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EFFECTS OF INSULIN, HISTAMINE AND VASOPRESSIN ON PITUITARY-ADRENOCORTICAL SECRETION WITH OR WITHOUT DEXAMETHASONE PRETREATMENT

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ABSTRACT

Adrenal venous 17-hydroxycorticosteroid (17-OHCS) output in response to insulin, histamine and synthetic lysine vasopressin (L-8-V) intravenous administration was determined in nembutal-anesthetized and adrenal-vein cannulated dogs. Dexamethasone pretreatment prevented the adrenal cortical activation provoked by insulin. But when the doses of insulin were increased adrenal cortical response appeared, whereas the larger doses of dexamethasone pretreatment suppressed the adrenal cortical response caused by the increased doses of insulin administration. The quantitative correlationship between the intensity of stress which was represented by the amount of insulin administered and the amount of corticosteroids needed for blocking the stress-induced adrenal cortical activation was observed. Such correlation was also observed between histamine-induced adrenal cortical activation and dexamethasone pretreatment. In hypophysectomized dogs, neither insulin nor histamine could cause adrenal cortical response. On the other hand, dexamethasone pretraetment did not prevent the adrenal cortical activation caused by L-8-V administration, but only the shortening of responding time was observed. L-8-V infused in hypophysectomized dogs did not cause appreciable adrenal cortical activation except for some minimal rise in 17-OHCS output. The enormous doses of dexamethasone did seemingly suppress the adrenal cortical activation caused by less doses of L-8-V. This suggests that massive doses of glucocorticoids might affect pituitary itself.

INTRODUCTION

Since the report by Ingle *et al.*[1] in 1938 that chronic administration of adrenal cortical extract results in atrophy of the adrenal cortex, the inhibitory effect of adrenal cortical hormone on the release of corticotropin has been firmly established both clinically and experimentally. However, the site on which corticosteroids exert their suppressing action and their mode of action are still obscure. Sayers and Sayers[2] offered that there exists quantitative relationship between the amount of administered corticosteroids and the degree of inhibition of pituitary adrenocorticotropic activity in the rat. Yates *et al.*[3]

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proposed the reset hypothesis to explain the negative feedback control of plasma corticosteroid concentration. Leeman *et al.*[4] showed that the site of negative feedback inhibition of corticotropin release by corticosteroids is in the central nervous system in their rat experiment.

Most of the results concerning with the effect of corticosteroids on the release of corticotropin have been obtained in experiments using adrenal ascorbic acid depletion or corticosterone level in peripheral blood in the rat as the assay procedure of circulating corticotropin. The mechanism of adrenal ascorbic acid depletion and its exact relation to corticosteroidgenesis is not well understood, and the suggestion has been made that adrenal ascorbic acid depleting factor is not always related to corticotropin production[5]. The corticosteroid level, on the other hand, in peripheral blood can not necessarily be a direct indicator for adrenal cortical activation following corticotropin release as well[6].

In 1955, Nelson and Hume[7] reported an assay method for corticotropin in hypophysectomized and adrenal-vein cannulated dogs. Such a procedure would give a direct measure of corticosteroids secreted in response to corticotropin.

In the present study changes of 17-OHCS output in the adrenal vein were measured over a period of one hour or so in the dog, following subjection of an animal to stress with or without corticosteroid pretreatment. And the aim of this study was to elucidate some aspects of interrelationship between the dose of glucocorticoid pretreated and that of medical stress agents. As a blocking a gent, highly potent synthetic glucocorticoid, dexamethasone was used. As medical stress insulin, histamine and synthetic vasopressin were infused respectively. Because histamine has been used frequently as one of the representative medical stress agents, whereas insulin has not, and vasopressin is agreed to act differently from other noxious stimuli.

METHODS AND PROCEDURES

Materials: 46 animals were used in the experiments reported here; they were healthy adult mongrel dogs of both sexes ranging in weight from 6 to 12 kg. Food and water were provided *ad lib.* at all times.

Operations for adrenal vein cannulation: For studying the pituitary-adrenocortical secretion mechanism, it is first of all necessary to avoid interference with the various stresses which accompany the experiments as much as possible. Therefore, the operation for adrenal-vein cannulation was performed on the day preceding the experimental protocol except for the hypophysectomized dogs. It was possible with this preparation to obtain samples of adrenal venous blood which was free from acute operative stress.

Adrenal vein cannulation was carried out according to a slight modification of the technique of Nelson and Hume[7]. The left lumbo-adrenal vein was ex-

posed through the abdominal route and after ligating all branches of lumbo-adrenal vein a polyethylene catheter (Fr No. 6, 2 mm in diameter) was inserted into the vein just lateral to the left adrenal gland and tied securely into vein. The distal end of the polyethylene cannula was led to the outside through a stab wound in the lateral abdominal wall. A thin vinyl tube was loosely passed around the adrenal vein between the adrenal gland and the inferior vena cava, and was conducted exteriorly. This was used as a choker from the outside at the time of blood collection.

