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ROLE OF THE NEUROHYPOPHYSIS IN THE RELEASE OF ADRENOCORTICOTROPHIC HORMONE IN THE RAT

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Considerable evidences have been presented as to the hypothalamic neurohumor regulating adrenocorticotrophic hormone, (ACTH). The neurosubstances so called corticotrophin-releasing factors (CRF) have been stated to relate to lysine vasopressin or melanocyte-stimulating hormone (MSH) in chemical structure[1].

In the lower vertebrates like teleost, the neurohypophysial tissue is irregularly inlaid into the adenohypophysis and the hypothalamic neurohumors reached to the former are directly secreted into the latter, thus controlling the adenohypophysial function. In the mammalian the neurohypophysis well developed forms a neural lobe which is clearly separated from the adenohypophysis[2].

With regard to vasopressin and oxytocin the neural lobe is considered as their storage site and the hormones stored can readily serve for acute use[3]. CRF as well as vasopressin and oxytocin is found not only in the hypothalamus, but also in the neural lobe[1]. It might be possible that CRF stored in the neural lobe is also used to meet acute demand by the adenohypophysis, though the route through which CRF is transported to the adenohypophysis from the neural lobe is not yet clearly known.

It is generally accepted that CRF reached to the nerve endings of the hypothalamic cells is secreted into the primary plexus of the hypophysial portal vessels located in the median eminence and transported to the adenohypophysis. This conception may imply that the neural lobe itself is not absolutely essential for the adenohypophysial activities, but may not exclude a possibility that the former plays a role in the regulatory mechanism of trophic hormones secretion as a by-player to make the adenohypophysial response take place smoothly.

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Several papers concerning ACTH secretion in the neurohypophysectomized rats appeared, presenting evidences of impairment of this function in some stimuli[4,5,6,7,8]. These observations were made using adrenocortical responses like adrenal ascorbic acid depletion or corticosterone secretion in the experimental animals. Since the parameters used are rather sluggish in character, and may or may not accurately reflecting ACTH release.

Adrenocortical response to ACTH was also reported to be modified by the presence of some other factors[9]. Further studies on ACTH secretion in the neurohypophysectomized rat may be necessary to perform by directly measuring levels of blood ACTH in the animals, which may vividly reflect the release of ACTH, because of its short biological half life. The present study was undertaken in an attempt to understand the role of the neurohypophysis in ACTH release mechanism under various kinds of stresses by measuring blood ACTH concentration.

MATERIALS AND METHODS

Animals: Wistar strain female rats weighing 150 to 220 g. were used throughout the study both in the experiments and ACTH assay.

They were kept at constant temperature of 20°±2°C and fed rat's biscuits prepared at the Department of Zoology, Hokkaido University Faculty of Science and tap water ad libitum prior to the experiments for at least 1 week.

Assay of blood ACTH: Rats subjected to various experimental procedures were killed by means of a guillotine. Blood was collected from the trunk in a beaker containing 0.2 ml. of heparine solution, 1000 U/ml, (Heparin Sodium Novo, Novo Industri A/S) and 2.0 ml. of the blood was immediately assayed for ACTH. ACTH activity was measured by determining a net increase in corticosterone secretion rate in a hypophysectomized assay rat after intravenous infusion of the test material. The method was reported in detail elsewhere[10].

Experimental procedures: The procedures used as stresses and the experimental conditions are described with the results.

Posterior lobectomy: Under anesthesia with hexobarbital, 10 mg/100 g. i.p., (Oltopan-Sodium, Dainippon Pharm. Co.), the rat was posterior-lobectomized via parapharyngeal approach using a biocular dissecting microscope[11]. After the operation the animal was injected with 5000 U. of aqueous penicillin (Penicillin G Kalium, Takeda Pharm. Co.) s.c. and given 0.5% of Aureomycin solution (Veterinary Aureomycin, Lederle Japan) ad libitum for 3 days to prevent infection.

Completeness of posterior-lobectomy and the damage of the anterior pituitary were macroscopically examined as carefully as possible at autopsy. The animal in which posterior-lobectomy was incomplete or the anterior lobe was damaged was discarded.

Total hypophysectomy: The pituitary gland was removed by means of a 18 gauge needle attached to a 2 ml.-syringe containing approximately 1 ml of water through the external auditory canal according to the method described by TANAKA[12].

Measurement of pituitary blood flow: A radioactive tracer method using ^{86}Rb described by GOLDMAN and SAPIRSTEIN[13] was applied for measurement of hypophyseal blood flow in the rat anesthetized with Nembutal, 4 mg./100 g. i.p. The principle of the method was described elsewhere[14].

Briefly, it can be applied to an organ having the same extraction ratio toward an indicator, ^{86}Rb, as the body as the whole during the first minute after injection. In such conditions, the ratio between organ uptake and body uptake of ^{86}Rb is equal to the ratio between organ blood flow and cardiac output. The pituitary gland of rat was shown to meet this requirement by the fact that this organ maintains a relatively constant level of indicator as a function of time after its initial arterial delivery[13].

