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Blockade of Hypothalamo-Hypophyseal-Adrenocortical Mechanism Caused by Dexamethasone

Keishun NAKASONE and Takeshi SHIMIZU *

Department of Pathophysiology, Atomic Disease Institute, Nagasaki University School of Medicine, Nagasaki, Japan
(Director: Prof. K. YAMASHITA)

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An effort has been made to elucidate the effect of dexamethasone on adrenocortical secretory function in dogs. The secretory activity was determined by measuring 11-hydroxycorticosteroids (11-OHCS) in adrenal venous blood.

In dogs under sodium pentobarbital anesthesia (25 mg/kg, injected i. v.), dexamethasone-21-phosphate was administered subcutaneously in doses of 50–200 µg/kg before the stress. This pretreatment inhibited largely the adrenocortical stimulation resulting from the surgical stress or X-irradiation of the adrenal. The most effective suppression was obtained with a single injection of 100 µg/kg.

Intravenous injections of 5–500 mU/kg of ACTH to dogs pretreated with 100 µg/kg of dexamethasone responded by showing definite increases in adrenal 11-OHCS secretion, the degree of the increase depended on the dosage administered.

In adrenocortical response to exogenous ACTH, double treatments with 100 µg/kg of dexamethasone resulted in lesser secretion rates of 11-OHCS than a single treatment. These findings would suggest the adrenal cortex as one of the sites of inhibition.

INTRODUCTION

Following the experimental demonstrations that dexamethasone has a depressing effect on the hypothalamo-pituitary-adrenocortical axis in rats, treatment with dexamethasone has been applied both experimentally and clinically to assays of adrenocorticotrophin or assays of corticotrophin releasing factor as a substitutive technique for hypophysectomy. On this favor, the evidence in the canine is still meager. The present investigation was, therefore, undertaken to determine the effect of glucocorticosteroid dexamethasone on adrenocortical activity of dogs subjected to the stress of surgery or to X-
irradiation of the adrenal, and also the effects of graded doses of ACTH in dexamethasone-treated dogs.

MATERIALS AND METHODS

Studies were performed on mongrel adult dogs, 8.8–14.8 kg in weight.

Lumboadrenal Vein Cannulation

The lumboadrenal vein cannulation was carried out by use of the lumbar route method of SATAKE, SUGAWARA & WATANABE17), with a minor modification. Each animal was anesthetized with 25 mg/kg of sodium pentobarbital, injected intravenously, and received intravenous supplements of the drug as required. Within 30 minutes after anesthetization, the left lumboadrenal vein was approached through a longitudinal incision in the left lumbar area. After accessory branches of the vessel were ligated, a small glass cannula fitted with a rubber tube was inserted into the vein just lateral to the adrenal gland. The cannula was filled with a heparin solution, diluted with physiological saline, and clamped. A soft silk thread was passed around the vein between the adrenal gland and the inferior vena cava. This procedure permits continuous or intermittent collection of the venous blood from the adrenal gland, by pulling at the thread and removing the clamp on a rubber tube.

Collection and Analysis of Adrenal Venous Blood Samples

The animals were divided into two groups in order to demonstrate: (1) the effect of dexamethasone on the increased corticoid secretion caused by the stress of surgery or by X-irradiation of the adrenal gland, and (2) the effects of graded doses of adrenocorticotrophin (ACTH) in dexamethasone-treated animals.

In the first group, the animals were given doses of 50–200 µg/kg of dexamethasone by a subcutaneous injection, once or twice. Approximately one hour after the injection, the animals were subjected to the left lumboadrenal vein cannulation under sodium pentobarbital anesthesia. Timed adrenal venous effluent was sampled at 0, 30 and 60 minutes after the end of the lumboadrenal vein cannulation. In other experiments, within 60 minutes after the end of operation, the exposed adrenal gland was irradiated with 2,000 R of X-ray beam by an X-ray machine operated at 180 KVP and 20 mA, with 0.5 mm Al and 0.5 mm Cu filtration (HVL 1.0 mm Cu). The dose-rate to the adrenal area was approximately 130 R/min. Adrenal venous blood samples were collected at the selected intervals before and after X-irradiation.

In the second group, dexamethasone was given subcutaneously once in doses of 100 µg/kg. One hour later, the animals were anestheti-
zed with sodium pentobarbital (25 mg/kg, injected i.v.) and the left lumboadrenal vein was cannulated through the lumbar route. Approximately 3 hours after treatment with dexamethasone, collection of adrenal venous samples was begun. After two control samples of adrenal venous blood were collected, ACTH was injected into the saphenous vein in a single dose. And then adrenal venous blood was sampled at 15, 30 and 60 minutes after ACTH injection.