Operations for hypophysectomy: Hypophysectomy was performed according to the temporal transdural unilateral method[8] on the day preceding adrenal-vein cannulation. In hypophysectomized dogs, the experiments were started soon after adrenal-vein cannulation was over. And the low 17-OHCS levels in adrenal venous blood proved that the hypophysis had been taken out completely.

Blood sampling: The dog was anesthetized with nembutal (sodium pento-barbital) 30 mg per kg body weight (mg/kg) intravenously (i.v.) injected into femoral vein through the cannula which had been placed on the day of operation. By tightening the choker blood from the adrenal gland could flow back through the polyethylene cannula the end of which had been placed into a collection tube, and after injection of stress agent the timed samples were collected for generally 60 minutes or so. Through the course of experiment, heparin sodium was used to prevent blood clotting and blood loss was carefully replaced with Ringer's solution. During the period of time that blood was not being collected, the polyethylene cannula was washed with Ringer's solution releasing the vinyl choker and a small plug was placed in the end. Thus during that time the adrenal venous effluent flowed into the inferior vena cava. The collected blood samples were kept in a refrigerator at 4°C adding heparin sodium until determination of 17-OHCS.

Corticoid studies and blood sugar estimations: 17-OHCS content of the adrenal venous blood was determined by the method of Peterson *et al.*[9], using the whole blood. The blood sugar level was estimated by the method of Hagedorn and Jensen[10].

Substances tested:

Saline.....0.9% solution (for control).
Insulin.....Isujilin 'Shimizu', 20 insulin units per ml.
Histamine.....As histamine dihydrochloride, 0.5 mg per ml.
ACTH.....Bovine corticotropin (N.V. Organon), 25 IU per vial.
Vasopressin.....Lysine-8-vasopressin, synthetic (Sandoz), 10 units per ml.
Dexamethasone..Dexamethasone-21-phosphate disodium salt, 4 mg per ml.

All drugs for intravenous solution were dissolved in 3 to 4 ml isotonic

saline solution, prewarmed to about 35°C and injected i.v. within 90 seconds through femoral vinyl cannula At the conclusion of each experiment, 10 to 25 IU of ACTH was administered to show each preparation responded to exogenous corticotropin. To prepare corticoid-pretreated animals, dexamethasone was injected subcutaneously (s.c.) 4 to 5 hours prior to the experiment, as the pilot experiment revealed that resting adrenal venous 17-OHCS outputs remained low from 2 to 6 hours after dexamethasone s.c. injection.

RESULTS

1) Basal adrenal venous 17-OHCS output and the evaluation of adrenocortical response: On the day of operation, the amount of adrenal venous 17-OHCS outputs just after adrenal-vein cannulation was generally high. The values were 3.5 to 6.5 μg per minute depending on the case, which correspond to the maximal amount obtained after corticotropin injection (Fig. 1 to 6). On the other hand, at the resting state amounts of adrenal venous 17-OHCS outputs were usually low and valued about 1 μg per minute or less. The low value of 17-OHCS output was essential for these experiments, and when resting values were high the experiments were considered as invalid.

Adrenal venous 17-OHCS outputs following administration of the stress agent were compared with the resting adrenal venous 17-OHCS output and the maximal adrenal venous 17-OHCS output after ACTH. And the value obtained after medical stress was referred to as positive response when it exceeded a half of maximal output value after ACTH plus resting value.

2) Adrenal venous 17-OHCS output following i.v. administration of saline or insulin: As seen in Fig. 1 (a), administration of 4 ml physiological saline did not lead to corticotropin release under nembutal anesthesia and adrenal venous 17-OHCS outputs and blood sugar levels remained pretty much the same for the following 60 minutes. However, soon after ACTH administration 17-OHCS output increased and the maximal level continued for the following 30 minutes or more.

Insulin, on the other hand, given in a dose of 1 unit per kg body weight (U/kg) caused marked rise in adrenal venous 17-OHCS outputs (Fig. 1 (b)). Such an activation of the pituitary-adrenocortical axis was also observed in the cases with 2 and 3 U/kg insulin administration (Table 1). In each case the increment of adrenal venous 17-OHCS output began within 15 minutes after insulin i.v. before the blood sugar level fell down and reached its maximal amount that persisted for the following 60 to 100 minutes studied.