The femoral artery and vein of pentobarbitalized rat were cannulated respectively with a polyethylene tubing filled with heparine solution and plunged with a straight pin as a stopper at the free end. The rat was placed on a rack so as to the free end of the arterial catheter to locate just above the shallow hollows regularly engraved

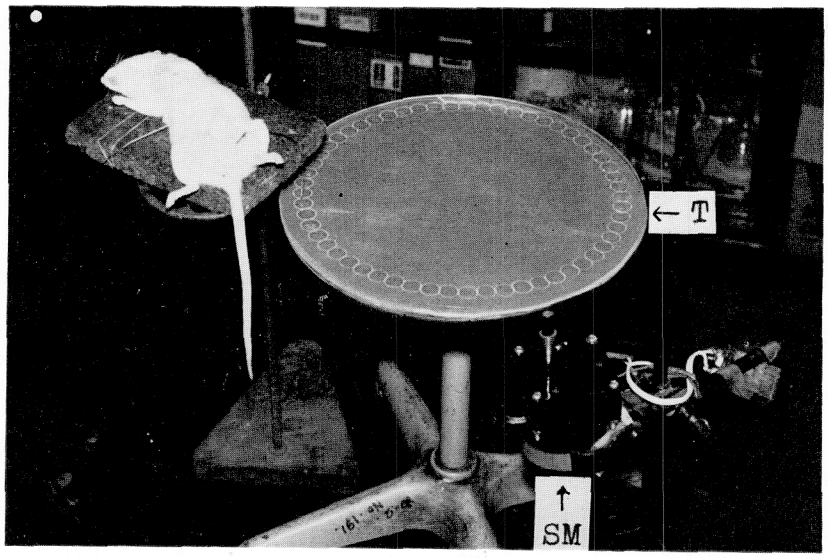

FIG. 1. Blood collector for measurement of cardiac output.
T; plastic turning table. SM; Synchronize motor.

along the rim of the plastic turning table (FIG. 1). Two tenth ml. of ^{86}Rb solution, usually containing 10–20 μc, was carefully introduced into the venous catheter the capacity of which was approximately 0.3 ml. Thus the solution of the indicator was retained in the catheter. A syringe containing 0.5 ml. of physiological saline was connected to the venous catheter. Then the table was started turning at a constant rate of 1.5 r.p.m. by rim driving method by means of a synchronize-motor. The free end of the arterial catheter was cut to allow the arterial blood to freely drop down on the hollows of the turning table. As soon as the blood started flowing down physiological saline was rapidly injected into the femoral vein through the catheter, extruding the whole ^{86}Rb solution in the cannule into circulation. The time of injection of the indicator was set as 0 time. At the 30 th second the arterial catheter was pinched with an artery forceps to stop the blood flow, and at the same time 0.2 ml. of saturated potassium chloride was quickly injected into the femoral vein through the cannule to arrest the heart beat. The position of the blood drop on the turning at the 30 th second was marked, thus the time when any blood sample was obtained could be known.

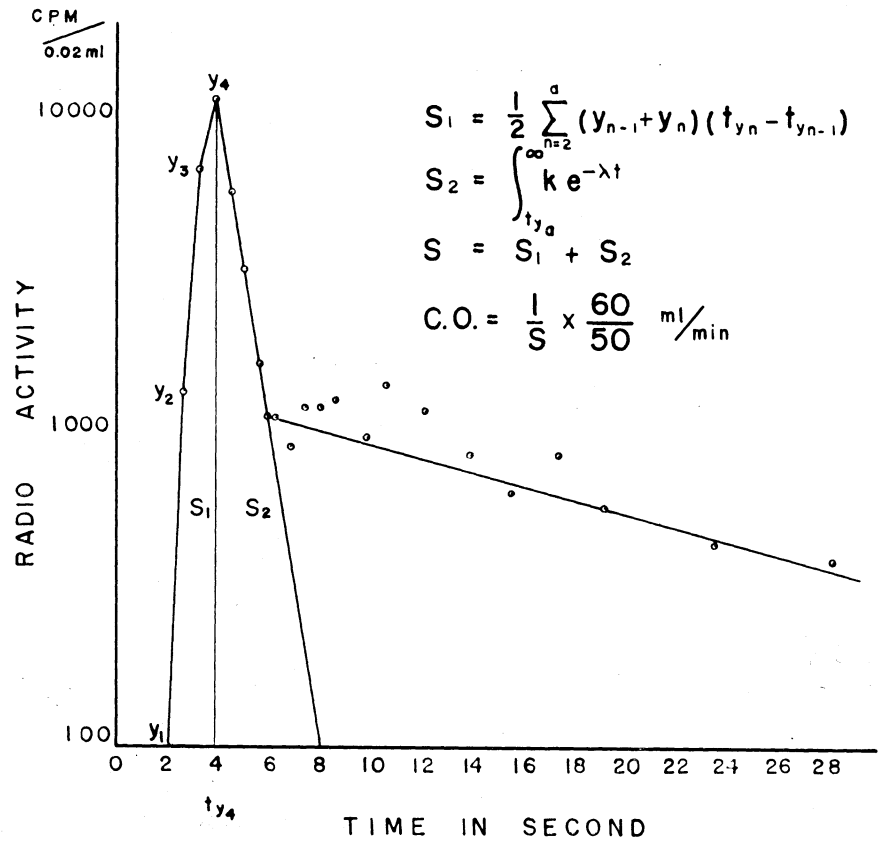

FIG. 2. Change in radio activity of 0.02 ml. of arterial blood against time in second. The area of S_1 was obtained in its approximate value by dividing it to a triangle and trapezoids using the first formula shown in the figure. a: cardiac output, I: total radioactivity injected, 60: conversion factor for seconds to minutes, 50: conversion factor for 0.02 ml. to ml.

Aliquot 0.02 ml. of blood taken from that collected on the table was placed in a small test tube containing 1 ml. of water and its radioactivity was measured by means of a Toshiba Well-type Scintillation Counter.

The radioactivity of each sample was plotted on a semilog graph paper against time in second as illustrated in FIG. 2. Thus cardiac output could be calculated.

The pituitary gland was removed as quickly as possible, weighed by a microtorsion balance (Shimadzu) to 0.01 mg. and determined for radioactivity. The body uptake of ^{86}Rb as a whole was considered to be equal to the total amount of the indicator injected. The pituitary blood flow was calculated using the formula,

$$\text{pituitary blood flow } (\mu l./min./mg.) = \frac{\text{cardiac output } (\mu l./min.) \times \text{pituitary radioactivity (cpm)}}{\text{total radioactivity} \times \text{pituitary weight (mg.) injected (cpm)}}$$

Pressor assay: Pressor activity of the material was performed using Dibenzyline-treated rats at 2 dosage levels according to the method described in Pharmacopeia U. S. P.[15]. In our hand, however, blood pressure of the femoral artery was measured

by means of a Nihonkoden Electromanometer. The standard used in this study was synthetic lysine vasopressin (Sandoz Ltd.).

