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Suppression by Metyrapone of the Adrenal Response to Exogenous Corticotrophin

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The effect of metyrapone on the augmented adrenal 17-hydroxycorticosteroid (17-OHCS) secretion induced by the administration of adrenocorticotrophin (ACTH) was investigated in dogs.

A single intravenous injection of ACTH (1 i.u./kg) resulted in the markedly increased secretion of adrenal 17-OHCS; the peak was attained within 60 minutes, and sustained over 3 hours. At the time when the secretion rate caused by ACTH was at a maximum, an intravenous injection of metyrapone (40 mg or 60 mg/kg) produced a rapid and marked decrease in adrenal 17-OHCS secretion. This diminution occurred within 15 minutes and the effect was reversible.

INTRODUCTION

There are several compounds which can inhibit the synthesis of certain adrenocortical hormones. Among them, the effect of amphenone B has been provided by the convincing data of ROSENFELD & BASCOM[8], indicating that amphenone B suppress 11β-, 17α- and 21-hydroxylation and the oxidation of a $\triangle^5$-3β-hydroxyl groups to $\triangle^4$-3-ketone in the adrenal cortex. A similar effect was found in the biological activity of methylenedianiline[11–13] and of perthane[10]. Metyrapone also is an amphenone analogue,[2] but unlike amphenone and methylenedianiline it has been shown by LIDDLE, ISLAND, LANCE & HARRIS[6] to inhibit selectively the enzymatic system responsible for 11β-hydroxylation of steroids.

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Therefore, in the present study it seemed desirable to test how the 17-hydroxycorticosteroid secretory response of the adrenal gland in response to the administration of adrenocorticotrophin is influenced by metyrapone. This was demonstrated by measuring quantitatively the level of 17-hydroxycorticosteroids in adrenal venous blood.

MATERIALS AND METHODS

Mongrel male adult dogs, weighing 7.9 to 15.3 kg, were used in these experiments. On the previous day of observation, each animal was anesthetized with 25 mg/kg of sodium pentobarbital given into the left saphenous vein. Immediately after anesthetization, the left lumboadrenal vein was approached through a longitudinal incision in the left lumbar area, in a fashion similar to the method of SATAKE, SUGAWARA & WATANABE[9], and then accessory branches of the vessel were ligated and sectioned. A silk thread was passed loosely around the vein which connects the adrenal gland to the inferior vena cava. After systemic administration of heparin, a small glass cannula fitted with a rubber tube was inserted into the exposed lumboadrenal vein. By pulling at a ligature, the entire blood flow through the adrenal gland is obtainable. When a ligature is released, blood can flow from the adrenal gland to the inferior vena cava during periods when it is not being collected. After refilling a cannula with a heparin-saline solution, the animal was allowed to recover for approximately 18 hours prior to the collection of adrenal blood samples.

On the day of observation, the animal was again reanesthetized with sodium pentobarbital (25 mg/kg, injected intravenously). Approximately 2 to 3 hours later, the collection of control samples of adrenal venous blood was begun. A single injection of 1 i.u. adrenocorticotrophin (ACTH)/kg body weight was made into the left saphenous vein for 15 seconds. Timed adrenal venous blood was sampled 15, 30 and 60 minutes after the injection of ACTH. Seventy-five minutes after the injection of ACTH, either metyrapone ditartrate (Ciba Ltd) (40 mg or 60 mg/kg) or isotonic saline only was injected into the left saphenous vein in a single dose of 2 ml for 30 seconds. Further samples of adrenal venous blood were collected 90, 120 and 180 minutes after the injection of ACTH.

The blood was chilled immediately and centrifuged under refrigeration. One milliliter of plasma was analysed for 17-hydroxycorticosteroids (17-OHCS) by the method of PORTER & SILBER[7].

Finally, the secretion rate of 17-OHCS by one adrenal gland (μg/kg/min) was calculated by multiplying the concentration of 17-OHCS in adrenal venous plasma(μg/ml) by the adrenal venous plasma flow (ml/kg/min).