3) Adrenal venous 17-OHCS output following insulin administration in dexamethasone pretreated dogs: Fig. 1 (c) depicts that 0.2 mg/kg dexamethasone pretreatment 260 minutes prior to the experiment prevented the increment of

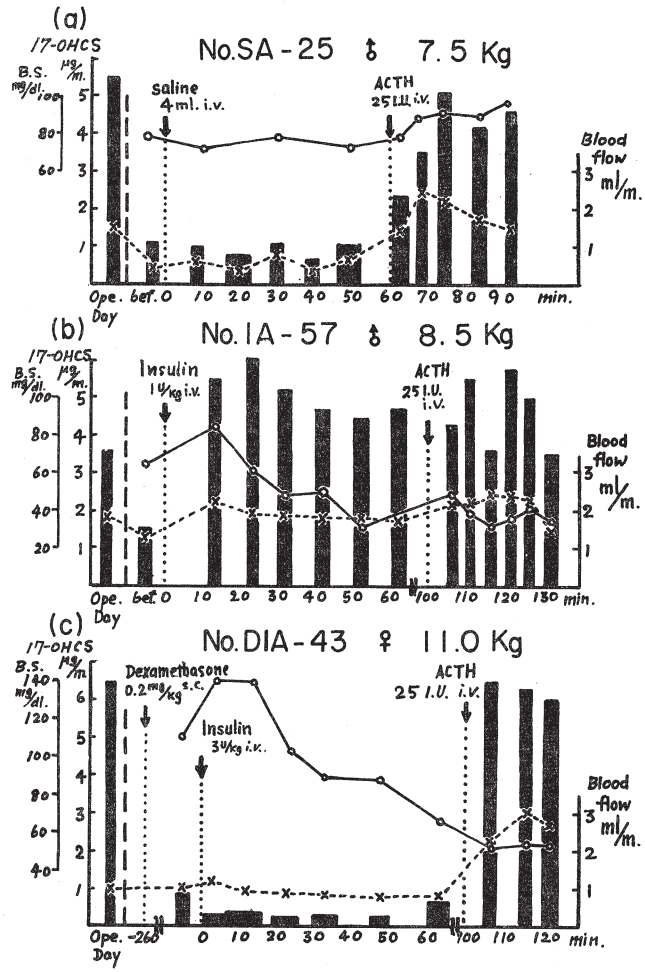

FIG. 1. (a) Dog SA 25. Time course of adrenal venous 17-OHCS outputs following saline and later ACTH administration.

(b) Dog IA-57. Increment of adrenal venous 17-OHCS outputs following insulin administration and later ACTH.

(c) Dog DIA-43. Dexamethasone pretreatment prevented insulin-induced adrenocortical activation.

* Broken line shows adrenal venous blood flow, and straight line blood sugar level in this and successive figures.

adrenal venous 17-OHCS output following 3 U/kg insulin administration, but did not interfere with the increment after ACTH. In the other cases, 0.2 mg/kg dexamethasone pretreatment did not prevent the adrenocortical response to 4 U/kg insulin i.v.. With increasing the dose of dexamethasone to 0.3 mg/kg (Fig. 2 (a)), adrenocortical response to 4 U/kg insulin did not appear. In the next case (Fig. 2 (b)), which was pretreated with 0.3 mg/kg dexamethasone,

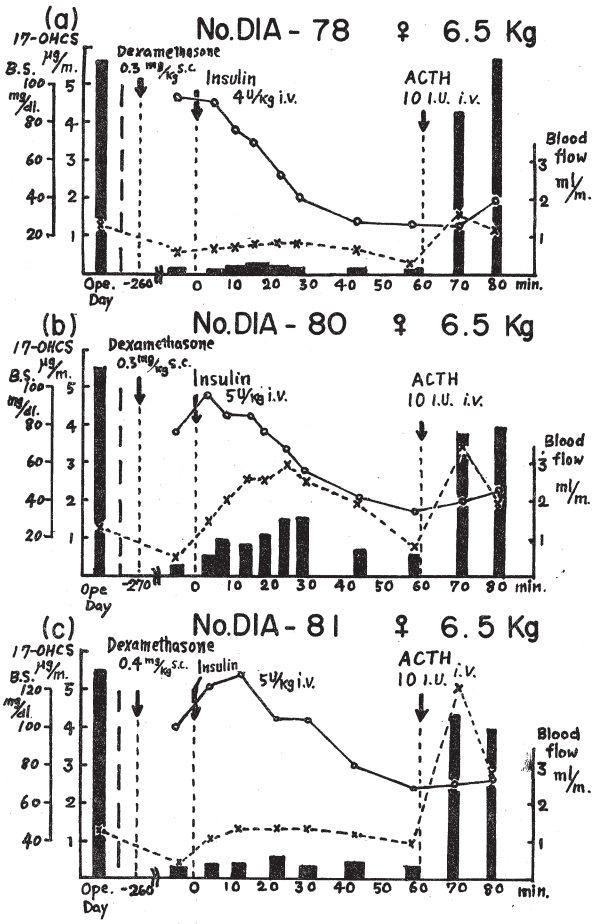

FIG. 2. (a) Dog DIA-78. Dexamethasone pretreatment (0.3 mg/kg) suppressed adrenocortical response to insulin (4 U/kg).

(b) Dog DIA-80. With the same dose of dexamethasone pretreatment, 5 U/kg insulin caused definitive increment of 17-OHCS outputs.