Extract of the neural lobe: To make the neurohypophysial extract, 5 to 8 neural lobi of rats obtained at the time of removal of the total pituitary gland for ACTH assay were pooled, extracted in cold 0.5 ml. 0.3% acetic acid and homogenized in ice. The extract was kept in a deep freezer until it was used, but not longer than for 3 days. Before the experiment it was adequately diluted with 0.3% acetic acid in physiological saline and determined for its pressor activity.

RESULTS

Blood ACTH after painful stimuli on the paw of rat: When the rates of corticosterone secretion at a 15 minute interval without injecting any material were measured in the hypophysectomized assay rats, the mean difference between the first and second measurements of 9 observations was -2 mμg./min. with standard error of ± 5.6. Blood ACTH of normal intact rats in terms of an increase in corticosterone secretion rate in the recipient rats was negligible (6 ± 2.2 mμg./min.).

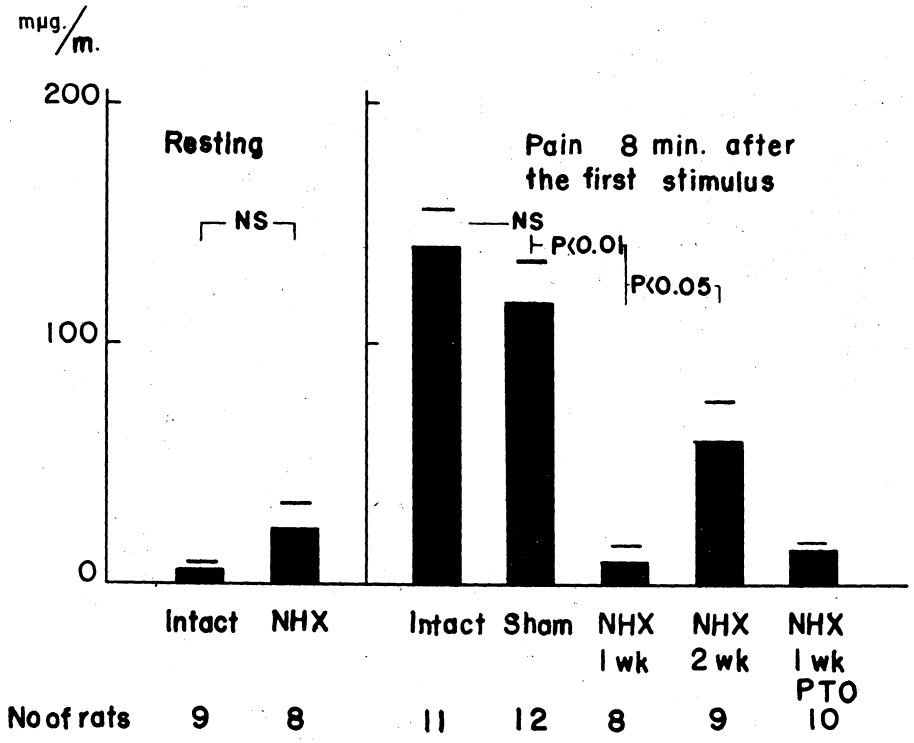

FIG. 3. Blood ACTH in terms of increase in corticosterone secretion rate in hypophysectomized recipient rats. NHX: neurohypophysectomized rats, PTO: pitressin tannate in oil, Sham: sham-operated rats.

Blood of the neurohypophysectomized rats 1 week after the operation showed ACTH activity of 23±9.1 mμg./min. which was not significantly higher than that of the intact animals (FIG. 3). A rat was placed in a plastic case the bottom of which consisted of brass grids. Fine rods of brass forming the grid were insulated each other but connected alternately by a wire respectively which was connected to the output plug of a Porter-type inductorium (FIG. 4). The inductorium was driven by a battery of 6 volt. Rats

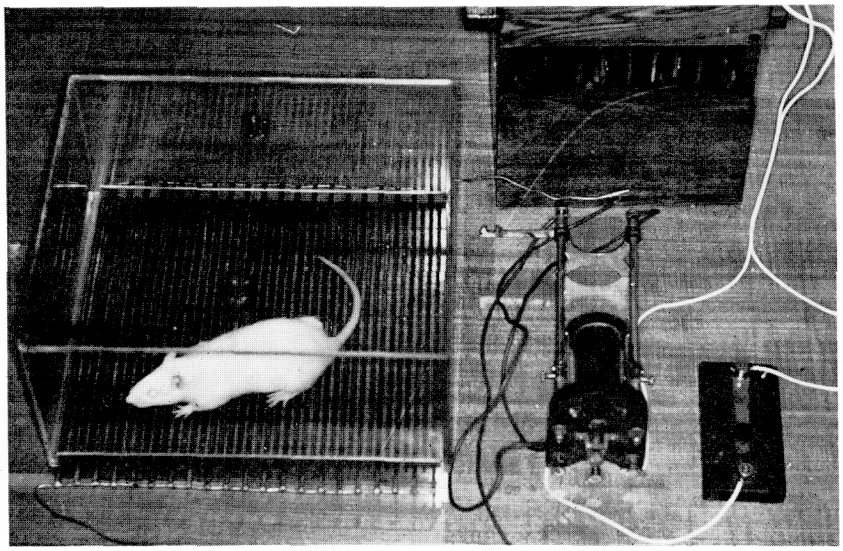

FIG. 4. The apparatus for stimulating the paws of rats.

