EFFECT OF POSTERIOR PITUITARY EXTRACTS AND STIMULATION OF MIDBRAIN RETICULAR FORMATION ON ACTH IN RATS WITH HYPOTHALAMIC LESION

By
R.N. Sen and B. Singh

Department of Physiology, V.S.S. Medical College, Burla (Sambalpur), Orissa

ABSTRACT

Median eminence has long been thought to be the probable site of any neurohumoral link in adenohypophysis mechanism. Our previous studies indicate that stimulation of medial parts of midbrain reticular formation produced an increased ACTH release from the adenohypophysis and this response was blocked after a median eminence lesion. Pituitary extracts were introduced into intact rats, rats with median eminence lesions and rats with anterior hypothalamic lesions. Our findings based on adrenal ascorbic acid depletion, corticosterone secretion rate, eosinophil response and adrenal histology suggest that the hypothalamic lesions without involvement of the median eminence do not block ACTH secretion following stimulation of medial midbrain reticular formation. The effect of posterior pituitary extracts (1 U/rat) on ACTH release in rats with median eminence lesions is not significant. However, large doses of posterior pituitary extracts do increase ACTH release in the absence of median eminence.

INTRODUCTION

A humoral link has been suggested between the hypothalamus and the adenohypophysis through the hypophysial portal vessels. Fibres of the supra-optico-hypophysial tract are in close apposition with the portal vessels in the region of the median eminence (2,8). Studies after lesion in this area have proved the significant role of these portal vessels (13) in carrying to the anterior pituitary a neuro-humoral substance which stimulate ACTH release from the adenohypophysis. De Groot and Harris (3) and many others have shown that certain hypothalamic areas are responsible for ACTH release through neurosecretory mediation of median eminence. It has further been shown that the release of ACTH is dependent on the integrity of the supra-optic-hypophysial tract up to the median eminence.

Kovacs and coworkers (4,6) felt that no relationship exists between the hypothalamic nuclei and control of ACTH release. They observed that ACTH release was evoked by high doses of posterior pituitary extracts. Me Cann (8) and Me Cann et al (9) observed the failure of secretion of ACTH following certain operation upon the hypothalamus in rats and cats especially when median eminence was injured. Sen and Singh (12) did not find any significant ACTH release on stimulation of the reticular formation in cases with a lesion of the median eminence. The present experiments were, therefore, designed to find out whether the brainstem areas described by our previous work function independently of the earlier described hypothalamic

*Received on 16.10.1966
*Aided by grant-in-aid from Indian Council of Medical Research, New Delhi,
Distribution of corticotropin releasing activity projected on dorsal surface of the brain stem of albino rat (cornea removed).

(scale 1 mm = distance)

+ = Excitation,
O = No significant change,
* = Either ↓ or ↑
↑ = first inhibition and then excitation.

Csu = Superior colliculus,
ci = Inferior colliculus,
fsu = Fovea superior,
f = Facial colliculus,
f = Funiculus teres,
bc = Brachium conjunctivum,
bp = Brachium pontis,
dc = Tuberculum acousticum,
f = Fovea inferior,
c = Calcarine,
f = Facial cuneatus,
ev = Vestibular area,
o = Obex.
nuclei. Further the action of the posterior pituitary extract was studied in intact rats and in rats with lesions of the median eminence and the hypothalamus.

MATERIAL AND METHODS

Adult male albino rats, 200-250 gms. in weight were used for the experiment. Bilateral electrolytic lesions were produced in the hypothalamic areas using stereotaxic instrument and a unipolar electrode which was insulated except at its tip. The indifferent electrode was placed in the rectum. Pentobarbital (30 mg./kg) anaesthesia was given. Co-ordinates ranging from 3-9 mm. anterior to ear plugs, 0.5-1 mm. lateral to the midline and 0.3-1 mm. from the base of the skull were used for positioning the electrode. Lesions were made with a high frequency alternating current (6 milli-ampere passed for ten seconds) delivered by the stereotaxically placed electrodes. This produces a lesion of 1-1.5 mm. in diameter. The lesions were made to destroy the supraoptic and paraventricular nuclei anteriorly, the mammillary bodies posteriorly or the median-eminence.

