4 and 6 hours.

ACTH used in our experiments was manufactured by Polfa Jelenia Gora, Polan exported by Ciech Warszawa. This was diluted with double glass distilled water to g desired concentration. For calculation of the working dose, gradually increasing d

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starting from 0.1 I.U. to 0.35 I.U. were injected intraperitoneally into ten male guineapigs of approximately the same size and weight at intervals of 4 days. Following the administration of increasing doses of ACTH, it was seen that the steroidogenic responses as measured by the blood cortisol assay were identical at the end of 4 hours and 6 hours for a wide range of doses. The most convenient dose which produced optimal steroidogenic response was 0.25 I.U. as shown in Fig. I, Therefore, this dose was employed throughout the experiment.

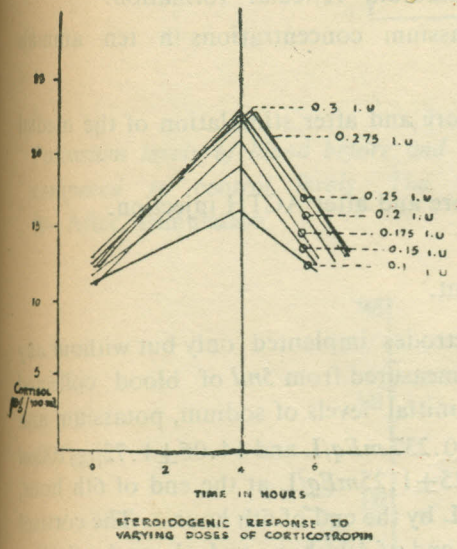

Fig. 1.

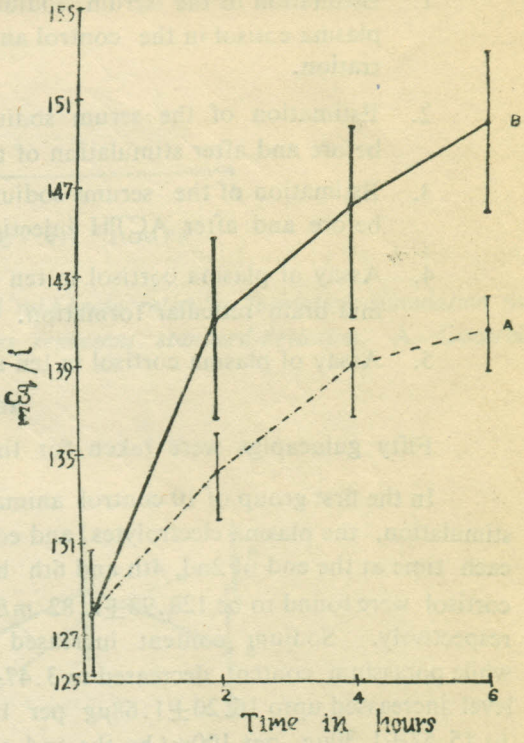

Fig. 2. *Sodim levels of blood before and after medial mid-brain reticular formation stimulation as compared to control levels. The vertical lines represent standard deviation.* A—Control B—After stimulation

In ten control animals electrodes were kept in situ without any stimulation.

The estimation of serum sodium and potassium was done with the EEL flame photometer. Cortisol assays were carried out by the method of Silber and Porter (11).

As 6-β hydrocortisol and 2-alpha hydroxy cortisol occur in the guineapig, there is likelihood their interference with the method used in this experiment to determine cortisol. However, t partition coefficients into dichloromethane are very low as suggested by Burstein(2), At end of eight hours animals were sacrificed and the brains were removed from the bony c of the skull and placed in 10% formalin; the brains were cut as 20 μ and stained with thio for localisation of the exact site of the lesion. The experiment was divided into following s tions :—

1. Estimation of the serum sodium and potassium concentrations and assay of plasma cortisol in the control animals, without any stimulation or ACTH admi tration.
2. Estimation of the serum sodium and potassium concentrations in ten anim before and after stimulation of the medial midbrain reticular formation.
3. Estimation of the serum sodium and potassium concentrations in ten anim before and after ACTH injection.
4. Assay of plasma cortisol in ten animals before and after stimulation of the me mid-brain reticular formation.
5. Assay of plasma cortisol in ten animals before and after ACTH injection.

RESULTS

Fifty guineapigs were taken for the experiment.