were stimulated in their paw by faradic stimulation 11 times at intervals of 29 seconds. The duration of one stimulus was 1 second. Eight minutes after the beginning of stimulation blood sample was obtained and assayed for ACTH activity. Blood ACTH significantly increased both in the intact (141±16.2 mμg./min.) and the sham-operated rats (118±17.5 mμg./min.) following the stress, while no increase was induced in the neurohypophysectomized rats (9±7.9 mμg./min.) 1 week after the surgery. Two weeks after the removal of the neural lobe the response to the stress was slightly restored (54±17.5 mμg./min.), but still significantly lower than that in the intact ($P<0.05$) or the sham-operated rats ($P<0.05$) (FIG. 3). De WIED reported that using plasma free corticosterone as an index of pituitary adrenal activity chronic administration of Pitressin tannate in the neurohypophysectomized rats resulted in a near-complete restoration of the corticotropic effect of neurogenic stimuli[6]. In order to reconfirm his finding the neurohypophysectomized rats were subcutaneously injected with Pitressin tannate in oil in a dose of 0.5 U/100 g. daily for 5 consecutive days starting on the third day after the surgical operation. The last injection

was made on the day preceding experiment. The control rats were administered with peanut oil in the same fashion as that of Pitressin tannate in oil.

In our hand, however, no restoration of the response was obtained by treatment with Pitressin tannate. The mean blood ACTH in 10 observations of neurohypophysectomized Pitressin-treated rats after the stress was 13.7± 2.4 mμg./min. and that in the control neurohypophysectomized rats in 18 observations was 18±5.1 mμg./min., both of which were significantly lower than the blood ACTH after the stress in the intact or the sham-operated rats ($P<0.005$).

Blood ACTH after prolonged pain: Stimulation by faradic current for a short duration using the apparatus described caused sensation of pain.

Therefore the stress was considered to be of neurogenic with little metabolic effect. In order to know whether the absence of neural lobe abolishes ACTH release following this kind of stress regardless its duration, the same stimulation but for a prolonged period was applied to the rat, e. g. 16 times of stimuli of 5 second duration each at 25 second intervals. Blood was collected 8 minutes after the first stimulation.

A significant increase in blood ACTH was demonstrated in the neurohypophysectomized rats (107±20.7 mμg./min.), however the value was still smaller than that in the sham-operated control animals (208±31.5 mμg./min.) ($P<0.05$). (FIG. 5). During the stimulation of 5 second duration the legs of rat were tonically extended, which might probably cause some metabolic or systemic changes in the body. Because no one knows enough about grading stress to rule out the possibility that the observed result is simply due to differences in stimulus strength, it is difficult to make a conclusion that neurohypophysectomy does not abolish the corticotropic effect of neurogenic stress if it is intense one.

Blood ACTH after laparotomy: Laparotomy was also used as a stress which was considered to induce severe metabolic changes as well as neurogenic ones. Rats were inhalated ether for 1 minute and underwent laparotomy after the animals were completely anesthetized. Eight minutes after the beginning of ether inhalation blood was collected by decapitation.

Blood ACTH was considerably elevated in the normal intact rats (216± 30.9 mμg./min.) and the sham-operated animals (230±21.0 mμg./min.).

In the neurohypophysectomized rats it was also increased (120±23.2 mμg., min.), but the value was significantly lower than those of both the intact and the sham-operated rats ($P<0.01$) (FIG. 5).

Blood ACTH after histamine injection: Histamine was employed by many investigators as a representative agent having metabolic effect. One mg. of histamine dihydrochloride per 100 g. was intraperitoneally injected into the

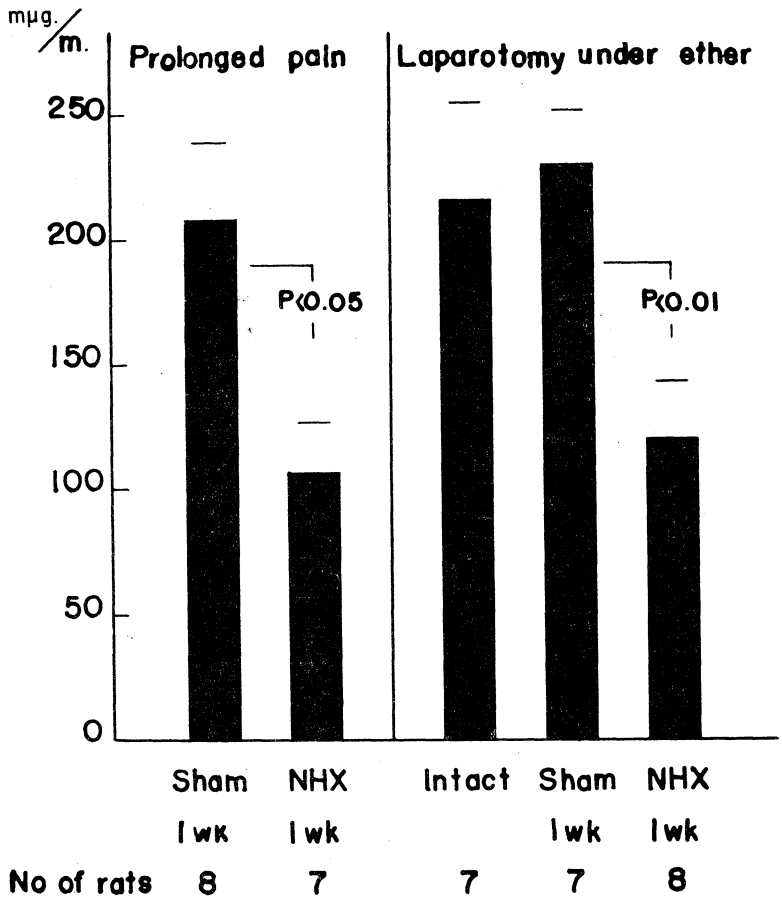

FIG. 5. Blood ACTH in terms of increase in corticosterone secretion rate in hypophysectomized recipient rats after prolonged pain or laparotomy.