Seven days after this operation two sets of multilead electrodes were permanently implanted bilaterally in different areas of the midbrain reticular formation with the help of modified Horsley-Clarke Stereotaxic instrument improvised for implantation of electrodes in rats. The technique of Delgado and Anand (5) was employed.

Fifteen days later the implantation of electrodes the animals were tested for ACTH release from the anterior pituitary, both consequent to stimulation of the midbrain reticular formation and following the administration of Pitressin intravenously (1U/rat). For the stimulation studies, bipolar stimulation was carried out by means of a square wave stimulator and the parameters of the stimulation used were usually of 50 cycles per sec. 2-4 msec. pulse duration and an intensity of 0.2 to 0.3 volts. The animals were stimulated for 3-5 minutes per sitting. The effect of pitressin (5 Units/rat) was noted in the group of animals with lesions of the median eminence. These observations were made under the same conditions of anaesthesia as the lesions. The experimental animals were divided into eight groups of fifteen each as under:

1. Control animals without any hypothalamic lesions plus stimulation of midbrain reticular formation.
2. Animals with anterior hypothalamic lesion plus stimulation of midbrain reticular formation.
3. Animals with median eminence lesion plus stimulation of midbrain reticular formation.
4. Animals with posterior hypothalamic lesion plus stimulation of midbrain reticular formation.
5. Pitressin (1 U/rat) treated in intact animals.
6. Pitressin (1 U/rat) treated animals with lesions of anterior hypothalamic areas.
7. Pitressin (1 U/rat) treated animals with lesions of median eminence.

8. Pitressin (5 U/rat) treated animals with lesions of the median eminence.

To assess the ACTH release adrenal ascorbic acid depletion, the rate of corticosterone secretion in adrenal venous blood and the eosinopenic response were determined; the adrenal histology was also investigated. All these investigations were made in the same rat.

Adrenal ascorbic acid depletion was determined by the method of Midlin and Butler (15). The amount of depletion was measured from the adrenal collected one hour after stimulation.

Adrenal venous blood was collected for five minutes by a polythene cannula introduced in the adrenal vein and the heparinized plasma was assayed for corticosterone by the method of Silver and Porter (14). Corticosterone secretion has been expressed in micro-gram per kilogram of body weight per hour.

The eosinophil count was done by the method of Spiers (11). Histological examination of the brains of the operated animals was carried out to find out the exact sites of the lesions and the stimulations.

RESULTS

Controls:

15 animals subjected to stimulation of the medial midbrain reticular formation showed a mean decrease of 160 mg. in concentration of ascorbic acid in right adrenal (Table I) and increased corticosterone secretion rate (Table II). The eosinophilic count (Table III) was 70% of the prestimulation level.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison of Ascorbic acid depletion in various animals</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of animals</th>
<th>Initial Ascorbic acid level in mg. per 100 gm. adrenal</th>
<th>Changes in ascorbic acid level in mg. after stimulation</th>
<th>Changes in ascorbic acid level in mg. after pitressin injection and without stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
<td>350 ± 10*</td>
<td>-160 ± 8*</td>
<td></td>
</tr>
<tr>
<td>Anterior Hypothalamic lesion</td>
<td>15</td>
<td>355 ± 12</td>
<td>-180 ± 12</td>
<td></td>
</tr>
<tr>
<td>Posterior Hypothalamic lesion</td>
<td>15</td>
<td>369 ± 20</td>
<td>-182 ± 10</td>
<td></td>
</tr>
<tr>
<td>Median eminence lesion</td>
<td>15</td>
<td>370 ± 20</td>
<td>-20 ± 10</td>
<td></td>
</tr>
<tr>
<td>Pitressin injection in intact rats (IU/rat)</td>
<td>15</td>
<td>350 ± 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior Hypothalamic lesion plus pitressin injection (IU/rat)</td>
<td>15</td>
<td>352 ± 10</td>
<td>-173 ± 10</td>
<td></td>
</tr>
<tr>
<td>Median eminence lesion plus pitressin injection (IU/rat)</td>
<td>15</td>
<td>358 ± 12</td>
<td>-180 ± 3</td>
<td></td>
</tr>
<tr>
<td>Median eminence lesion plus pitressin injection (5 IU/rat)</td>
<td>15</td>
<td>358 ± 10</td>
<td>-170 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

*Standard error of mean
January 1970
Ind. J. Physiol. & Pharmacol.