The blood was centrifuged immediately under refrigeration and the plasma was stored in a freezer at −20°C until analyzed for 11-hydroxycorticosteroids (Silber, Busch & Oslapas, 1958).

**Materials**

The following preparations were used: dexamethasone-21-phosphate (Decadron, Nippon Merck-Banyu Co.); ACTH (Cortrophine, Organon). Dexamethasone (stock concentration 4 mg/ml) was diluted with physiological saline to bring final injection volumes to 0.5 ml. Intravenous injection of ACTH was made in 1.0 ml. The dose of ACTH varied between 5 mU and 2,000 mU/kg.

**RESULTS**

The Secretion of Adrenal 11-Hydroxycorticosteroids in Response to the Lumboadrenal Vein Cannulation in Dogs with and without the Prior Administration of Dexamethasone

Fig. 1 shows adrenal 11-OHCS secretion rates obtained in dexamethasone-treated (50 μg–200 μg/kg) and control animals at 0, 30 and 60
minutes after the end of a surgical treatment. These estimations were made approximately 3 to 4 hours after dexamethasone had been injected. Eleven-OHCS secretion rates from one adrenal gland of control animals varied from 0.25 µg to 0.38 µg/kg/min. Pretreatment with 50 µg/kg of dexamethasone was not sufficient to block the corticotrophic effect of the surgical stress and reduced by 66–90%. However, the corticotrophic effect of such a stress could be blocked completely by the prior administration of dexamethasone in doses up to 100–200 µg/kg.

Inhibitory Effect of Dexamethasone on Adrenal 11-Hydroxycorticosteroid Secretion Produced by a Stress Consisting of the Lumboadrenal Vein Cannulation and X-Irradiation of the Adrenal

To ascertain the effect on a stress consisting of the lumboadrenal vein cannulation and X-irradiation of the adrenal, the adrenal gland was irradiated with 2,000 R without pretreatment with dexamethasone. A marked adrenocortical stimulation was elicited by a direct irradiation and the increased secretion persisted over 60 minutes following exposure, the peak being attained at 15 minutes. However, this response to the irradiation-operation stress was completely nullified by the prior administration of dexamethasone, twice at an interval of 18

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Fig. 2. Average secretory responses of the adrenal cortex to X-irradiation of the adrenal with and without dexamethasone. The secretory response is expressed in µg 11-OHCS/kg/min. Radiation to the adrenal gland was 2,000 R and the dose of dexamethasone 200 µg/kg. ○—○, without dexamethasone; ●—●, with dexamethasone.
hours in doses of 100 μg/kg. The results are shown in Fig. 2.

Effects of Graded Doses of Adrenocorticotrophin in Dexamethasone-Treated Dogs

The experiments were performed to determine whether or not the stimulating effect of ACTH on the secretory activity is affected by large doses of dexamethasone. As can be seen in Table 1, the results were obtained that the 100 μg/kg-treated animals gave a greater response to ACTH stimulation than did the 200 μg/kg-treated ones. This shows that the feedback action of dexamethasone is, in part, exerted on the adrenal cortex.

Since 100 μg dexamethasone/kg was considered to be sufficient to block the hypothalamo-pituitary-adrenal activation resulting from the surgical stress, without any effects to the basic function of the adrenal gland, the following experiments were carried out on dogs pretreated with 100 μg/kg of dexamethasone. The control adrenal 11-OHCS secretion rate before the injection of ACTH was within or below the measurable limit. This again shows that 100 μg dexamethasone/kg blocks the secretory response of the adrenal cortex to the surgical stress. With the intravenous injection of the lowest dose of ACTH, i.e. 5 mU/kg, a slight but definite increase in the secretion of adrenal 11-OHCS was found. On increasing the dose of ACTH from 10 mU to 500 mU/kg, more marked increase in the secretory activity occurred, the extent of which depended on the dosage administered.

