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STEROID ANTAGONISM OF THE HYPERTENSINOGENIC ACTIVITY OF ADRENOCORTICAL STEROIDS

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Summary—Previous studies in sheep have provided evidence for a separate "hypertensinogenic" class of adrenocortical steroid activity which is not simply related to their classical mineralocorticoid (MC) and/or glucocorticoid (GC) actions. This study investigated the structure–activity relationships of the effects of structural analogues of prednisolone on mean arterial pressure (MAP), and MC and GC actions in sheep. Infusions of these synthetic GC at 0.6 and 24 mg/day produced variable pressor effects which were dissociated from their MC and GC actions.

In other experiments, the minimum adrenocortical steroid requirement to reproduce the onset of ACTH-dependent hypertension was determined. Infusion of cortisol, aldosterone, 17α-hydroxy progesterone and 17α,20α-dihydroxy-4-pregnene-3-one was found to be sufficient to reproduce the hypertensive response to ACTH administration in sheep. A subsequent experiment showed that substitution of cortisol by the more potent synthetic GC, prednisolone had no effect on MAP. Therefore, cortisol appears to exert an essential action in ACTH hypertension which is not dependent on its GC activity. Other studies have found that prednisolone (100 mg/day) antagonized 9α-fluoro-prednisolone (0.6 mg/day) induced hypertension but not its MC effects.

The effect of progesterone (500 mg/day) and the progesterone analogues, norethisterone, medroxy-progesterone and 16α-methyl progesterone on ACTH (5 μg/kg per day) hypertension was investigated. Progesterone completely blocked the hypertension and MC effects of ACTH infusion, while medroxy-progesterone partially blocked the increase in MAP. These data support our concept of a "hypertensinogenic" class of steroid activity.

INTRODUCTION

Much evidence has accumulated to support our concept that adrenocortical steroids raise arterial blood pressure by a novel "hypertensinogenic" mechanism of action which can be distinguished from their classical mineralocorticoid (MC) and glucocorticoid (GC) actions [1, 2]. Studies investigating the mechanism of ACTH-induced hypertension in sheep provided the first evidence for a "hypertensinogenic" class of steroid activity. ACTH hypertension in sheep was found to be adrenally dependent; however, individual or combined i.v. infusion of the five major adrenocortical steroids, cortisol, corticosterone, 11-deoxycortisol, deoxycorticosterone and aldosterone at rates appropriate for ACTH treatment reproduced the MC and GC features but not the pressor effects of ACTH administration [3]. Later it was shown that infusion of two steroids identified in adrenal venous blood of ACTH-treated sheep, 17α-hydroxyprogesterone (17αOHP) and 17α,20α-dihydroxy-4-pregnene-3-one (17α20αOHP) in combination with the steroids referred to above were necessary to reproduce the pressor effects of ACTH [1]. Neither 17αOHP nor 17α20αOHP when infused alone had an effect on blood pressure or exhibited any *in vivo* MC and/or GC actions, consistent with the observation *in vitro* that these steroids had low affinity for ovine renal cytosol binding sites for aldosterone, dexamethasone or cortisol [4]. Therefore, it was concluded on the basis of these studies that these steroids exert a "hypertensinogenic" type of steroid action which is distinct from the classical MC and GC activities [1].

Further support for this concept was obtained from subsequent studies investigating the relationship between pressor activity and the MC and/or GC action of a number of synthetic steroids [2, 5, 6]. These studies showed a dissociation between the hypertensive potency of steroids and their MC and/or GC activities. For example, administration of aldosterone at a pharmacological dose (2 mg/day) produced urinary Na retention and hypokalaemia, but had no effect on blood pressure [6] while the less potent MC, DOC and MC 19-nor-DOC produced significant hypertension. Further, 9α-fluorocortisol-induced hypertension in sheep could not be reproduced by combined infusions of aldosterone and cortisol at rates calculated on the basis of ovine receptor binding studies to be equivalent in terms of MC and GC potency [5].