(c) Dog DIA-81. With increasing the dose of dexamethasone (0.4 mg/kg), 5 U/kg insulin did not provoke any increment of adrenal venous 17-OHCS outputs.

administration of insulin 5 U/kg increased adrenal venous 17-OHCS outputs slightly but definitely. However, this response was suppressed with 0.4 mg/kg dexamethasone pretreatment (Fig. 2 (c)). In each case adrenocortical response to ACTH was sufficient. The increase in the doses of insulin provoked pituitary-adrenocortical activation in dexamethasone pretreated dogs. But when the doses of dexamethasone were increased to the amount which exceeds the previous study, the same dose of insulin did not provoke pituitary-adrenocortical activation (Table 1).

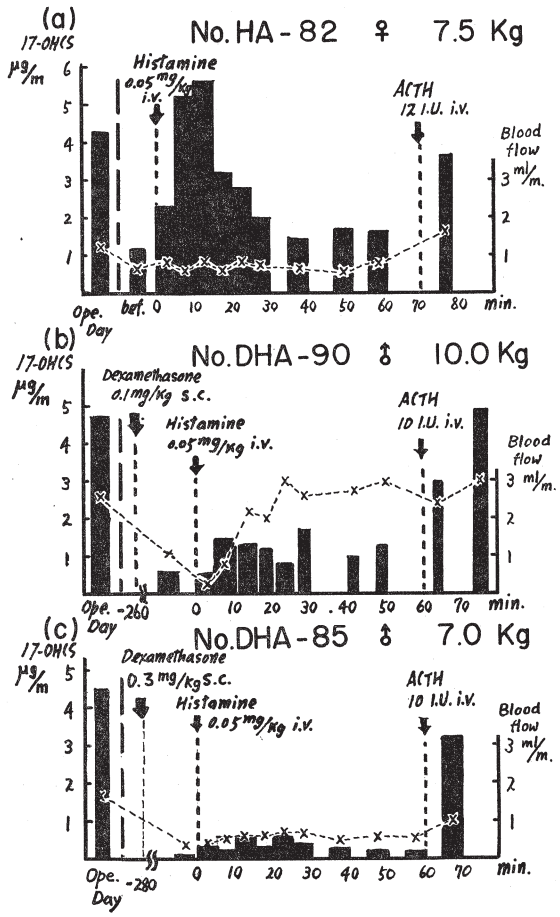

FIG. 3. Correlation between histamine stress and dexamethasone pretreatment.

(a) Dog HA-82. Increment of adrenal venous 17-OHCS outputs after 0.05 mg/kg histamine i.v..

(b) Dog DHA-90. Dexamethasone pretreatment 0.1 mg/kg partially blocked adrenocortical response to 0.05 mg/kg histamine.

(c) Dog DHA-85. Dexamethasone pretreatment 0.3 mg/kg completely blocked adrenocortical response to 0.05 mg/kg histamine.

4) *Adrenal venous 17-OHCS output following histamine administration with or without dexamethasone pretreatment:* As seen in Fig. 3 (a), following i.v. administration of 0.05 mg/kg histamine adrenal venous 17-OHCS output reached its maximal level within 15 minutes and decreased to its resting level within 40 minutes. Fig. 3 (b) shows that with dexamethasone pretreatment 0.1 mg/kg adrenocortical response to 0.05 mg/kg histamine was definitive, but far less than non-dexamethasone pretreated group. By increasing the doses of dex-

amethasone to 0.2, 0.3 (Fig. 3 (c)), 0.4 mg/kg, histamine 0.05 mg/kg administration could not provoke any increment of adrenal venous 17-OHCS outputs (Table 1), whereas the adrenocortical response to ACTH was sufficient.

Such correlationship as seen in insulin administration to dexamethasone pretreated animal could also be observed, using histamine instead of insulin (Table 1).

TABLE 1. Adrenocortical Response to Insulin, Histamine, Vasopressin and ACTH Administration with or Without Dexamethasone Pretreatment in the Dog

		Dexamethasone pretreatment (mg/kg)						Hypophysectomized
		0	0.1	0.2	0.3	0.4	1.0	
Saline (control)		−(3)		−(2)				
Insulin (U/kg)	1.0	+(3)						
	2.0	+(1)		−(1)				−(1)
	3.0	+(1)		−(2)				−(1)
	4.0			+(2)	−(1)	−(2)		
	5.0				±(1)	−(1)		
Histamine (mg/kg)	0.05	+(2)	±(2)	−(2)	−(1)	−(1)		−(2)
L-8-V (U/kg)	0.05	+(2)					−(3)	
	0.1	(A)	(B)					−(2)
	0.25	+(3)			+(1)	+(2)	+(2)	
	0.5	+(4)	+(2)	+(1)	+(1)	+(2)	+(1)	−(2)
ACTH (IU/animal)	10 to 25	+	+	+	+	+	+	+

* Numerals in parentheses are the number of experiments. (A) and (B) surrounded rectangulary with dotted line show each group calculated for the responding time.