In the first group of 10 control animals, with electrodes implanted only but without a stimulation, the plasma electrolytes and cortisol were measured from 5*ml* of blood collect each time at the end of 2nd, 4th and 6th hour. The initial levels of sodium, potassium a cortisol were found to be 128.98±2.82 *mEq*/L, 3.96±0.237 *mEq*/L and 14.06±1.72μg/10 respectively. Sodium content increased upto 140.25±1.25*mEq*/L at the end of 6th ho while potassium content decreased to 3.47±0.15*mEq*/L by the end of 6th hour. The corti level increassed upto 16.20±1.68μg per 100*ml* by the end of 4th hour and showed a decl to 15.52±1.30μg per 100*ml* by the end of 6th hour.

In the second group of animals the serum sodium and potassium levels were determin before and after stimulation of the medial mid-brain reticular formation, values bei obtained every two hours for a total period of 6 hours after stimulation. The deter nations are plotted in Figs. 2 and 3. In these animals the sodium content of blo increased from the pre stimulation mean value of 127.75±2.71 *mEq*/L to 149.38±3 *mEq*/L at the end of the 6th hour. As there was chance of death due to decre in blood volume blood could not be collected from the animlas after the 6th hour. case of potassium there was a tendency of the prestimulation mean value to fall after t stimulation. The prestimulation level was 3.91± 0.23 *mEq*/L. But after stimulation, t sixth hour sample showed a value of 3.07±0.10 *mEq*/L (Fig. 3).

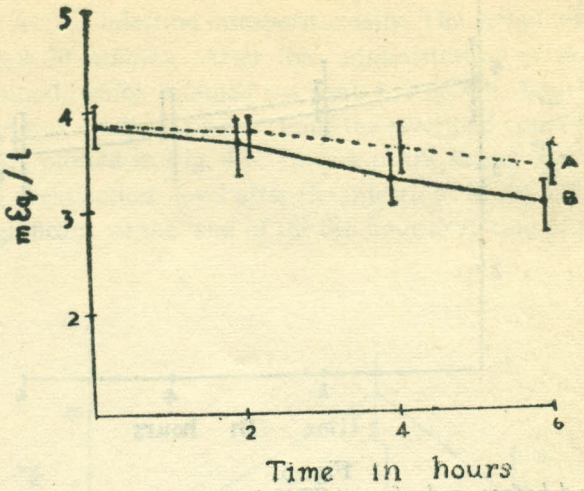

Fig. 3.

Potassium levels of blood before and after medial mid-brain reticular formation stimulation as compared to control levels. The vertical lines represent standard-deviation. A—Control B—After stimulation

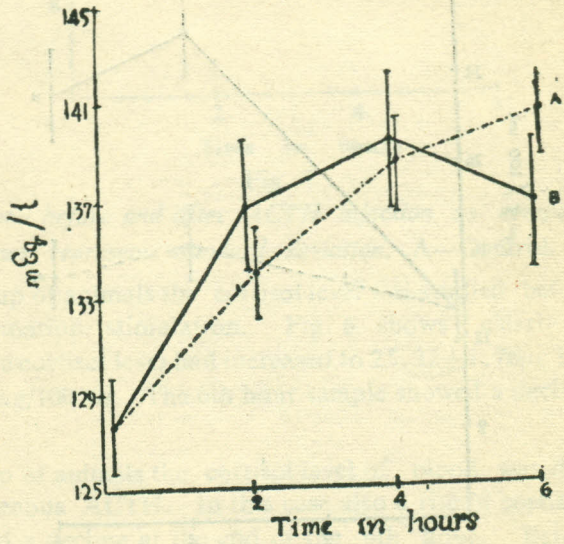

Fig. 4.

Sodium levels of blood before and after ACTH injection as compared to control levels The vertical lines represent standard deviation. A—Control. B—After injection

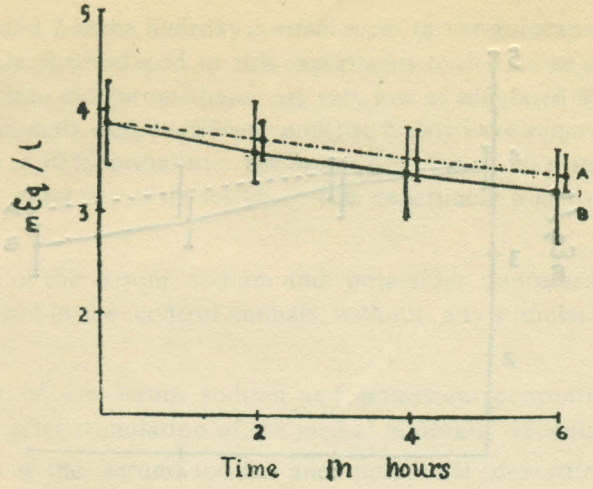

Fig. 5.