These data provide strong evidence for a separate "hypertensinogenic" class of steroid activity; however, despite many studies in sheep [7] the precise mechanism remains to be elucidated. In this

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paper, we report our findings of studies investigating:
(a) structure–activity relationships of the effect of structural analogues of prednisolone on blood pressure and MC and GC activities;
(b) the glucocorticoid requirement for ACTH-dependent hypertension and;
(c) antagonism of adrenocortical steroid-induced hypertension.

In these studies MC activity was assessed *in vivo* by measurement of changes in urinary Na^+ excretion, and *in vivo* GC activity was assessed by measurement of increases in fasting plasma glucose concentration.

EXPERIMENTAL

Adult cross-bred Merino ewes, body wt 35–45 kg, were used in these studies. Animals were housed in individual metabolism cages allowing separate collection of urine and faeces. Each sheep was offered daily 0.8 kg of lucerne–oaten chaff (containing 80–140 mmol/kg Na^+ and 200–300 mmol/kg K^+) and water *ad libitum*. Bilateral carotid arterial loops were prepared at least 2 months prior to experimentation. Water, food intake, urine output, blood pressure and cardiac-rate were recorded daily at 10.00 h. Blood and urine samples were taken at 10.00 h. Na^+ and K^+ concentrations in urine and plasma were analysed by a Corning flame photometer or a Technicon autoanalyser. Plasma glucose concentration was determined using a Technicon sequential multiple analyser.

ACTH (Ciba-Geigy) and steroids (Steraloids) were administered by continuous infusion into a jugular vein. Following a 3-day pre-infusion period for control observations, infusions were commenced and terminated at 11.00 h on the day prescribed by the various protocols (see Results).

Data were analysed by 2-way analysis of variance and are expressed as the mean and SEM. Student's *t*-test modified for repeated measures by the least significant difference method was employed to determine the statistical significance of multiple comparison data.

RESULTS

(a) *Structure–activity relationships for the effect of prednisolone and analogues on blood pressure*

Figure 1 summarizes the structure–activity relationships of the effect of 5-day infusions of various structural analogues of prednisolone on mean arterial pressure (MAP) [8]. The parent compound in this series, prednisolone, had a small effect on MAP (+5 mm Hg, $P < 0.05$) at a large dose (100 mg/day). 9α-Fluoro substitution greatly enhanced both pressor and MC activities while the 16α,17α-butylidenedioxy derivative, budesonide also displayed enhanced pressor activity. However, 16α-methyl or 16α-hydroxyl substitutions in the cases of dexamethasone and triamcinolone, respectively, abolished the pressor activity conferred by 9α-fluoro substitution; MC activity was abolished in the case of triamcinolone but not dexamethasone. In contrast, 16β-methyl substitution (betamethasone) did not greatly diminish the pressor or MC activity. 16α,17α-Acetonide substitution increased the pressor activity of triamcinolone similar to the effect of 16α,17α-acetal substitution of prednisolone in the case of budesonide. However, the increased pressor activity conferred by 16α,17α-acetal substitution was not associated with either increased MC or GC activity. Furthermore, despite their disparate effects on MAP, the analogues in this series all increased fasting plasma [glucose] by approximately the same degree at the 24-mg/day dose.

(b) *Studies investigating the glucocorticoid requirement for the onset of ACTH-induced hypertension* (*Fig.* 2)

Administration of ACTH (5 μg/kg per day) increased MAP by 21 mm Hg and fasting plasma [glucose] by 3.2 mmol/l.

The infusion for 5 days of combined cortisol (5 mg/h), aldosterone (3 μg/h), 17αOHP (1 mg/h) and 17α20αOHP (0.5 mg/h) increased MAP by 19 mm Hg similar to the 21 mm Hg rise with ACTH. However, when aldosterone was omitted from the infusion, the MAP rise was only 7 mm Hg [9]. Thus cortisol and aldosterone appear to be essential components, i.e. the minimum combined infusion.

Both of these infusions reproduced the increase in fasting plasma [glucose] obtained with ACTH administration. In contrast, substitution of prednisolone (4 mg/h) for cortisol in the minimum combined infusion completely abolished the hypertensive response, despite reproducing the increased plasma [glucose].

