ACTH CONTENT IN THE NEUROHYPOPHYSIS OF RAT

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Role of the neurohypophysis in the release of ACTH has been studied by a number of investigators. Posterior pituitary hormone, particularly vasopressin, was proposed as a neurohormone which stimulates the secretion of ACTH from the anterior lobe¹⁻⁴. A group of workers⁵⁻⁷, however, have shown that commercial posterior pituitary hormone preparations are contaminated with corticotrophin-releasing factor, CRF. It was also reported that the neurohypophysis contains considerable amount of ACTH. According to MIALHE-VOLOSS⁸ and ROCHEFORT et al.⁹, ACTH content of the anterior lobe is diminished by systemic stimuli, while a fall in ACTH of the posterior lobe is caused by neurogenic stimuli. The present experiments were carried out to confirm the occurrence of ACTH activity in the neurohypophysis of rat.

METHODS

Male Holtzman rats, weighing 170 to 220 g, were used throughout the experiments. Rats were quickly killed by manual separation of the cervical vertebrae or decapitation. Anterior and posterior lobes of pituitary gland were dissected out employing a dissecting microscope. Three to six glands were pooled and extracted with 0.1 N HCl. The extracts were neutralized and diluted with Krebs-Ringer bicarbonate solution at pH 7.4.

These samples were assayed for corticosterone-releasing capacity *in vitro*¹⁰ and for adrenal ascorbic acid depleting activity in hypophysectomized rats¹¹. For the *in vitro* assay, eight rats were sacrificed for one assay. The adrenals obtained from these rats were freed from adhering tissue, and the glands quartered. The eight quarters obtained from two glands of each rat were incubated in 1.5 ml Krebs-Ringer bicarbonate buffer (pH 7.4) at 37.5°C, for 1 hour under oxygen containing 5 per cent carbon dioxide in a Dubnoff incubator. Glucose was added into the above medium in a concentration of 200 mg per cent. After 1 hour preincubation, the medium was poured off and discarded. An equal quantity of fresh medium was added with 0.1 ml of test sample. After 2 hour incubation corticosterone discharged by the adrenal tissue into the medium was determined by fluorometric method¹². For the ACTH assay by adrenal ascorbic acid depletion method, hypophysectomy was performed through the external auditory canal. Ascorbic acid concentration was determined by the method of ROE and KUETHER¹³. The activity was expressed in term of milli-units of standard ACTH. ACTH preparation used in this experiment as standard was provided by Armour Co. (Lot # 216-172-5).

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RESULTS AND DISCUSSION

1. *Release of corticosterone in vitro.* FIG. 1 shows dose-response curve; ordinate indicates the amount of corticosterone released by 100 mg adrenal tissue during 2 hour incubation in Krebs-Ringer bicarbonate solution with glucose, and abscissa represents the amount of ACTH added into the incubation medium.

FIG. 1. Dose-response curve.

ACTH activity of the anterior lobe was 1242 ± 101 mU per gland and 283 ± 21.0 mU per mg fresh tissue. The activity of the posterior lobe was 53 ± 4.8 mU per gland and 88 ± 7.6 mU per mg. The ACTH content per mg posterior lobe tissue was approximately one-third as much as that of the anterior lobe tissue. This value of the anterior lobe ACTH was almost the same as the values reported by BIRMINGHAM et al.¹⁴⁾ and ROBERTS¹⁵⁾, but considerably higher than those by FORTIER^{16,17)} and KITAY et al.¹⁸⁾. According to ROCHEFORT et al.⁹⁾, ACTH content of the anterior lobe was roughly 150 mU per mg and that of the posterior lobe about 35 mU. ROBERTS¹⁵⁾ claimed that the release of corticosterone by rat adrenal tissue *in vitro* was markedly responsive to rat ACTH and much less responsive to ACTH from other sources. This might be the case. However, no explanation can be afforded for such large differences in those values obtained by different investigators.

To know the direct effect of vasopressin on the adrenal gland, HILTON et al.^{19,20)} performed an arterial perfusion experiment in dogs, and found that vasopressin stimulated the secretion of hydrocortisone. High value might be obtained by the posterior pituitary hormone which is possibly contained in the

TABLE 1.
ACTH content of rat pituitary gland.