The rate of corticosterone was determined; the adrenal
in the same rat.

Histological examination showed the exact sites of the lesions
of median eminence.

Midbrain reticular formation showed an increase in right adrenal (Table I) and
eosinophil count (Table III) was 70% of normal.

| TABLE II

Comparison of absolute secretion rates of corticosterone in various animals

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of animals</th>
<th>Initial corticosterone rate in ug/kg/hour</th>
<th>After stimulation of midbrain reticular formation corticosterone rate in ug/kg/hour</th>
<th>After pitressin injection corticosterone secretion rate in ug/kg/hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
<td>46 ± 10*</td>
<td>160 ± 18*</td>
<td>50 ± 10*</td>
</tr>
<tr>
<td>Anterior Hypothalamic lesion</td>
<td>15</td>
<td>70 ± 10</td>
<td>165 ± 16</td>
<td>58 ± 10</td>
</tr>
<tr>
<td>Posterior Hypothalamic lesion</td>
<td>15</td>
<td>65 ± 10</td>
<td>158 ± 12</td>
<td>45 ± 10</td>
</tr>
<tr>
<td>Median eminence lesion</td>
<td>15</td>
<td>42 ± 10</td>
<td>45 ± 10</td>
<td>45 ± 10</td>
</tr>
<tr>
<td>Pitressin injection (1 U/rat) in intact rats</td>
<td>15</td>
<td>45 ± 10</td>
<td>150 ± 10*</td>
<td>150 ± 10*</td>
</tr>
<tr>
<td>Anterior hypothalamic lesion plus pitressin injection (1 U/rat)</td>
<td>15</td>
<td>62 ± 10</td>
<td>160 ± 10</td>
<td>160 ± 10</td>
</tr>
<tr>
<td>Median eminence lesion plus pitressin injection (1 U/rat)</td>
<td>15</td>
<td>42 ± 10</td>
<td>58 ± 10</td>
<td>58 ± 10</td>
</tr>
<tr>
<td>Median eminence lesion plus pitressin injection (5 U/rat)</td>
<td>15</td>
<td>42 ± 10</td>
<td>152 ± 10</td>
<td>152 ± 10</td>
</tr>
</tbody>
</table>

*Standard error of mean

| TABLE III

Comparison of eosinopenic response in various animals

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of animals</th>
<th>Change in eosinophil % after reticular formation stimulation</th>
<th>Change in eosinophil after pitressin injection and without stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
<td>−70 ± 6*</td>
<td>−68 ± 10*</td>
</tr>
<tr>
<td>Anterior Hypothalamic lesion</td>
<td>15</td>
<td>−60 ± 12</td>
<td>−68 ± 10*</td>
</tr>
<tr>
<td>Posterior Hypothalamic lesion</td>
<td>15</td>
<td>−68 ± 10</td>
<td>−68 ± 10*</td>
</tr>
<tr>
<td>Median eminence lesions</td>
<td>15</td>
<td>+ 8 ± 2</td>
<td>−68 ± 10*</td>
</tr>
<tr>
<td>Pitressin injection in intact rats (1U/rat)</td>
<td>15</td>
<td>−68 ± 10*</td>
<td>−68 ± 10*</td>
</tr>
<tr>
<td>Anterior hypothalamic lesions plus pitressin injection (1 U/rat)</td>
<td>15</td>
<td>−65 ± 10</td>
<td>−58 ± 8</td>
</tr>
<tr>
<td>Median eminence lesions plus pitressin injection (1 U/rat)</td>
<td>15</td>
<td>−10 ± 8</td>
<td>−58 ± 8</td>
</tr>
<tr>
<td>Median eminence lesions plus pitressin injection (5 U/rat)</td>
<td>15</td>
<td>−10 ± 8</td>
<td>−58 ± 8</td>
</tr>
</tbody>
</table>

*Standard error of mean
Adrenal histology showed lipid depletion with loss of granules and loss of zonal difference in adrenocortical cells. In two of these animals haemorrhagic foci with degenerative changes were also seen.