Although combined cortisol, aldosterone, 17αOHP and 17α20αOHP infusion was associated with urinary Na retention and hypokalaemia, the infusion in which cortisol was substituted with prednisolone produced no MC effects.

(c) *Antagonism of 9α-fluoro-prednisolone-induced hypertension by prednisolone* (*Fig.* 3) [10]

The effects of separate 2-day infusions of prednisolone (100 mg/day) ($n = 6$) and 9α-fluoro-prednisolone (0.6 mg/day) ($n = 4$) on MAP, plasma [glucose], urinary Na excretion and fasting plasma [K] were studied in sheep. In the same sheep which received prednisolone alone for 2 days, 9α-fluoro-prednisolone was given for a further 2 days while continuing the prednisolone infusion ($n = 6$).

Prednisolone had no significant effect on MAP but increased fasting plasma [glucose] from 3.4 ± 0.1 to 5.8 ± 0.4 mmol/l ($P < 0.001$). Prednisolone administration had no effect on either plasma [K] or urinary Na excretion.

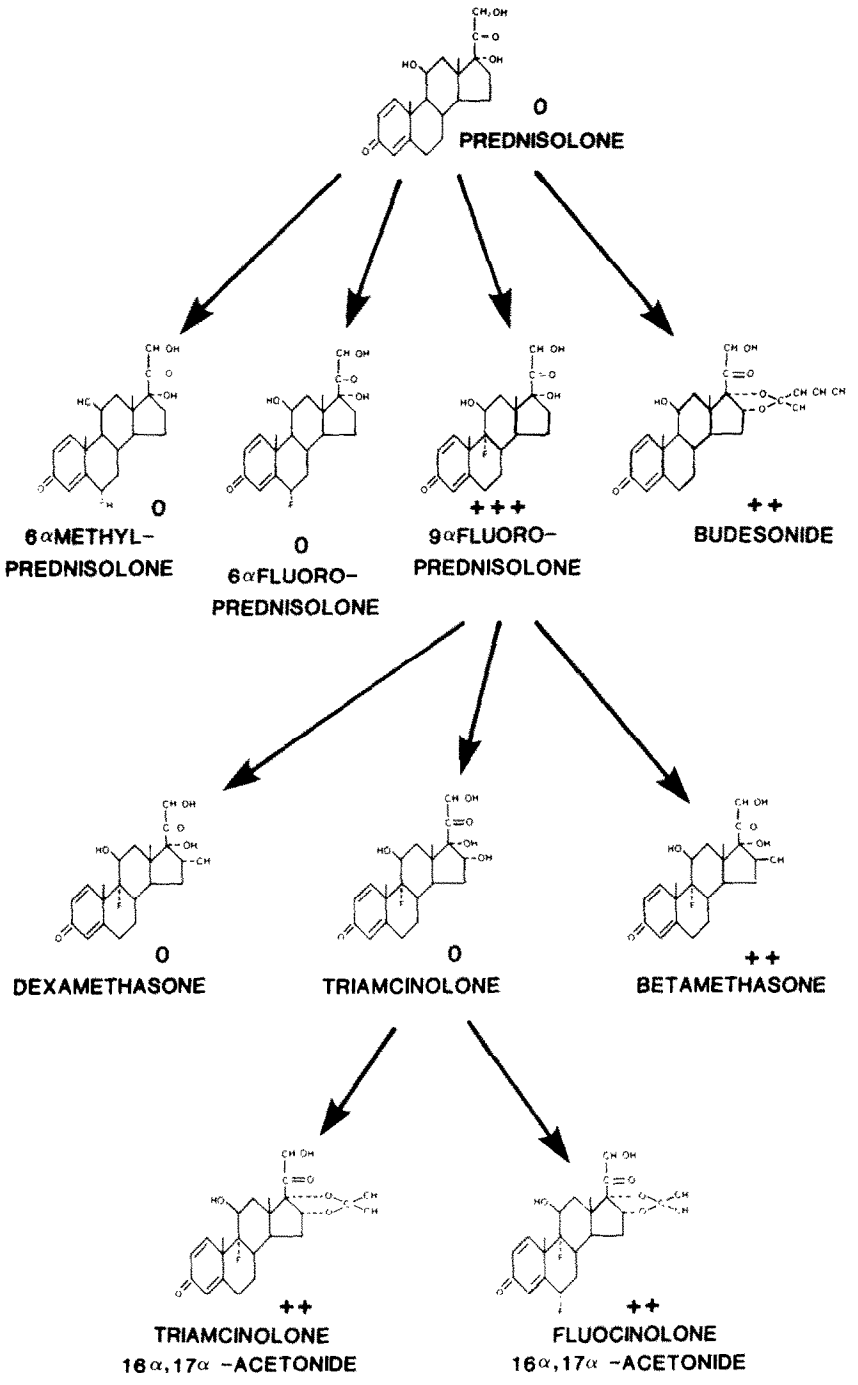

Fig. 1. Structure–activity relationships of the effect of structural analogues of prednisolone on blood pressure in sheep.

Pressor Activity	Increase in MAP
0	<5 mm Hg
+	5–10 mm Hg @ 24 mg/day
++	10–20 mm Hg @ 24 mg/day
+++	>20 mm Hg @ 0.6 mg/day

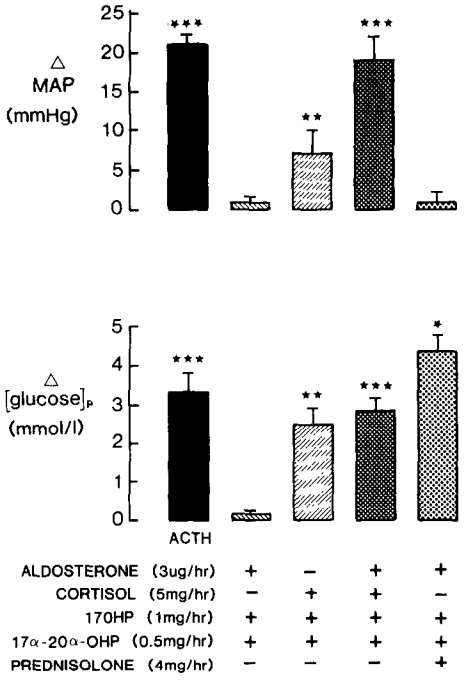