5) *Adrenal venous 17-OHCS output following synthetic lysine vasopressin administration with or without dexamethasone pretreatment:* After i.v. administration of L-8-V 0.5 (Fig. 4 (a)) and 0.25 (Fig. 5 (a)) U/kg, adrenal venous 17-OHCS outputs increased. With dexamethasone pretreatment ranging in the doses of 0.1 to 0.4 mg/kg, adrenocortical response to 0.5 and 0.25 U/kg L-8-V failed to be suppressed (Fig. 4 (b), (c) and Fig. 5 (b), (c)) in contrast to the response to insulin or histamine administration. In subjection to L-8-V administration, however, dexamethasone pretreated dogs showed the shortening of responding time as compared with non-pretreated group. The pattern that the peak of maximal adrenal venous 17-OHCS output was the same and the persistence of the peak shortened could not be observed in the cases administered insulin or histamine. In the present study, each blood sample was collected every 5 minutes or so and it would not be appropriate to determine the responding time accurately. However, gross calculation showed that in dex-

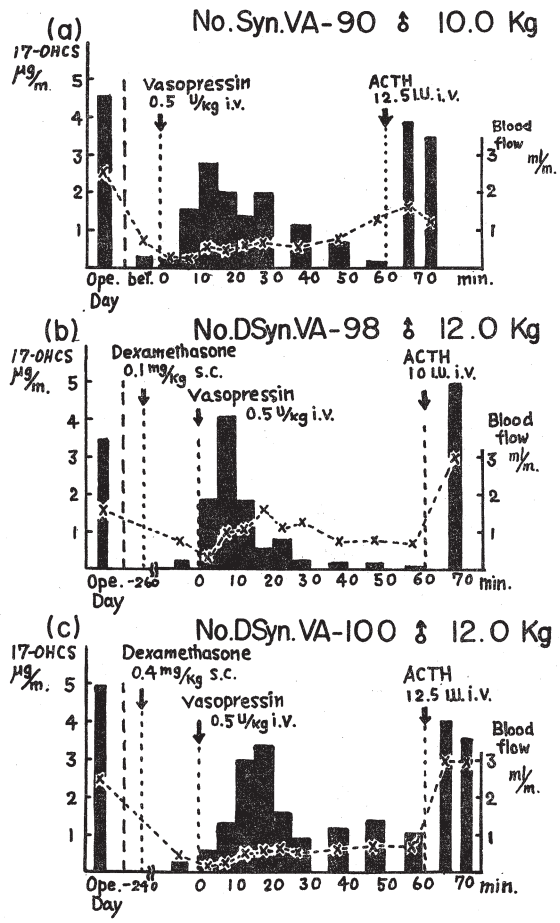

FIG. 4. (a) Dog Syn. VA-90. Increment of adrenal venous 17-OHCS outputs after 0.5 U/kg L-8-V administration. Note the peak of response lasted for about 30 min or so.

(b) Dog DSyn. VA-98. Dexamethasone pretreatment 0.1 mg/kg did not block the adrenocortical response to 0.5 U/kg L-8-V. Note the peak of response appeared shortened.

(c) Dog DSyn. VA-100. With dexamethasone pretreatment 0.4 mg/kg, adrenocortical response to 0.5 U/kg seemingly unchanged as compared with above (b) pattern.

amethasone nonpretreated dogs adrenocortical response had lasted for about 20 to 40 minutes and the mean of 7 cases (Table 1, Group A) was 23.6 minutes. On the other hand, in dexamethasone 0.1 to 0.4 mg/kg pretreated dogs adrenocortical response lasted for about 5 to 15 minutes and the mean of 9 cases (Table 1, Group B) was 12.3 minutes.

To clear up the relation between exceeding doses of dexamethasone and

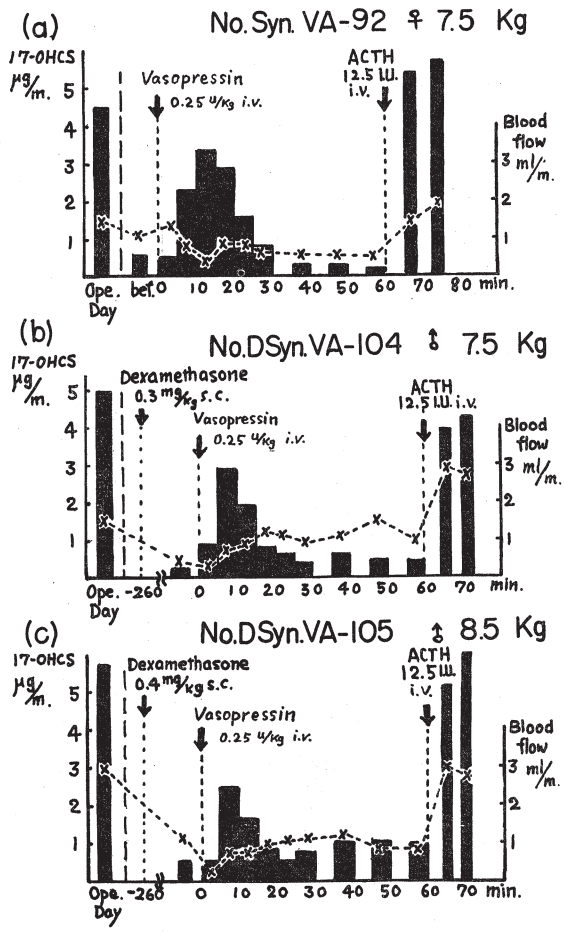

FIG. 5. Nearly the same pattern of adrenocortical response to 0.25 U/kg L-8-V as FIG. 4.