rats. Blood ACTH levels 5 and 10 minutes after the injection were 49±15.2 mμg./min. and 80±22.1 mμg./min. respectively in the normal intact rats. In the sham-operated rats it was 55±15.1 mμg./min. 10 minutes after histamine. In the neurohypophysectomized rats the values 5 and 10 minutes after the injection were 59±17.6 mμg./min. and 57±22.5 mμg./min. respectively (FIG. 6). In some rats, no matter they were intact, sham-operated or neurohypophysectomized, no demonstrable increase in blood ACTH was detected by the method used, e. g. a fairly large variation in the responses as shown by large standard errors of mean. As illustrated in FIG. 6, no difference in blood ACTH was observed between the intact or the sham-operated control rats and the neurohypophysectomized rats following histamine, though the increase in blood ACTH by histamine was much less in extent than that seen after pain stimuli and laparotomy in the control animals.

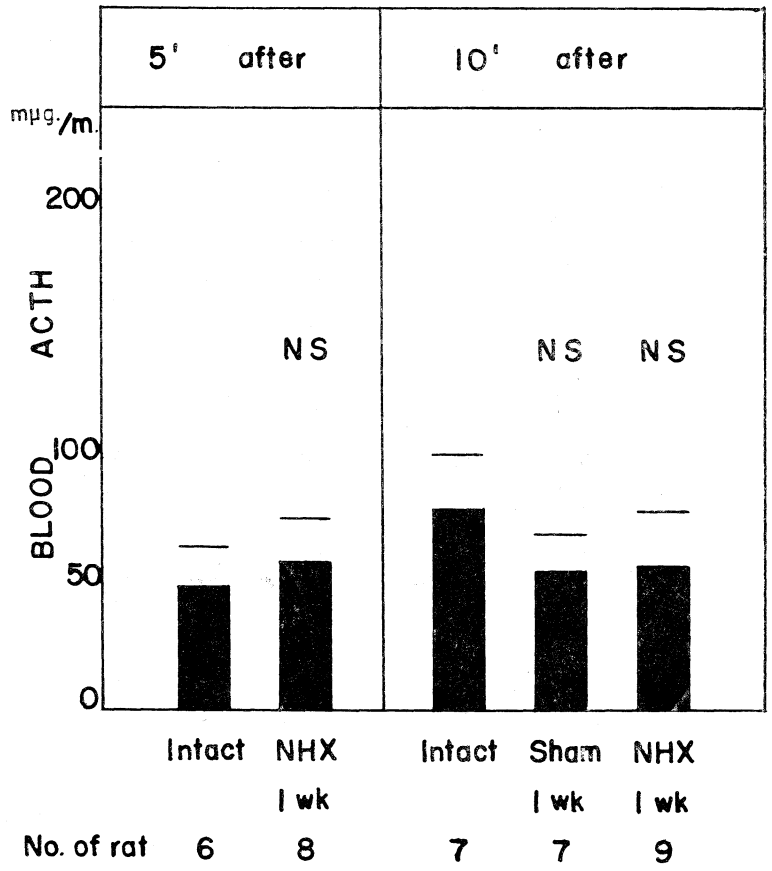

FIG. 6. Blood ACTH in terms of increase in corticosterone secretion rate in hypophysectomized recipient rats after intraperitoneal injection of histamine.

Blood ACTH after an intraperitoneal injection of a large dose of synthetic lysine vasopressin: Vasopressin was known to stimulate the adenohypophysis to release ACTH[16-23]. If vasopressin's corticotropic effect in vivo is provoked merely by its direct action on the anterior pituitary, ACTH secretion following injection of vasopressin may be observed to the same extent both in the intact and the neurohypophysectomized rats, if the blood supply to the adenohypophysis is not impaired in the latter group.

Synthetic lysine vasopressin in a dose of 100 mU./100 g. was intraperitoneally injected into rats. Blood ACTH 10 minutes after an intraperitoneal injection of vasopressin in the intact and the sham-operated rats were 233± 25.4 mμg./min. and 233±45.7 mμg./min. respectively. However, it was nearly not detectable level in the rats in which the posterior lobe was removed 1 week previously (13±3.6 mμg./min.).