Fig. 2. The effect of ACTH infusion (5 μg/kg per day) and combined infusions of aldosterone, cortisol, prednisolone, 17αOHP and 17α20αOHP on mean arterial pressure (ΔMAP) and Δplasma [glucose] after 5 days administration.

9α-Fluoroprednisolone infusion increased MAP from 66 ± 1 to 80 ± 5 mm Hg ($P < 0.01$) reduced urinary Na^+ excretion from 120 ± 12 to 87 ± 20 mmol/day ($P < 0.05$) during the first 24 h and reduced plasma [K^+] from 4.4 ± 0.1 to 3.6 ± 0.2 mmol/l ($P < 0.001$) but had no effect on fasting plasma [glucose].

In the experiment where sheep received the combined prednisolone plus 9α-fluoroprednisolone, there was no significant effect on MAP; control 71 ± 2 mm Hg compared with 74 ± 2 mm Hg after 2 days of prednisolone alone and 72 ± 3 mm Hg after 2 days of prednisolone plus 9α-fluoroprednisolone. Therefore, prednisolone blocked the increase in MAP normally produced by 9α-fluoroprednisolone.

The urinary Na^+ retention and hypokalaemia normally produced by 9α-fluoro-prednisolone infusion was not blocked by prednisolone; control values of 92 ± 12 mmol/day were unaltered by prednisolone alone (115 ± 8 mmol/day) but fell to 45 ± 12 mmol/day ($P < 0.05$ during the first 24 h of combined infusion. Plasma [K^+] fell from 4.4 ± 0.1 to 3.8 ± 0.1 ($P < 0.001$). Fasting plasma [glucose] was increased from 3.4 ± 0.1 to 6.9 ± 0.6 mmol/l ($P < 0.001$) with the combined infusion.