(a) Dog Syn. VA-92. Adrenocortical response to 0.25 U/kg L-8-V lasted for about 20 min or so.

(b) Dog DSyn. VA-104. 0.3 mg/kg dexamethasone pretreatment shortened the adrenocortical response to 10 min or so.

(c) Dog DSyn. VA-105. With 0.4 mg/kg dexamethasone pretreatment adrenocortical response to L-8-V lasted for 10 min or so.

less doses of vasopressin, the dose of L-8-V was decreased to 0.05 U/kg and that of dexamethasone pretreated was increased to 1.0 mg/kg. As Fig. 6 (a) shows, following i.v. administration of L-8-V 0.05 U/kg adrenal venous 17-OHCS outputs increased. After dexamethasone pretreatment 1.0 mg/kg, 0.05 U/kg L-8-V i.v. did not cause any appreciable rise in adrenal venous 17-OHCS output (Fig. 6 (b)), except for the minimal rise as seen in hypophysectomized dogs. On the other hand, 0.25 U/kg L-8-V to 1.0 mg/kg dexamethasone pretreated

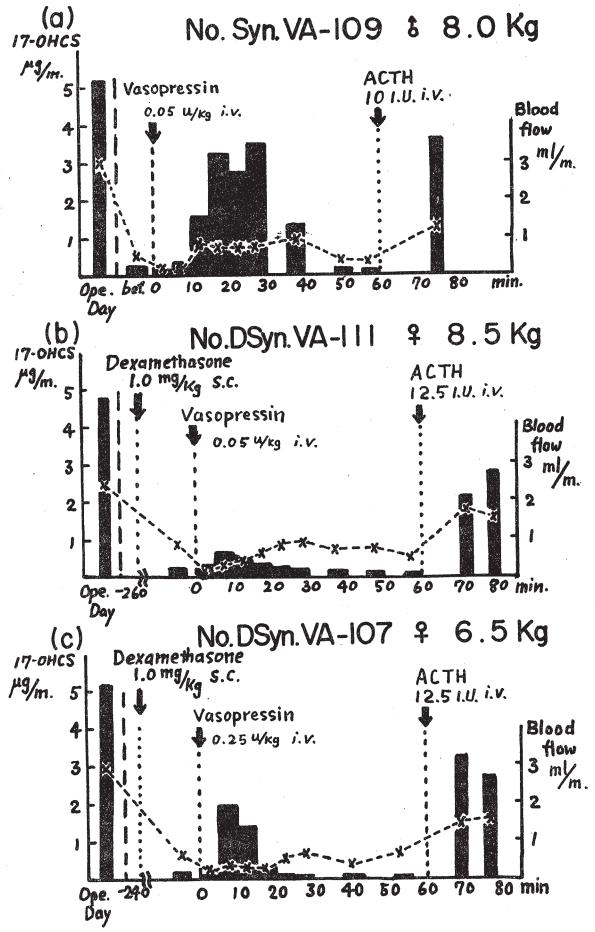

FIG. 6. (a) Dog Syn. VA-109. 0.05 U/kg L-8-V i.v. caused increment of adrenal venous 17-OHCS outputs.

(b) Dog DSyn. VA-111. 1.0 mg/kg dexamethasone pretreatment prevented the increment of adrenal venous 17-OHCS outputs.

Note some minimal rise as seen in hypophysectomized dog (FIG. 7 (c)).

(c) Dog DSyn. VA-107. With 1.0 mg/kg dexamethasone pretreatment, adrenocortical response to 0.25 U/kg L-8-V was definitive.

dog caused definitive rise in adrenal venous 17-OHCS output which far exceeds the minimal rise in hypophysectomized dogs (Fig. 6 (c)). Massive doses of dexamethasone did not appear to interfere the adrenal cortical activation by ACTH administration (Fig. 6 (b) and (c)).