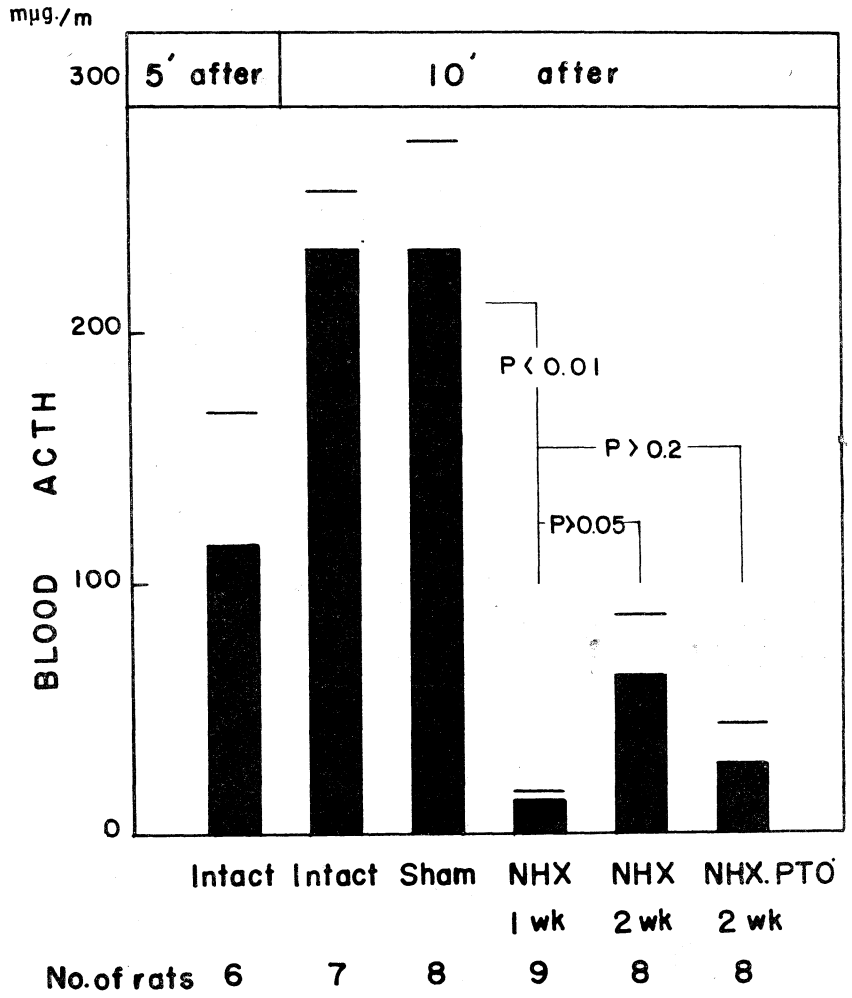

FIG. 7. Blood ACTH in terms of increase in corticosterone secretion rate in hypophysectomized recipient rats after intraperitoneal injection of synthetic lysine vasopressin.

Two weeks after neurohypophysectomy the response to exogenous vasopressin seemed to be somewhat restored (63±25.9 mμg./min.) though statistically not significant. Treatment of the neurohypophysectomized rats with Pitressin tannate in oil, 0.5 U./100 g. s.c. daily for 5 consecutive days preceding the experment, failed to restore the vasopressin's corticotropic effect (FIG. 7).

Blood ACTH after an intravenous injection of synthetic lysine vasopressin and the neurohypophysial extract: Rats were anesthetized with Nembutal, 4 mg./100 g. i. p. Fifteen minutes after the injection of Nembutal, 400 mU./100 g.

of synthetic lysine vasopressin was infused into the jugular vein of the rat over 2 minutes. Blood was collected 5 minutes after the beginning of infusion of the hormone. Mean blood ACTH in the 8 sham-operated control animals was 164 mμg./min. with standard error of ±16.2 in terms of increase in corticosterone secretion rate in the assay rat.

An intravenous administration of vasopressin induced only a very slight increase in blood ACTH in the absence of the neural lobe (41±15.6 mμg./min.)

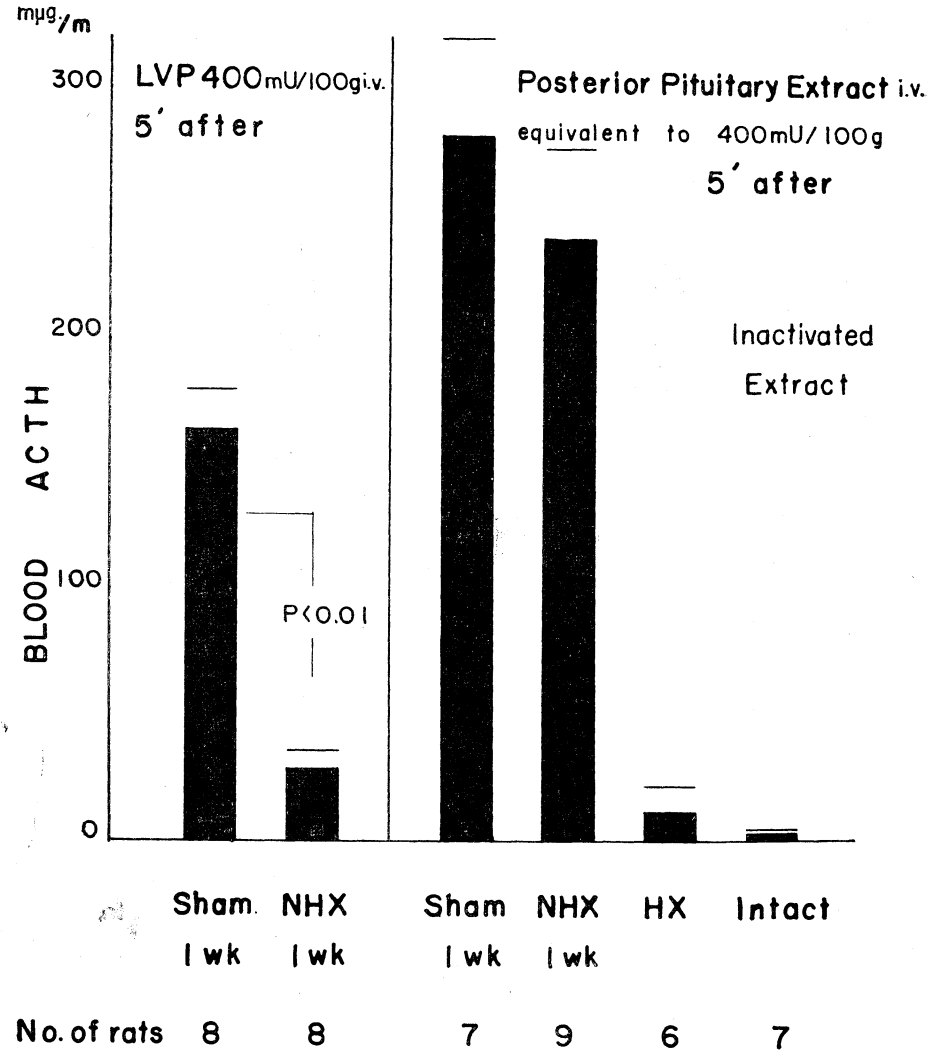

FIG. 8. Blood ACTH in terms of increase in corticosterone secretion rate in hypophysectomized recipient rats after intravenous injection of synthetic lysine vasopressin or the extract of the posterior pituitary gland of rat over a 2-minute period. Inactivated extract: thioglycollate-treated extract.