(d) *Antagonism of ACTH-induced hypertension by progesterone* (*Fig.* 4)

In this study, sheep received separate infusions of progesterone (500 mg/day) and ACTH (5 μg/kg per day) for 3 days and combined infusion of progesterone plus ACTH for 3 days ($n = 5$).

Control infusions of ACTH increased MAP from 70 ± 1 to 91 ± 3 mm Hg ($P < 0.001$), reduced urinary Na^+ excretion from 58 ± 5 to 37 ± 13 mmol/day ($P < 0.05$) during the first 24 h, increased fasting plasma [glucose] from 3.2 ± 0.1 to 6.4 ± 0.6 mmol/l) ($P < 0.001$) and produced hypokalaemia, control of 4.4 ± 1 compared to 3.5 ± 0.2 mmol/l ($P < 0.001$).

Control infusions of progesterone had no effect on MAP, plasma [K^+] and plasma [glucose] but increased urinary Na^+ excretion from 60 ± 8 to 97 ± 7 mmol/day ($P < 0.05$) during the first 24 h.

In the sheep which received the combined infusion, progesterone completely blocked the increase in MAP normally produced by ACTH. Progesterone also blocked the urinary Na^+ retention and hypokalaemia associated with control ACTH administration. Fasting plasma [glucose] was increased from 3.6 ± 0.1 to 6.9 ± 0.6 mmol/l ($P < 0.001$) after 3 days of combined infusion.

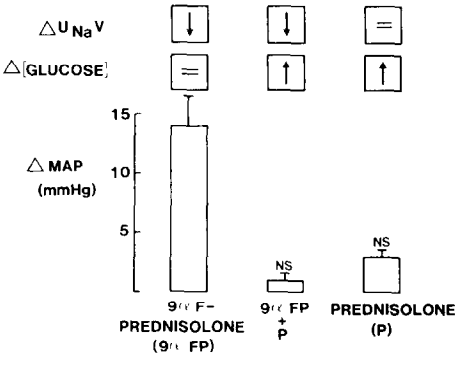

Fig. 3. Summary of the effect of prednisolone (100 mg/day) on 9α-fluoro-prednisolone (0.6 mg/day) induced hypertension. The effect of separate and combined infusions of prednisolone and 9α-fluoroprednisolone on mean arterial pressure (ΔMAP), urinary Na^+ excretion (ΔUNaV) and fasting plasma [glucose].

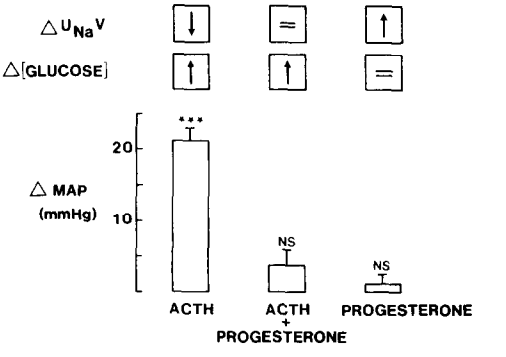

Fig. 4. Summary of the effect of progesterone (500 mg/day) on ACTH (5 μg/kg per day) induced hypertension. The effect of separate and combined infusions of progesterone and ACTH on mean arterial pressure (ΔMAP) on urinary Na^+ excretion (ΔUNaV) and fasting plasma [glucose].

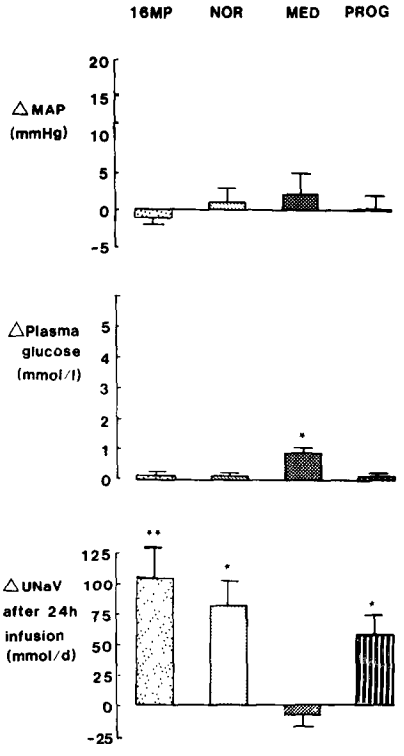

Fig. 5. The effect of 4-day infusions of 16α-methyl progesterone (16MP) (400 mg/day), norethisterone (NOR) (100 mg/day) and medroxyprogesterone (MED) (400 mg/day) on mean arterial pressure (MAP), fasting plasma [glucose], urinary Na^+ excretion (ΔUNaV) and plasma $[K^+]$ ($P^* < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