	No. of expts.	Average tissue weight (mg)	ACTH content (mU)	
			per gland	per mg
Anterior lobe	14	4.44	1242 ± 101	283 ± 21.0
Posterior lobe	15	0.61	53 ± 4.8	88 ± 7.6

extracts. Therefore, the direct effect of vasopressin on rat adrenal tissue was tested *in vitro*, by adding 20 to 200 mU Pitressin (Parke-Davis Co.) in each incubating flask. As shown in TABLE 2, we failed to find any stimulatory effect of this hormone on the release of corticosterone.

TABLE 2.
Effect of Pitressin on the release of corticosterone
by rat adrenal tissue *in vitro*.

Pitressin added (mU)	ACTH added (mU)	No. of expts.	Corticosterone released*		
			with	without	diff.
20	—	5	13.2	13.0	+ 0.2
50	—	5	12.5	13.0	— 0.5
100	—	5	13.1	13.7	— 0.6
200	—	5	12.7	13.1	— 0.4
—	10	9	22.8	12.3	+10.5
20	10	7	19.9	10.4	+ 9.5
50	10	8	21.1	10.9	+10.2
100	10	7	20.8	10.4	+10.4

* microgram per 100 mg adrenal tissue.

According to ROYCE and SAYERS²¹⁾, Pitressin potentiates the action of ACTH in hypophysectomized rats. In the present experiment 20 to 100 mU Pitressin was added with 10 mU ACTH into the flask. The amount of corticosterone released was the same in every case, either absence or presence of Pitressin. From these results it would be inferred that inclusion of vasopressin in the pituitary extracts does not impede the assay of ACTH *in vitro*.

Effect of brain extracts on the release of corticosterone was also observed as control experiment. For the assay of ACTH content the amount of anterior lobe tissue added into each flask was approximately 0.1 mg and that of posterior lobe tissue 0.1 to 0.2 mg. In this control experiment the tuber cinereum and brain cortex tissues in a quantity of 1 mg or more were added in the flask. It

TABLE 3.
Effect of brain tissue on the release of corticosterone
by rat adrenal tissue *in vitro*.

	No. of expts.	Average weight of tissue added (mg)	Corticosterone released*		
			with extract	without extract	diff.
Tuber cinereum	10	1.25	12.7	12.6	+0.1
Brain cortex	4	1.55	14.4	14.8	—0.4

* microgram per 100 mg adrenal tissue.

is well known that hypothalamic tissue contains various kinds of substances such as histamine, serotonin, norepinephrin, substance P, and so on. However, as shown in TABLE 3, these extracts had no activity in releasing corticosterone *in vitro*.

2. *Adrenal ascorbic acid depletion in hypophysectomized rats.* ACTH activity of the pituitary extracts was reexamined by the method of SAYERS et al.¹¹. As shown in FIG. 2, intravenous administrations of standard ACTH in doses from 0.5 mU to 2.0 mU per 100 g body weight into previously hypophysectomized rats caused a marked depletion in adrenal ascorbic acid content, while both anterior and posterior lobe extracts had no effect at all, if dose of the extracts was less than 2 mU equivalent ACTH activity as determined by the *in vitro* method.

FIG. 2. Adrenal ascorbic acid depletion due to standard ACTH (S), anterior lobe extract (A), posterior lobe extract (P), and vasopressin (V). C: control, 0.85% NaCl.

FIG. 3. Dose-response curve. S: standard ACTH, A: anterior lobe extract, P: posterior lobe extract.

When the extracts were injected more than 5 mU (determined by the *in vitro* method), adrenal ascorbic acid concentration decreased markedly to similar extent as observed after one-tenth dose of standard ACTH. FIG. 3 shows log dose-response curves of these three samples. Slope of the curve of the anterior lobe extract was almost the same as that of the standard preparation, but the curve of the posterior lobe extract was steeper than the curves of the formers. From this figure it will be found that 1 mU standard ACTH activity is equivalent to 10.9 mU anterior lobe extract and 7.6 mU posterior lobe extract as determined by the *in vitro* method. If calculation was done from these values, ACTH content of the anterior lobe will be 114 mU per gland and 25.9 mU per mg, and that of the posterior lobe 7.0 mU per gland and 11.6 mU per mg.