RESULTS

The secretory response of adrenal 17-hydroxycorticosteroids to the administration of adrenocorticotrophin

Representive results are shown in Fig. 1. The basal rates of 17-hydroxycorticosteroids (17-OHCS), established by samples taken 20 and 10 minutes prior to the

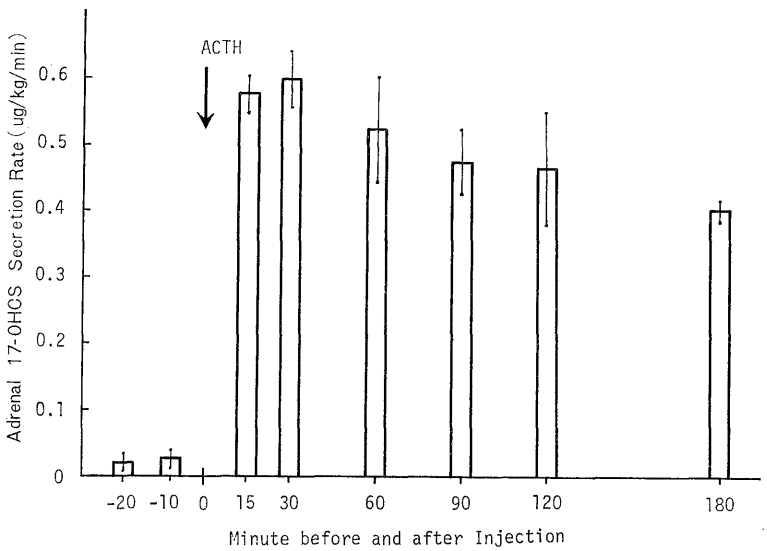

Fig. 1. Effect of corticotrophin (ACTH) stimulation on adrenal 17-hydroxycorticosteroid (17-OHCS) secretion in dogs. ACTH was given intravenously in a dose of 1 i.u. /kg. Additionally, isotonic saline was injected intravenously in 2 ml 75 minutes after the injection of ACTH. Each column represents the mean±SE in adrenal 17-OHCS secretion of five dogs at various times after the injection of ACTH.

injection of adrenocorticotrophin (ACTH), were extremely low. Following an intravenous injection of ACTH in a dose of 1 i.u./kg, there was the markedly increased secretion of adrenal 17-OHCS. The maximum rate occurred within 60 minutes. Seventy-five minutes after the injection of ACTH, isotonic saline was injected via the intravenous route, ACTH-induced increase in the secretion was not influenced by this treatment and lasted over the 3-hour period of observation. These results show that the stimulating effect of ACTH on adrenal secretion of 17-OHCS was not affected by treatment with isotonic saline.

Effects of metyrapone on the increased secretion of adrenal 17-hydroxycorticosteroids produced by the administration of adrenocorticotrophin

As can be seen in Fig. 2, an intravenous administration of metyrapone, performed at 75 minutes after the injection of ACTH effected a prompt and marked decrease

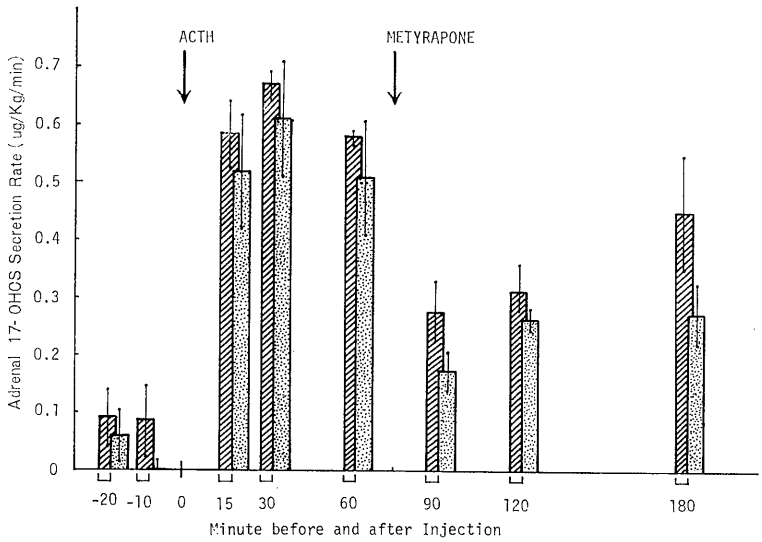

Fig. 2. Inhibitory effect of metyrapone on the increased secretion of adrenal 17-hydroxycorticosteroids caused by corticotrophin (ACTH). Metyrapone was injected into the left saphenous vein 75 min after the injection of ACTH: (metyrapone, 40 mg or 60 mg/kg; ACTH, 1 i.u./kg). Each bar shows the mean±SE of 5 dogs in adrenal 17-OHCS secretion at various times after the ACTH injection. ▨ : ACTH 1 i. u./kg, metyrapone 40 mg/kg; ▩ : ACTH 1 i.u./kg, metyrapone 60 mg/kg.