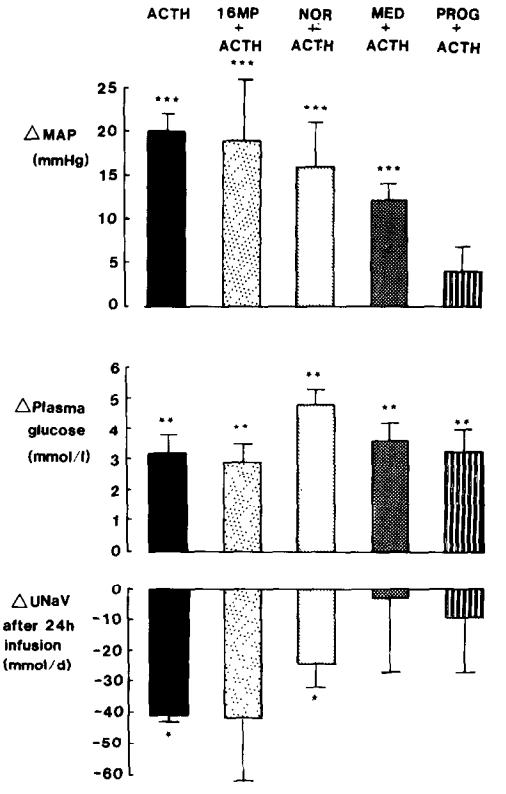

Fig. 6. The effect of 16α-methylprogesterone (16MP) (400 mg/day) norethisterone (NOR) (100 mg/day) medroxy progesterone (MED) (400 mg/day) and progesterone (PROG) (500 mg/day) on ACTH-induced hypertension. Shown is the effect of combined 3-day infusion ACTH and progesterone analogue on mean arterial pressure (ΔMAP), plasma [glucose] and urinary Na^+ excretion (ΔUNAV) ($^*P < 0.05$; $^{**}P < 0.01$).

(e) *The effect of synthetic progesterone analogues on ACTH induced hypertension. (Figs 5 and 6)*

This study examined the effect of the synthetic progesterone analogues, norethisterone (100 mg/day) ($n = 4$), medroxyprogesterone (400 mg/day) ($n = 4$) and 16α-methylprogesterone (400 mg/day) ($n = 4$) on ACTH hypertension. In control experiments sheep were given 4-day infusions of the progesterone analogues. The same sheep received 1 day of progesterone analogue alone followed by 3-days infusion in combination with ACTH.

None of the three synthetic progesterone analogues had any effect on MAP (Fig. 5). Plasma [glucose] was unchanged, with the exception of the medroxyprogesterone infusion, which increased plasma [glucose] by 0.9 ± 0.1 mmol/l ($P < 0.05$). Both 16α-methylprogesterone and norethisterone infusions caused a significant natriuresis during the first 24 h, while medroxyprogesterone had no effect on urinary Na^+ excretion.

Norethisterone and 16α-methylprogesterone did not affect the onset of ACTH hypertension while medroxyprogesterone partly blocked the pressor response ($+12 \pm 2$ mm Hg compared with $+21 \pm 2$ mm Hg, $P < 0.05$) (Fig. 6). This partial blockade was associated with blockade of urinary Na^+ retention normally produced by ACTH infusion. None of the three progesterone analogues affected the increase in plasma [glucose] or the hypokalaemia produced by ACTH, although norethisterone augmented the increase in plasma [glucose] ($+4.8 \pm 0.5$ mmol/l compared with $+3.2 \pm 0.6$ mmol/l in ACTH control infusions).