Potassium levels of blood before and after ACTH injection as compared to control levels. The vertical lines represent standard deviation. A—Control B—After injection

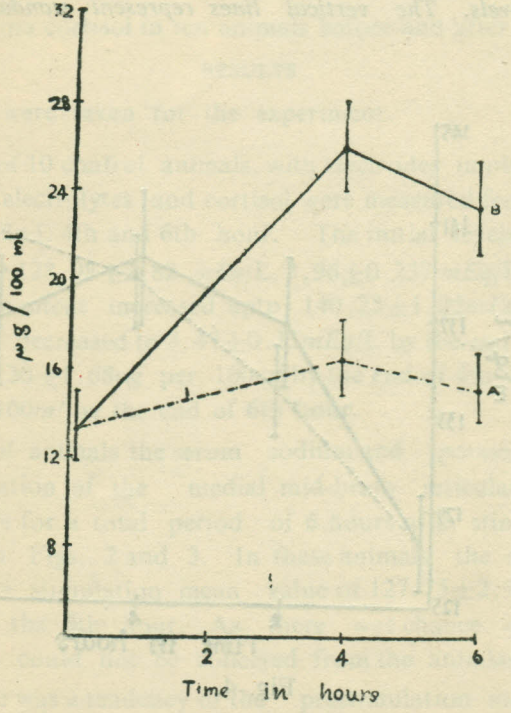

Fig. 6.

Cortisol levels of blood before and after medial mid-brain reticular formation stimulation as compared to control levels. The vertical lines represent standard deviation. A—Control B—After stimulation.

In the third group of animals the sodium and the potassium levels of blood were estimated before and after ACTH injection intraperitoneally. The initial level of sodium before the injection, was 127.88±2.30 *mEq*/L. After the administration of ACTH, there was a rise in sodium content of blood, which attained the peak by the 4th hour (140.41 2.45 *mEq*/L). But after 4 hours, there was a fall. By 6th hour the level had come down to 136.62±2.59 *mEq*/L. The results are plotted in Fig. 4 . In case of the serum potassium also, there was a tendency to fall from preinjection level after the injection of ACTH (Fig. 5). But the fall detected was not as significant at the end of the 6th hour as in case of the reticular formation stimulation.

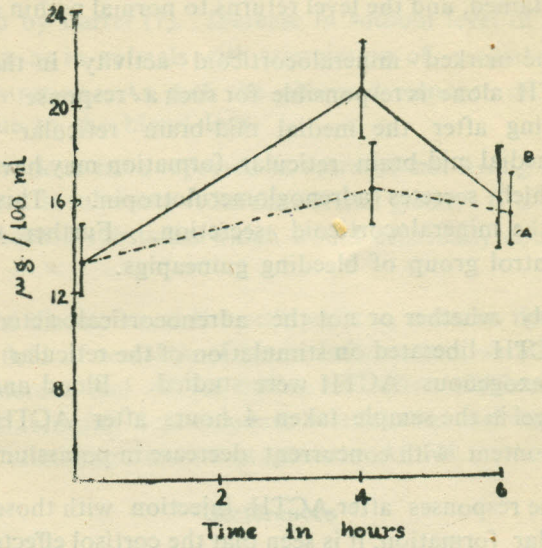

Fig. 7

Cortisol levels of blood before and after ACTH injection as compared to control levels. The vertical lines represent standard deviation. A—Control. B—After injection.

In the fourth group of animals the cortisol level was studied before and after the medial mid-brain reticular formation stimulation. Fig. 6 shows clearly that by the 4th hour after the stimulation, the cortisol level had increased to 25.22±1.76µg 100 *ml.*, prestimulation level being 13.14±1.69 *ug*/100 *ml.* The 6th hour sample showed a decline in the level of serum cortisol.