(FIG. 8). An intravenous injection of the acid extract of the neurohypophysis in a dose of 400 mU./100 g. in terms of pressor unit of synthetic lysine vasopressin induced a considerable rise in blood ACTH in the sham-operated control rats (281±33.4 mμg./min.). Blood ACTH in the neurohypophysectomized rats following injection of the same material was also considerably high (241±39.5 mμg./min.). Since the neural lobe contains ACTH also[24-26], the increase in blood ACTH found in the absence of the neural lobe following injection of the neurohypophysial extract might be due to the neurohypophysial ACTH contaminated in the extract. Although it is diluted by the circulating blood, the sensitive assay method used might detect the exogenous ACTH. In order to clarify this problem, the extract was injected into the hypophysectomized rats and its blood was assayed for ACTH. As illustrated in FIG. 8, the blood ACTH level was negligibly small (12±5.2 mμg./min.), which ruled out the possibility as raised above.

Since vasopressin did not, but the neurohypophysial extract did induce ACTH release in the neurohypophysectomized rats the extract was considered to contain some active substance or substances other than vasopressin which probably directly stimulate the adenohypophysis to release ACTH. It is likely that this substance may be CRF stored in the neurohypophysis.

The extract was treated with thioglycollate as recommended by Walker[27]. More than 98% of the original pressor activity was assured to be lost using Dibenzyline-treated rats. As shown in FIG. 8, the inactivated neurohypophysial extract in a dose of 400mU./100 g. i. v. of the original potency did not induce ACTH release (3±1.8 mμg./min.) even in the intact rats (FIG. 8).

Measurement of hypophysial blood flow: Removal of the neural lobe resulted in nearly complete abolishment of ACTH release in response to pain stimuli for a short duration or vasopressin. ACTH secretion following other stresses such as laparotomy under ether anesthesia and prolonged pain was also reduced. Since the rise in blood ACTH after histamine injection, though moderate in

Table 1.
Regional Blood Flow in the Pituitary Glands of Rats.

Procedure	B. W.±SE g	No. of Rats	Adenohypophysis		Neurohypophysis	
			μl/m/gl.±SE	μl/m/mg±SE	μl/m/gl.±SE	μl/m/mg±SE
Intact	153±3.3	9	6.22±0.50	0.99±0.17	6.99±1.54	7.81±1.35
Sham 1 wk	151±7.7	7	8.94±1.90	2.65±0.35**	15.21±4.23	15.73±3.81*
NHX 1 wk	154±4.7	10	9.25±0.98*	2.42±0.40**		

Levels of significance: vs Intact *<0.05, **<0.01 No significant difference between sham & NHX.

its extent, was not affected by neurohypophysectomy, impairment of hypophysial blood supply might not fully account for the inhibition or suppression of ACTH release by neurohypophysectomy. However, it is necessary to examine whether blood supply to the adenohypophysis is impaired by the surgery of neurohypophysectomy, or if any, to what extent the blood flow is disturbed. Blood flow of the adenohypophysis as well as the neurohypophysis was measured using the method originally described by SAPIRSTEIN[14]. As shown in Table 1 the adenohypophysial blood flow was not diminished in the neurohypophysectomized rats 1 week after the operation, but rather increased in these animals as well as in the sham-operated ones. The neurohypophysial blood flow was also remarkably increased in the sham-operated animals.

DISCUSSION

Close association between the discharge of neurohypophysial vasopressin and ACTH in response to noxious stimuli led to a postulation that vasopressin of the hypothalamo-hypophysial system may be involved in the ACTH release mechanism. Many papers have indicated that natural or synthetic lysine and arginine-vasopressin induced ACTH secretion from the adenohypophysis in vivo[17-23] and in vitro[16, 28]. Vasopressin was reported to be effective in the blocked rats with hypothalamic lesion or treated with steroid such as corticosterone[29], hydrocortisone[30, 31], prednisolone[32], dexamethasone[23, 33] or drugs like morphine[18], Nembutal-morphin[34, 35]. Although most of these observations were made using a large dose of vasopressin, some reports recently appeared indicated that a minor dose of vasopressin within physiological ranges was also effective when the hormone was injected into the carotid artery of the dexamethasone-treated rats[23], the third ventricle of conscious dogs[36] or the artery perfusing the ACTH-producing pituitary tumor implanted in a leg of hypophysectomized rats[22]. These reports support the conception that vasopressin may be the physiological mediator to provoke ACTH release.

However the experiments presented here demonstrated a marked reduction in ACTH secretion following vasopressin injection after ablation of the neurohypophysis. If vasopressin acts directly on the adenohypophysis of rats under the experimental condition used ACTH release should be produced to the same extent regardless presence or absence of the neural lobe, as far as the adenohypophysis itself is not damaged by the surgical operation. Our experiment on the hypophysial blood flow indicated no impairment but rather an increase in blood supply to the adenohypophysis 1 week after the surgery of neurohypophysectomy. It implies that vasopressin injected reaches the adenohypophysial tissue of the neurohypophysectomized rats in the same or higher concentration than in the intact or the sham-operated animals. Reduction or near-complete abolishment of ACTH release following vasopressin injection in the neurohypo-

physectomized rats, therefore, must be accounted for by other mechanism than an impairment of blood supply to the adenohypophysis. Therefore a considerable increase in ACTH release following vasopressin injection i. p. or i. v. observed in the intact or the sham-operated control rats may be resulted from its indirect effect. Although vasopressin's direct effect on the adenohypophysis can not be absolutely ruled out, the amount of ACTH released due to its direct action in the rats must be much smaller in extent than that due to the indirect effect.