**Anterior Hypothalamic lesion:**

Bilateral anterior hypothalamic lesion did not impair the response to stimulation of the medial midbrain reticular formation. The mean ascorbic acid depletion corticosterone secretion rate, eosinophilic count and adrenal histology in this group of 15 animals were similar to that obtained on medial midbrain reticular formation stimulation in control animals.

Mean adrenal ascorbic acid depletion remained at $180 \pm 12 \text{ mg.}$ on stimulation of medial midbrain reticular formation after anterior hypothalamic lesions (Table I). In six of these rats, mainly the supraoptic nuclei and in other nine the paraventricular nuclei were destroyed. Intragroup differences were not observed and the results were quite comparable.

**Posterior Hypothalamic lesion:**

In animals with lesion in the posterior hypothalamic nuclei stimulation of the medial midbrain reticular formation gave rise to an adrenal ascorbic acid depletion of $182 \pm 10 \text{ mg.} \%$ (Table I). A major portion of the mammillary bodies were injured in nearly all of the cases. Corticosterone secretion rate also increased (Table II). The “Eosinopenic response” showed definite eosinopenia of $68 \pm 10 \text{ \%}$ (Table III). Adrenal histology was almost the same as was found following stimulation of medial midbrain reticular formation of animals with anterior hypothalamic lesions, and showed haemorrhagic foci with degenerative changes of cortical cells.

**Median Eminence lesion:**

15 animals with extensive lesions in the median eminence (Fig.1) did not show much changes in content of ascorbic acid of the right adrenal after stimulation of medial midbrain reticular formation. Intragroup differences were not observed and the results were quite comparable.

![FIG. 1(a)](Transverse section through normal hypothalamus of rat at the level of maximum development of median eminence.)
of the response to stimulation of the
acid depletion corticosterone secre-
tion group of 15 animals were similar
stimulation in control animals.
100±12 mg. on stimulation of
lesions (Table I.). In six
the paraventricular nuclei were
results were quite comparable.
Injection of the medial
acid depletion of 182±10 mg. %
injured in nearly all of the cases.
"Eosinopenic response" showed
histology was almost the same as was
formation of animals with anterior
degenerative changes of cortical cells.
ence (Fig. 1) did not show much
stimulation of medial midbrain
reticular formation, and there was slight decrease in the ascorbic acid level of the right adrenal
(Table II.) Animals with median eminence lesion were found to have a marked suppressed rate of
corticosterone secretion (Table I) as compared to the control group, after stimulation of the
medial midbrain reticular formation. There was a mean rise in eosinophil count of 8±2 %
(Table III). Adrenal histology did not show any changes.

Pitressin :
When posterior pituitary extract was injected intravenously in single doses of 1 U/rat
in one control animal, there was a mean ascorbic acid depletion of 173±10 mg % after 5
hours (Table 1), with an increased corticosterone secretion rate (Table 1I) and a definite eosino-
penia (Table 1II). Adrenal histology showed haemorrhagic foci with degenerative changes
in adrenocortical cells (Fig 2.).

FIG. 1(b)
Lesion of rat 40 showing extent of damage at the level of maximum development of median eminence.

Histology of adrenal cortex after intravenous injection of pitressin in rat 23. H.E. Stain, x 400. Note haemor-
hagic foci and degenerative changes in adrenocortical cells.
The administration of pitressin (1 U/rat, I.V.) to rats with hypothalamic lesions by excluding the median eminence area, produced an increased ACTH release as evidenced by increase in corticosterone secretion rate. There was a significant eosinopenia (Table III) and the adrenal histology showed degenerative changes with haemorrhagic foci. 1 U/rat of pitressin injected to animals with median eminence lesion however, caused only $25\pm10\text{mg}\%$ ascorbic acid depletion (Table I) and produced no marked change in the corticosterone secretion rate (Table II), and marked eosinopenic response (Table III) and very little change in adrenal histology.