In the fifth group of animals the cortisol level of blood was studied before and after administration of exogenous ACTH. In this case also a rise in cortisol level was seen at the end of the 4th hour and a decline at the end of the 6th hour. Before injection, the mean average cortisol value was 13.13±1.5µg/100 *ml.* and at the end of the 4th hour after injection, it went upto 21.05±1.6µg/100 *ml.* At the end of the 6th hour the mean value was 15.49± 1.4*ug*/100 *ml.* (Fig. 7).

DISCUSSION

The present study clearly revealed that on stimulation of the medial mid-brain reticul formation there was an increased sodium retention and a diminution in the potassium le of the blood, with concurrent increase in the cortisol level. It seems likely, then, that su stimulation increases both mineralocorticoid and glucocorticoid activity of the adrenal cort The marked rise in the sodium level with concurrent lowering of the level of potassium in th plasma can, in accordance with the views of Leutscher, *et al* (9) and of Dunc *et al* (6) be taken as evidence for aldosterone secretory activity. In this connecti it is worth while to mention that ACTH causes aldosterone secretion to a very small exte this slight rise is not sustained, and the level returns to normal within a very short time.

So considering the marked mineralocorticoid activity in the present study, it ca not be claimed that ACTH alone is responsible for such a response. Some other factor factors may be operating after the medial mid-brain reticular formation stimulati to bring it about. The medial mid-brain reticular formation may have regulatory connecti with the pineal body which secretes adrenoglomerulotropin. This adrenoglomerulotrop exerts some control on the mineralocorticoid secretion. Further, these observed respon were not seen in the control group of bleeding guineapigs.

To know definitely whether or not the adrenocortical activity observed in the pr sent work was due to ACTH liberated on stimulation of the reticular formation, the respons following injection of exogenous ACTH were studied. Blood analysis showed a defin increase in the cortisol level in the sample taken 4 hours after ACTH injection and a slig increase in the sodium content with concurrent decrease in potassium was also observed.

On comparing the responses after ACTH injection with those after stimulation of t medial mid-brain reticular formation, it is seen that the cortisol effects are more or less simil but that the sodium and potassium responses, while qualitatively similar, show a quantitat difference.

After ACTH injection, the sodium level was no doubt increased, but it seemed to be le sustained and showed a tendency towards a fall later on as evidenced in the sixth hour samp In case of medial mid-brain reticular formation stimulation, the degree of rise in the sodi level was greater; the level showed an ascending pattern and there was no tendency to fall the end of the 6 th hour. Potassium showed comparatively a more marked fall after t stimulation of the reticular formation. Thus the present study reveals a differential behavi after the exogenous administration of ACTH and the stimulation of the mid-brain reticu formation, so far as the minerolocorticoid activity is concerned. This differential behavi can have the following explanations.

The marked rise and the sustained sodium level after the stimulation of the me mid-brain reticular formation may not be due to ACTH activity lone. Koritz and P

(8) suggested that even under conditions of maximum glucocorticoid secretion, if the rate limiting factor NADP-H is available in plenty the steroid precursors can be converted to aldosterone by passing the corticosterone pathway. It is possible then that stimulation of the mid-brain reticular formation provides, from some unknown sources, excess of the rate limiting factor NADP-H. In some studies in vitro the existence of a cholesterol independent pathway for corticoidogenesis has been reported. Stimulation of the mid-brain reticular formation possibly influences the mineralocorticoid sysnthesis and release through this independent pathway. Lastly, the mid-brain reticular formation may influence the activity of the pineal body and consequently cause increased secretion of adrenoglomerulotropin; this, in turn, could account for the marked elevation of the sodium level and the concurrent potassium loss as postulated by Farrel (7). Increase in sodium level in control group of bleeding animals was not as high as in animals with stimulation of medial mid-brain reticular formation or ACTH administration. As such the observed response of increased mineralocorticoid activity is not due to the blood loss.

However some workers have reported in cases of salt losing syndrome, overproduction of an aldosterone antagonist substance from the adrenal gland. It is quite likely that exogenous ACTH could produce such a substance which would eventually counteract the mineralocoricoid activity.

Before closing the discussion, it is worth while to mention that the present studies indicated the need in this field of research of better information, as may be gained from direct assays of plasma aldosterone and ACTH before and after mid-brain reticular formation stimulation and from establishing dose-response relationship making allowance for the lapse of time in the case of administration of exogeneous ACTH.

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