6) Adrenal venous 17-OHCS output following administration of insulin, histamine and synthetic lysine vasopressin in hypophysectomized dogs: In hypophysectomized dogs, 2 or 3 U/kg insulin i.v. administration did not provoke the

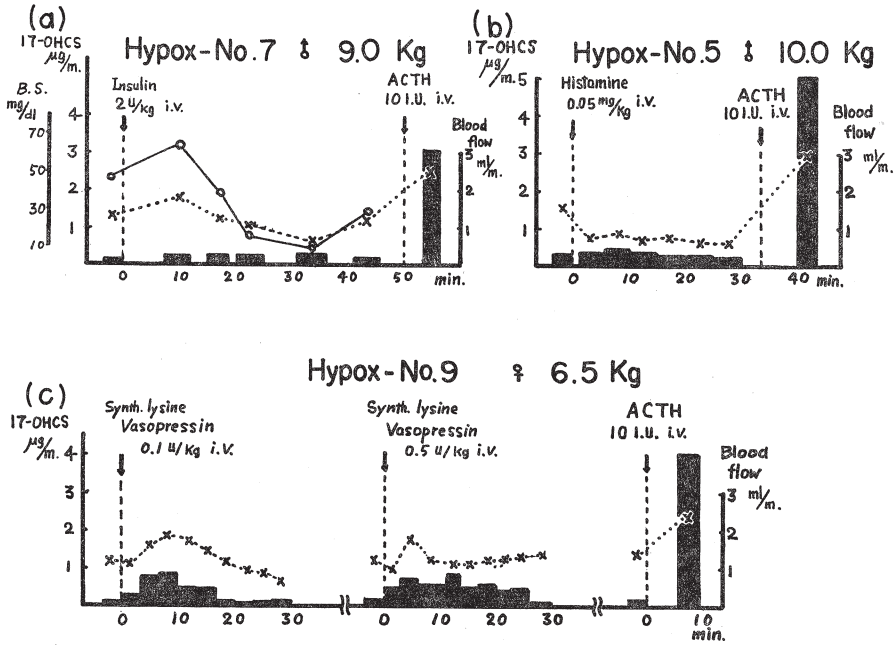

FIG. 7. Adrenal venous 17-OHCS outputs in hypophysectomized dogs.

(a) Dog Hypox-7. Insulin 2 U/kg administration did not cause any increment of adrenal venous 17-OHCS outputs. Note the response to ACTH.

(b) Dog Hypox-5. Histamine 0.05 mg/kg administration did not result in the increment of adrenal venous 17-OHCS outputs, whereas ACTH provoked marked response.

(c) Dog Hypox-9. L-8-V 0.1 and 0.5 U/kg administration did not cause appreciable increment of adrenal venous 17-OHCS outputs, but for some minimal rise.

increment of adrenal venous 17-OHCS outputs, while blood sugar level fell down markedly, and ACTH administration caused marked adrenocortical response (Fig. 7 (a)). And 0.05 mg/kg histamine administration also failed to provoke adrenocortical response (Fig. 7 (b)). Administration of L-8-V 0.1 and 0.5 U/kg i.v. did not show appreciable stimulatory effects except for some minimal rise in adrenal venous 17-OHCS outputs (Fig. 7 (c)).

DISCUSSION

In 1951, Vogt found that normal and adrenal-denervated rats showed adrenal ascorbic acid depletion following injection of insulin[11]. Arner *et al.*[12] offered the increased plasma corticosteroid concentration in man following hypoglycemia induced by injection of insulin. The present investigation demonstrated that i.v. injection of insulin 1 to 3 U/kg in nembutal-anesthetized dogs produced marked activation of the pituitary-adrenocortical axis accompanying with hypoglycemia indicated by the increment of adrenal venous

17-OHCS output. This adrenal cortical activation was not observed in hypophysectomized dogs, thus illustrating that adrenal cortical activation by insulin administration is caused mediating through the hypophysis. The insulin-induced 17-OHCS increment was suppressed by the pretreatment of dexamethasone. However, when the doses of insulin were increased adrenal cortical response to insulin appeared, that means the steroid block can be overcome by stronger stress. This adrenal cortical activation caused by increased doses of insulin could also be suppressed by increasing the doses of dexamethasone pretreated. It has been clearly demonstrated that there exists a quantitative correlationship between the intensity of stress which is represented by the amount of insulin and the amount of corticosteroids which is required for blocking the stress-induced adrenal cortical activation.

Histamine also provoked pituitary-adrenocortical activation not in the same way as seen in insulin administration. In hypophysectomized animals, there was no increment of adrenal venous 17-OHCS output after 0.05 mg/kg histamine administration, which illustrates that histamine also acts as stress mediating through the hypophysis. In dexamethasone pretreated dogs, the quantitative relationship between the doses of dexamethasone and histamine stress could be observed. Adrenal cortical activation caused by histamine 0.05 mg/kg was blocked completely by 0.2 to 0.4 mg/kg dexamethasone pretreatment, and partially by 0.1 mg/kg dexamethasone pretreatment. The relationships observed between the doses of insulin or histamine and corticosteroid pretreatment are in accord with those observed by Sayers *et al.*[2] in the rat using adrenal ascorbic acid depletion as an assay method of corticotropin. However, the present study with direct measuring of adrenal venous 17-OHCS outputs illustrated more concrete and definitive observation of the quantitative relationships between the intensity of stress and the inhibitory action of corticosteroids on corticotropin release. And this is the first observation of the quantitative relationships between insulin stress and corticosteroid block.