On administration of 5 U/rat of pitressin to rats with lesions of median eminence, there was a definite increase in blood corticosterone level and in the ascorbic acid depletion.

**DISCUSSION**

The presence of humoral link in activation of ACTH release has been proved by many workers. But it is not yet clear whether the adrenocortical activation is also dependent on other mechanisms in the form of a neural mechanism or a humoral link between the adenohypophysis and forebrain structure.

Our studies indicate that the median eminence is essential for increased ACTH release. No increased adrenal secretion is produced by reticular formation stimulation if the median eminence is damaged. The increase in the adrenal secretion on introduction of posterior pituitary extract after median eminence lesion is also not very significant. The adrenals of the rats with intact median eminence but with other hypothalamic lesions, retain the ability to respond to posterior pituitary extract administration, thought fail to respond effectively to posterior pituitary extract after the median eminence lesions as above. Probably the posterior pituitary extract in a dose of 1 U/rat induces ACTH release as does traumatic stress through the ascending neural pathways, leading the hypothalamus and to the median eminence through the dorsal mesencephalon as pointed out by Matsuda et al. (7).

Our observations indicate that there is loss of responsiveness of the adrenal after median eminence lesion as is evident from the lack of effect of 1 U dose of pitressin. This has been corroborated by Mc Cann(8) who found loss of responsiveness of adrenal after median eminence lesions, causing a reduction in sensitivity to ACTH and to vasopressin in rats with chronic lesions. The correlation between increased ADH release and increased ACTH release, which prompted Rothballer(14) and Mirsky(10) to suspect ADH as a possible neurohumoral transmitting agent may be true, but possibly an intact median eminence is essential for this type of correlation. In general our studies suggest that the median eminence is the final common path to the adenohypophysis for condition leading to ACTH release. It is felt that the presence of intact median eminence and its direct contiguity with the adenohypophysis is an essential factor in the increased ACTH release following either stimulation of the midbrain reticular formation or administration of posterior pituitary extract.
hypothalamic lesions by excluding ACTH release as evidenced by ant eosinopenia (Table III) and hemorrhagic foci. 1 U/rat of ever, caused only 25±10mg. % age in the corticosterone secretion (III) and very little change in ionsof median eminence, there ascorbic acid depletion.

Disease has been proved by many tion is also dependent on other link between the adenohypophysial for increased ACTH release. nation stimulation if the median significant. The adrenals of lesions, retain the ability fail to respond effectively to s above. Probably the posterior as does traumatic stress through to the median eminence ara et al. (7).

ness of the adrenal after median use of pitressin. This has been ef adrenal after median eminence pressin in rats with chronic lesions. ACTH release, which prompted neurohumoral transmitting agent al for this type of correlation.inal common path to the adenohypophysial the presence of intact median ion is an essential factor in the inmidbrain reticular formation or

The increase in the corticosterone level in the blood after administration of posterior pituitary extract in doses of 5 U/rat in animals with lesions of the median eminence was significant. Presumably this was an effect on the pituitary directly. Further studies on hypophysectomized rats are required for the elucidation of the cause of this rise of corticosterone level. All the above findings taken together mean that the hypothalamic areas mentioned did not play an essential part in ACTH release, but the median eminence played a definite role in liberation of ACTH from the adenohypophysis. From the analysis of the sections it was evident that the destruction of the median eminence was responsible for the block of ACTH release. However, damage to some fibre tracts related to the posterior hypothalamus can not be excluded.

It is postulated that the stimulation of medial midbrain reticular formation or the administration of posterior pituitary extract stimulate the release of ACTH from the adenohypophysis, through the median eminence acting as the final common path.

REFERENCES


ACKNOWLEDGEMENT

Our grateful thanks to Dr. S. M. McCann, Professor of Physiology; Southwestern School University of Texas for his valuable suggestions.