Table 1. Effect of Intravenous Administration of ACTH on the Secretion of Adrenal 11-Hydroxycorticosteroids (11-OHCS) in Dogs Pretreated with Dexamethasone

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Dose of ACTH (IU/kg)</th>
<th>Rate of adrenal 11-OHCS secretion (μg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before injection of ACTH (min)</td>
<td>After injection of ACTH (min)</td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>-10</td>
</tr>
<tr>
<td>14.8 ♀</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>10.8 ♀</td>
<td>100</td>
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<tr>
<td>12.5 ♀</td>
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<td>14.4 ♀</td>
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</tr>
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<tr>
<td>10.2 ♀</td>
<td>200</td>
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* Below the measurable limit
Fig. 3. The effect of graded doses of ACTH in dogs pretreated with dexamethasone (100 μg/kg). ACTH was given in amounts ranging from 5 mU to 500 mU/kg by an intravenous injection.
DISCUSSION

The present results, in dogs under sodium pentobarbital anesthesia, show that the adrenocortical stimulation (indicative of an increased rate of ACTH secretion) in response to the stimulus of surgery is suppressed by the prior administration of dexamethasone. However, in view of the demonstration of Suzuki, Yamashita, Kamo & Hirai\(^{23}\) that sodium pentobarbital lowered slightly the secretion rate of adrenal 17-hydroxycorticosteroids of conscious dogs, and of the finding of Guillemin, Dear, Nichols & Lipscomb\(^{6}\) that in rats treatment with dexamethasone-pentobarbital resulted in a lower level of corticosterone than dexamethasone, it would appeared that sodium pentobarbital may exert as least part of its blocking action on the hypothalamo-pituitary-adrenal mechanism in dogs too. In various doses of dexamethasone along with pentobarbital, at least 100 \(\mu\)g dexamethasone/kg had to be injected to block the effects of the stimuli employed in the present experiments. Previously, Kendall\(^{8}\) has demonstrated that in rats maximum suppression in surgery-induced corticosterone secretion increase occurred 4 to 8 hours after an administration of 100 \(\mu\)g/kg of dexamethasone. Therefore, the potentiating effect of sodium pentobarbital on the blocking action of dexamethasone appered to be extremely weak in dogs. Asano\(^{20}\), who employed 100—400 \(\mu\)g/kg of dexamethasone on dogs under pentobarbital anesthesia, also observed the blocking effect on the pituitary-adrenal mechanism.

Although there are controversial observations of the responsiveness of the adrenal cortex to radiation\(^{20,24}\), one\(^{12}\) of the present authors demonstrated that localized X-irradiation to the adrenal gland caused a remarkable augmentation of 11-OHCS by the canine adrenal, and noted that the response was pituitary-dependent. Further experiments were, therefore, performed on 200 \(\mu\)g/kg of dexamethasone on the adrenocortical stimulation arising by X-irradiation of the adrenal with doses of 2,000 R. After the administration of dexamethasone, X-irradiation of the adrenal failed to produce an increase in adrenal 11-OHCS, the effect being essentially the same as that of hypophysectomy\(^{12}\). It is obvious that dexamethasone also suppresses the activation of the pituitary-adrenal mechanism, resulting from X-irradiation of the adrenal gland.

In the present experiments, 100 \(\mu\)g dexamethasone/kg were necessary to inhibit the surgical stress-induced release of ACTH, as mentioned previously. Therefore, in dogs pretreated with 100 \(\mu\)g/kg of dexamethasone, the secretory response of the adrenal cortex to graded doses of ACTH was examined. The degree of the increase depended on the dosage administered.

The site of the blocking action of dexamethasone on the hypothalamo-pituitary-adrenal axis is controversial. There is a great number
of reports in this point, suggesting that the primary focus of the blockade with dexamethasone is on either the central nervous system\textsuperscript{4}\textsuperscript{10}\textsuperscript{11}\textsuperscript{14} or the anterior pituitary\textsuperscript{15}\textsuperscript{16}\textsuperscript{18}\textsuperscript{26}\textsuperscript{27}. And also evidence\textsuperscript{22}\textsuperscript{23}\textsuperscript{29}\textsuperscript{31}\textsuperscript{35} for the adrenal cortex as the site of inhibition has been obtained from experiments \textit{in vivo} and \textit{in vitro}. Since several workers failed to confirm an inhibitory effect in favor of an action on the adrenal cortex, an attempt was made to determine whether the secretory response of the adrenal cortex to ACTH would be affected by large doses of dexamethasone. Accordingly, 100 $\mu$g dexamethasone/kg was administered to dogs, twice at an interval of approximately 18 hours. Compared with the increase in 11-OHCS secretory response to ACTH observed on a single treatment with 100 $\mu$g/kg, ACTH appeared to have considerably the lesser potency in dogs subjected to this double dexamethasone treatment, as was seen in Table 1. This can be deduced that dexamethasone may, in part, exert a direct action on the adrenal cortex itself, if given relatively large doses by repeated administrations.

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REFERENCES