Briggs *et al.*[13] investigated the blocking effect of morphine on the release of corticotropin in rats exposed to stress, and found the existence of a proportional relationship between the doses of histamine and that of morphine. This suggests that some quantitative relationships between the intensity of stress and blocking mechanism on corticotropin release are generally observed.

Vasopressin is known to provoke corticotropin release in the animals with hypothalamic lesions[14][15]. Others[16][17] found that directly infused vasopressin to adrenal artery in hypophysectomized dogs had stimulated the adrenal hydrocortisone secretion. However, the present data yielded that intravenously infused L-8 V 0.1 and 0.5 U/kg in hypophysectomized dogs did not give appreciable rise to adrenal venous 17-OHCS output, but gave remarkable rise in non-hypophysectomized dogs. Therefore, intravenously infused L-8-V in non-hypophysectomized dog did not seem to act like corticotropin, but act mediat-

ing via hypophysis, though the minimal rise seen in hypophysectomized dogs might be resulted from direct stimulation on the adrenal cortex. On the other hand, dexamethasone pretreatment ranging in the doses 0.1 to 0.4 mg/kg failed to prevent the pituitary-adrenocortical activation caused by 0.25 and 0.5 U/kg L-8-V i.v. in contrast to the activation caused by insulin or histamine administration. From these results it is likely that vasopressin acts on the higher center than hypophysis and more adjacent to hypophysis than the site where dexamethasone blocks pituitary-adrenocortical activations caused by other stresses such as histamine or insulin infusion. Leeman *et al.*[4] offered that the blocking site of morphine is different from that of corticosteroids in their rat experiment from the following facts: *i.e.* 1) Morphine blocked vasopressin-induced adrenal cortical activation, while corticosterone did not. 2) Both morphine and corticosterone did not block hypothalamic extract-induced adrenal cortical activation. And they concluded that the location of morphine block is more adjacent to the hypophysis than that of corticosteroids and vasopressin acts between the sites where morphine and corticosteroids inhibit the hypothalamo-hypophysial axis. In this respect, the results presented here coincide with those obtained by Leeman *et al.*[4] and by Rerup[18]. It appears that the main blocking action of corticosteroids on corticotropin release is accomplished in the central nervous system, most probably in the hypothalamus.

The reason for the shortening of adrenocortical activation time after vasopressin in dexamethasone pretreated dogs remains unknown. The corticotropin releasing mechanism after vasopressin might have two factors, *i.e.* one is the direct stimulating action of octapeptide itself on the hypophysis, the other is the indirect stimulating action of vasopressin mediating through hypothalamus caused by its side effects such as vasopressor activity or antidiuretic activity. It seems likely that the former action is isolated and the latter action is suppressed under dexamethasone pretreatment.

De Wied proposed that massive doses of dexamethasone blocked the adrenal cortical activation after purified lysine vasopressin injection in the rat[19]. In the present experiments, the dose of dexamethasone pretreated was increased to 1.0 mg/kg and the dose of vasopressin was decreased to 0.05 U/kg. Under these conditions, dexamethasone suppressed the adrenal cortical activation by 0.05 U/kg L-8-V administration, while the same dose of dexamethasone did not completely block the adrenal cortical activation by 0.25 U/kg L-8-V administration. The possibility that massive doses of corticosteroids directly inhibited the steroid secretion of adrenal cortex[20][21] seems to be excluded from the present experiment, because exogenous corticotropin administration into 1.0 mg/kg dexamethasone pretreated dogs resulted in sufficient response of the adrenal cortex. The quantitative relationship could be observed between larger doses of dexamethasone and less doses of vasopressin administration. Schapiro *et al.*[22] studied the distribution of injected hydrocortisone 4-C^{14} in rats, guinea

pigs and rabbits and found that activity was higher in the posterior pituitary gland than in any other tissues and successively anterior pituitary, liver or kidney. On the other hand, Eik-Nes *et al.*[23] reported that (1, 2-$^3H_2$) cortisol uptake was higher in the hypothalamic nuclei than in the brain or in the hypophysis, and other investigators[24][25] observed that implantation of the corticoid into the pituitary did not interrupt the stress response. The present study shows that the blocking action of corticosteroids on corticotropin release is obviously related to the doses administered, and suggests that in exceeding doses even hypophysis can be involved.

CONCLUSIONS

Judging from the changes of adrenal venous 17-OHCS output in the dog, the following conclusions were obtained.

1) Insulin and histamine administration produces corticotropin release, which can be suppressed by the pretreatment of dexamethasone. There exists quantitative correlationship between the doses of dexamethasone pretreated and the intensity of stress.

2) Dexamethasone pretreatment does not suppress the corticotropin release provoked by vasopressin infusion. This indicates that vasopressin acts adjacent to the hypophysis than the site where dexamethasone exerts its suppress action in the hypothalamo-hypophysial axis, and the main blocking action of corticosteroids is accomplished in the central nervous system, probably in the hypothalamus.

3) Enormous doses of dexamethasone suppress corticotropin release by less doses of vasopressin. From these results it is suggested that massive doses of corticosteroids might affect the hypophysis itself.

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