DISCUSSION

The data reported in this paper are consistent with a distinctly separate "hypertensinogenic" class of adrenocortical steroid activity. First, the structure–activity analysis of the effect of structural analogues of progesterone on blood pressure and the metabolic indices of MC and GC activities confirmed the findings of other studies [2, 5, 6] that there is no simple relationship between pressor activity of steroids and their classical MC and GC actions.

Recent studies showed that ACTH-dependent hypertension can be reproduced by the minimum combined infusion of cortisol, aldosterone, 17αOHP and 17α20αOHP [9]. Infusion of individual steroids or infusions of cortisol, 17αOHP and 17α20αOHP failed to reproduce the pressor response to ACTH. Therefore a complex interaction between cortisol,

aldosterone, 17αOHP and 17α20αOHP at the putative "hypertensiongenic" receptor appears to mediate the pressor response to ACTH.

This study investigated the role of the GC component of the minimum adrenocortical steroid requirement to reproduce ACTH-dependent hypertension and demonstrated that the synthetic GC, prednisolone, could not replace cortisol. This observation is particularly important since cortisol and prednisolone differ structurally only by a double bond at C1 and C2 and because prednisolone is more potent than cortisol in terms of GC activity [11]. In sheep, 5-day infusions of cortisol and prednisolone at doses of 100 mg/day increased MAP by 10 mm Hg [6] and 5 mm Hg [8], respectively, while they raised fasting plasma [glucose] by 2.8 ± 0.1 and 2.1 ± 0.1 mmol/l, respectively (unpublished observation). Furthermore, competitive binding studies demonstrate that cortisol and prednisolone have similar affinities for renal aldosterone binding (type 1 receptor) sites [12]. This study shows that cortisol possesses an intrinsic ability to increase MAP which is not dependent on GC activity because prednisolone reproduces the GC but not the pressor effect of cortisol in the combined steroid infusion. Therefore, it would appear that cortisol but not prednisolone is an agonist at the putative "hypertensinogenic" receptor site, and indeed there is evidence that prednisolone is an antagonist of "hypertensinogenic" activity [10]. Prednisolone has been shown to block the onset of 9α-fluoroprednisolone-induced hypertension in sheep, where it selectively blocked the "hypertensinogenic" but not the MC effects associated with 9α-fluoroprednisolone infusion [10].

Progesterone was found to block both the pressor response and MC features of ACTH administration in sheep, but it was not possible in this study to dissociate the "hypertensinogenic" and MC activities of the adrenocortical steroids which are responsible for ACTH hypertension.

Because progesterone is a MC antagonist, the mechanism by which it blocks ACTH hypertension may involve antagonism of the MC features of ACTH administration or simply blockade of the aldosterone component of the adrenocortical steroid requirement for ACTH-dependent hypertension [7]. Previous studies have shown that infusions of aldosterone [6] or DOC [13] at pharmacological doses which reproduced the MC effects of ACTH administration had no effect on MAP or increased MAP by only 10 mm Hg, respectively. Other studies have shown that ACTH hypertension can be produced in sheep on low Na^+ dietary intake (<2 mmol/day over 3 weeks) [14] and in animals acutely depleted of Na^+ by uncompensated parotid salivary loss (Na^+ deficit > 500 mmol) [15]. Since these procedures are known to block the onset of DOC-induced hypertension [16, 17], it is quite clear that ACTH hypertension is not simply a type of MC hypertension.

The synthetic progesterone analogues, 16α-methylprogesterone and norethisterone, were not effective in blocking either the hypertension or urinary Na retention and hypokalaemia produced by ACTH administration. In contrast, medroxyprogesterone partially blocked the increase in MAP and antagonized the Na^+ retention but not the fall in plasma [K^+]. Therefore, only the progesterone analogues with MC antagonist properties appear to be capable of influencing the onset of ACTH hypertension, presumably by their action on the aldosterone component.

In conclusion, these studies provide more evidence for a separate "hypertensinogenic" class of steroid activity, describe antagonism of hypertensinogenic activity and demonstrate the essential role that cortisol plays in the development of ACTH hypertension.

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