It seems probable that the neurohypophysial factor, compared with the adenohypophysial ACTH, is more active to deplete adrenal ascorbic acid content than to cause the release of corticosterone. Contamination with vasopressin of the posterior lobe extract may cause such discrepancy. However, it was found that vasopressin in doses ranging 20 mU to 100 mU had no effect on the adrenal ascorbic acid content, nor 100 mU of Pitressin did cause any potentiation of the activity of anterior lobe extract. Posterior lobe extract may contain material(s) other than vasopressin, which potentiate(s) ACTH activity in depleting adrenal ascorbic acid concentration, or ACTH in the posterior lobe may be different in nature from that in the anterior lobe.

Employing neurohypophysectomized rats, ARIMURA and LONG²²⁾ found that there was no increase in blood ACTH level after painful stimuli, nor decrease in adrenal ascorbic acid concentration after epinephrine in such animals. Similar results were obtained by SMELIK²³⁾. According to him, the effect of psychic stress was completely abolished in neurohypophysectomized rats, whereas a somatic stress induced a normal ascorbic acid depletion. Since the effect of epinephrine was also entirely inhibited in neurohypophysectomized rats, he proposed a hypothesis that neurogenic stimuli act in the ACTH in the posterior lobe by the mediation of endogenous adrenaline, whereas in the case of somatic stress neither the posterior lobe nor adrenaline is involved. Observing plasma corticosterone levels, DE WIED²⁴⁾ studied the adrenocortical response to stresses in neurohypophysectomized rats, and found that the response to neurogenic stimuli was partially, but not entirely, blocked, and that exposure to systemic stress elicited an increase in the corticosterone levels similar to that found in controls. Results obtained by other investigators were not in agree with the above observations. NOWELL²⁵⁾ reported that in neurohypophysectomized rats adrenocortical response to cold exposure and injection of hypertonic saline was completely inhibited, whereas the response to exposure to a ringing bell was not inhibited. This result is reverse to that obtained by ARIMURA and SMELIK. Recent studies of MIALHE-VOLOSS²⁶⁾ and ROCHEFORT et al.²⁷⁾ have shown that neurogenic stimuli cause an ACTH release from the posterior lobe,

whereas somatic stimuli induce an ACTH depletion from the anterior lobe. Thus, physiological significance of neurohypophysial ACTH is now becoming apparent.

FISHER and DE SALVA²⁶⁾ claimed that neurohypophysectomy did not inhibit an increase in plasma corticosterone, but did block adrenal ascorbic acid depletion response following epinephrine. According to SLUSHER²⁷⁾, lesions in the posterior and mid-central portions of the hypothalamus specifically inhibited corticosterone discharge without altering adrenal ascorbic acid response and lesions in the basal tuberal region inhibited adrenal ascorbic acid depletion without affecting corticosterone response in response to somatic stress. These observations could be explained by the existence of two separate adrenocorticotrophic factors. To solve the problem, however, further experiments in which ACTH is isolated from the neurohypophysis should be carried out.

SUMMARY

1. ACTH activity of anterior and posterior lobe extracts obtained from adult male rats was determined by the *in vitro* method of SAFFRAN and SCHALLY and by the method of SAYERS et al.
2. In case ACTH activity was measured by the *in vitro* method, the activity of the anterior lobe was 1242 ± 101 mU per gland and 283 ± 21.0 mU per mg fresh tissue, and that of the posterior lobe 53 ± 4.8 mU per gland and 88 ± 7.6 mU per mg.
3. Markedly less activity was obtained by the method of SAYERS: 1 mU standard ACTH activity was equivalent to 10.7 mU anterior lobe extract and 7.6 mU posterior lobe extract as determined by the *in vitro* method.
4. Pitressin in doses used in the present experiments had no effect on rat adrenal tissue *in vitro* in releasing corticosterone, nor potentiating the activity of ACTH added together. Pitressin did not cause any depletion in adrenal ascorbic acid concentration in hypophysectomized rats, nor potentiate ACTH activity of the anterior lobe extract.
5. Brain extracts did not show any ACTH activity *in vitro*.

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