in adrenal 17-OHCS secretion. When 40 mg/kg of metyrapone was given (Fig. 2), a remarkable inhibition of secretion was observed 15 minutes later, but it tended to recover within approximately 2 hours. The secretory response to ACTH at 15 minutes after the injection of metyrapone showed a reduction of 39%, as compared with that of controls. With higher doses up to 60 mg/kg (Fig.2), there was much lowered secretion of adrenal 17-OHCS, showing an inhibition of 58% at 15 minutes after the administration. The inhibitory activity of this dose was more enhanced and prolonged than that with lower doses. The effect lasted over 2 hours.

DISCUSSION

In the present results, a marked increase in adrenal 17-OHCS secretion in response to exogenous ACTH could largerly be inhibited by treatment with metyrapone. An inhibition in the secretion occurred soon after the treatment, and the effect was reversible.

The present findings agree with the early work of CHART, SHEPPARD, ALLEN, BENCZE & GAUNT[2], in which it was shown that in the dog the administration of 10 to 30 mg of metyrapone ditartrate produced a reduction in adrenal venous corticoids from 25% to nearly 100% below pretreatment control values. In 4 endocrinologically normal individuals, LIDDLE, ISLAND, LANCE & HARRIS[6] have shown that intravenous treatment with metyrapone resulted in a rapid and marked fall in the level of hydro-

cortisone in adrenal venous blood. Such a decrease in the secretion may usually be elicited by either an impairment of release of hormone, a decreased hormone synthesis, or a combination of these possible alterations. For this favor, evidence for the enzymatic system in the adrenal cortex as the focus of inhibition by metyrapone has been obtained from experiments *in vivo* and *in vitro*. *In vitro* experiments performed by CALLOW, CALLOW & EMMENS[1], the activity of 11β-hydroxylase obtained from bovine adrenal tissue was almost totally inhibited by an addition of a 1.5×10^{-5} molar solution of metyrapone, and was 50% inhibited by a 2.5×10^{-6} molar solution. In contrast, 21-hydroxylase was not inhibited by a 1×10^{-3} molar. CHART et al.[2] have demonstrated in their *in vitro* experiments that metyrapone suppressed the release of corticoids when applied with ACTH to canine adrenal slices. These findings suggest a possible inhibition of 11β-hydroxylation reactions in the adrenal tissue as the site of action of metyrapone. Furthermore, LIDDLE et al.[6] have investigated systemically for the mechanism of action of metyrapone. They observed that in subjects having normal adrenocortical function the prolonged oral or intravenous administration of metyrapone caused a marked fall in the level of plasma 17-OHCS, urinary 17-OHCS and 17-ketosteroids. From these results they pointed out that metyrapone might act as a specific inhibitor of 11β-hydroxylation of steroids by the adrenal cortex. This hypothesis was also confirmed by JENKINS, MEAKIN & NELSON[5] in the dog, GOLD, BIGLER, NEWMAN, ANGERS & DIRAIMONDO[3] and JENKINS, MEAKIN & NELSON[4] in man.

From the results mentioned above it seems that the inhibitory effect of metyrapone observed in the present study is not due to an impairment of release of 17-OHCS by the adrenal, but to a block of biosynthesis of these hormones. Although 11-deoxycortisol and cortisol are the main 17-OHCS secreted by the adrenal, considering from pathways of biosynthesis of 17-OHCS the secretion of 11-deoxycortisol caused by ACTH is probably not inhibited by metyrapone. The finding of this study showed that the adrenal 17-OHCS secretory activity resulting from the administration of 1 i.u. ACTH/kg was inhibited approximately 39 to 58% by doses of 40 mg to 60 mg/kg of metyrapone.

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