Since the neural lobe contains a large amount of CRF as well as vasopressin, there is a likelihood that exogenous vasopressin induces release of CRF stored in the neurohypophysis. EEG pattern was reported to be modified by intracarotid injection of vasopressin[37] suggesting an activation of the neural system which in turn might lead to a reflex secretion of the neurohypophysial CRF. It may be also possible that exogenous vasopressin directly affects the neural lobe by modifying the permeability of cell membrance or other mechanisms causing the release of CRF stored. These conceptions do not conflict with the facts that a fairly small dose of vasopressin is effective in ACTH release when it is injected into the carotid artery[23] or into the third ventricle[36].

These routes of administration may be also favorable for CRF release from the neural lobe through the neural activation or by the direct effect on the neurohypophysial CRF. ANDERSON's observation on the effect of vasopressin on the ACTH-producing pituitary tumor[22] may not be adequate to discuss at the same time, until the tumor cells are shown to possess the same nature as that of the normal pituitary cells.

By measuring adrenal ascorbic acid depletion, NOWELL reported that exogenous Pitressin administered into the neurohypophysectomized rats intravenously in a dose of 300 mU. per rat caused ACTH secretion[38], which is contrary to ours. In our experiment the rise in blood ACTH level after an intravenous injection of vasopressin in these animals was very slight. But the level (41 ± 15.6mμg./min.) seemed to be somewhat higher than that of the non-treated neurohypophysectomized rats (23 ± 9.1 mμg./min.), though statistically insignificant. This implies that ACTH release following vasopressin was very much reduced in the absence of the neural lobe, but might not be completely abolished, probably due to its direct action on the adenohypophysis. The small increment in blood ACTH, if any, not detected by the method used might affect the adrenal ascorbic acid concentration in the experimental animal. Further, adrenal ascorbic acid depletion in the experimental animals may not necessarily accurately reflect ACTH secretion. Adrenal ascorbic acid response may be influenced by vasopressin, which stimulates directly the adrenal cortex[45] or potentiate ACTH[7, 46]. Several papers reported dissociation of adrenal ascorbic acid depletion and steroidogenesis[17, 39–44]. NOWELL injected Pitressin into the jugular vein of conscious rats through the polyethylene tube cannulated, while

we injected vasopressin into the animals under Nembutal anesthesia which may also lower the activities of the central nervous system. The difference in the parameters used as well as that in expermental conditions may be able to account for the discrepancy.

It is interesting to know the acid extract of the neural lobe with equipressor unit with vasopressin injected induced a considerable increase in blood ACTH in the Nembutalized-neurohypophysectomized rats.

Since the neurohypophysial extract did not increase blood ACTH in the hypophysectomized rats, the neurohypophysial ACTH itself can not account for this activity. This finding may suggest that the neural lobe contains active substance or substances which stimulate the adenohypophysis directly or through the hypothalamus without involvement of the neurohypophysis.

The most likely explanation is that the substance may be CRF stored in the neural lobe, probably β-CRF, because the activity was destroyed by thioglycollate treatment.

According to VERNIKOS[47] CRF activity in the neural lobe can be accounted for mainly by vasopressin. However, the distinct difference between vasopressin and the neurohypophysial extract in ACTH release in the neurohypophysectomized rats is strongly against her conception.

She assayed blood ACTH by measuring adrenal ascorbic acid depletion in the steroid-blocked recipient rats, while we used steroidogenic activity in the hypophysectomized rats. Again dissociation between these 2 activities as well as different experimental conditions used might explain the discordance. The failure in ACTH release following pain stimulus of a short duration in the absence of the neural lobe may be accounted for by the lack of CRF of the neurohypophysis to meet an acute demand.

Unmodified ACTH release following histamine injection in the neurohypophysectomized rats confirmed the observation made by de WIED who stated dual mechanisms in ACTH secretion[6]. Prolonged pain or laparotomy also induced a considerable rise of blood ACTH both in the normal and the neurohypophysectomized rats. But the response was somewhat smaller in the latter than the former, implying that two mechanisms in ACTH release are involved under these stresses and one which necessitates the neural lobe is blocked in its absence.

Although it may be impossible to clearly differenciate neurogenic and systemic stress, the ACTH release mechanism which involves the neurohypophysis seems to be mainly activated by the stress which induces chiefly neural excitation with little metabolic change as suggested by the present study and that of de WIED[6]. In our hand, substitution with Pitressin tannate in the neurohypophysectomized rats failed to restore the response to pain stimulus or vasopressin injection, which is contradictory to de WIED's finding. Two weeks after the removal of the neural lobe a slight restoration in the ACTH release

response was observed. It may be possible that the miniature neurohypophysis formed at this time also started storing CRF available for acute use, though insufficient in amount.

SUMMARY

The role of the neurohypophysis in ACTH release mechanism was investigated in the rat by measuring changes in blood ACTH levels under various kinds of stress. A remarkable increase in blood ACTH following repeated pain stimuli of 1 second duration on the paws or injection of synthetic lysine vasopressin, 400 mU./100 g. i. p. or i. v., was seen in the normal intact or the sham-operated rats, while it was absent or considerably reduced 1 week after the removal of the pituitary neural lobe. Prolonged pain or laparotomy induced a marked rise in blood ACTH both in the control and the neurohypophysectomized rats, but the elevation of blood ACTH was significantly less in extent in the absence of the neural lobe. Intraperitoneal injection of histamine induced a moderate rise in blood ACTH to the same extent both in the control and the neurohypophysectomized rats. The extract of the pituitary neural lobe of the rat, in a dose of 400 milli pressor units of lysine vasopressin, induced a considerable rise in blood ACTH regardless presence or absence of the neural lobe, but was not effective in the hypophysectomized rats. Since the ablation of the neural lobe resulted in a near-complete abolishment of vasopressin's corticotropic effect, the increase in blood ACTH following injection of the neurohypophysial extract may be due to CRF stored in the neural lobe. Blood supply to the adenohypophysis was not impaired, but rather increased 1 week after the removal of the neural lobe.

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