THE EFFECT OF COLCHICINE ON THE IN VIVO AND IN VITRO SECRETION OF ALDOSTERONE BY RAT ADRENOCORTICAL CELLS

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1. Introduction

Colchicine can induce metaphase arrest in dividing cells and also interfere with many biochemical events in a variety of cell types [1–8]. This antimicrotubular agent was found to inhibit hormone release from several endocrine tissues [2–5] or stimulate steroid production by Y-1 adrenal tumour cells [6] and in decapsulated adrenocortical cells obtained from adult male rats [8]. The effect of this agent on aldosterone production by adrenal zona glomerulosa is not known. We have reported that in vivo administration of ACTH increased mitotic activity in rat adrenal zona glomerulosa [9,10]. Rats treated with ACTH produced an increase in plasma corticosterone and aldosterone levels when compared to controls injected with saline [10]. However, when colchicine was administered to controls and to groups of rats treated with ACTH on purpose to block mitosis, no differences could be observed in plasma aldosterone and corticosterone levels between the groups studied [9]. To clarify discrepancies between results obtained when colchicine was included or omitted in our experiments, we have examined here the effect of the in vivo administration of colchicine, or ACTH, or colchicine with ACTH on rat plasma aldosterone and corticosterone levels. We have also compared the capacity of adrenal zona glomerulosa cells of these treated animals to produce aldosterone in vitro.

2. Materials and methods

General procedures used for this study were similar to those in [9,10]. Female Long Evans rats, ~2 months old, were obtained from our local breeding colony. Animals were kept in individual cages, and injected subcutaneously twice a day for 2 days with 3.4 IU ACTH (Synacthen, Ciba-Geigy Pharmaceutical, Montreal); controls received the vehicle only. Animals received their treatments at 09:00 h and 16:00 h and were sacrificed 16 h after the last injection. When used, colchicine (1 μg/g body wt) was administered 6 h before sacrifice by the subcutaneous route. Cell suspensions were prepared as in [11,14]. Aldosterone was determined by specific radioimmunoassay [9] and corticosterone as in [13].

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3. Results

Experiments were performed, with 4 groups of 6 rats. The first group received distilled water; the second group received ACTH; the third group received ACTH and colchicine; the fourth group received distilled water and colchicine. Table 1 shows results of a typical experiment.

Table 1
Effect of in vivo treatment with ACTH and/or colchicine on plasma aldosterone and corticosterone levels in female rats

Treatment	Aldosterone (ng/dl)[a]	Corticosterone (μg/dl)[a]
Distilled water	7.38 ± 1.22	13.73 ± 3.84
ACTH	19.86 ± 1.07[b]	71.86 ± 1.72[b]
ACTH–colchicine	16.79 ± 1.25[b]	62.40 ± 1.29[b]
Colchicine–distilled water	23.64 ± 1.25[b]	67.11 ± 5.4[b]

[a] Mean ± SEM of values from 6 rats
[b] Significantly different from control group, $p < 0.001$

3.4 IU ACTH were injected twice daily for 2 days and animals sacrificed 16 h after the last injection while controls received distilled water. Colchicine (1 μg/g body wt) was injected 6 h before sacrifice

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It can be seen that colchicine alone provoked significant increases in plasma aldosterone and corticosterone levels, compared to controls. These increases were in the same order of magnitude as those produced by ACTH. No additive effect could be observed when ACTH together with colchicine were injected to animals. To explore the relationship between plasma aldosterone and corticosterone levels and the secretory capacity of the adrenal cortex, in three experiments suspensions of cells from zona glomerulosa were prepared for each group of rats and incubated for two hours at 37°C. Steroids were analyzed in the supernatant.

As it can be seen in table 2, under the experimental conditions used, the basal capacity of zona glomerulosa cells to produce aldosterone in vitro was diminished by 83% with ACTH, by 81% with the combined treatment of ACTH and colchicine and not significantly modified with colchicine. The pattern of the basal capacity of the same glomerulosa cell suspensions to produce corticosterone was however different that in the case of aldosterone. Indeed, glomerulosa cells from adrenals of rats treated with ACTH produced 2.3-fold more corticosterone than controls. Colchicine alone stimulated the production of corticosterone by 2.8-fold while it prevented the full enhancing effect of the ACTH treatment; indeed the increased value observed was not statistically different from control.

Table 2
Effect of in vivo treatment with ACTH and/or colchicine on in vitro aldosterone and corticosterone production by rat adrenal cell suspensions

Treatment	Glomerulosa cells	
	Aldosterone (ng/50 000 cells)[a]	Corticosterone (ng/50 000 cells)[a]
Distilled water	4.42 ± 1.21	150.9 ± 15.3
ACTH	0.77 ± 0.18[c]	348.1 ± 41.8[b]
ACTH-colchicine	0.85 ± 0.24[c]	227.4 ± 69.1
Colchicine–distilled water	4.98 ± 1.95	431.2 ± 64.3[b]

[a] Mean ± SEM of three experiments with duplicate determinations
[b] $0.01 < p < 0.02$; [c] $0.02 < p < 0.05$, significantly different from control group

For details of treatments see table 1: ~50 000 cells/ml were incubated in duplicate in a Krebs-Ringer bicarbonate buffer containing 0.2% glucose, 0.2% bovine serum albumin and 5.6 mM K^+. Incubations were performed at 37°C for 2 h and steroids analyzed in the supernatant

4. Discussion

We show for the first time that in vivo administration of colchicine to rats interacts with the formation of aldosterone. This substance induced a 3-fold and 5-fold increase in plasma aldosterone and corticosterone levels, respectively (table 1); this response was similar to that obtained when rats were treated with ACTH for 2 days. When colchicine was injected to ACTH-treated animals, plasma steroid levels were only slightly diminished compared with groups treated with ACTH or with colchicine alone. Results of table 1 also clarify [11] where a 2-day treatment with ACTH had no stimulating effect on rat plasma steroid levels compared to controls which were treated with colchicine. This study clearly demonstrates that the colchicine-treated groups cannot be used as controls for such experiments, because colchicine alone injected 6 h before sacrifice stimulates plasma aldosterone and corticosterone, as does ACTH. Cholchicine injected in vivo into hypophysectomized rats prevented the full elevation of serum corticosterone by corticotropin [14]. The effect of colchicine when injected alone was not reported in [14]. In contrast to increased values found for plasma aldosterone, glomerulosa cells from groups of rats treated with ACTH or with ACTH and colchicine produced less aldosterone than similar preparations from controls treated with distilled water. The colchicine treatment did not modify aldosterone secretion in vitro (table 2). This low aldosterone secreting capacity by glomerulosa cells of rats treated with ACTH is in agreement with [10,15–17]. This could be an exhaustion of the proper substrates in those cells, necessary to sustain aldosterone production in vitro. This possibility is weakened by the fact that corticosterone concentrations in incubation media of preparations of treated groups were not found to be lower, but even higher than those of the control. In glomerulosa cells preparations the colchicine treatment produced a 2.8-fold increase of corticosterone concentration compared to control. Our results are in agreement with [8,12]. Using a different approach the effect of colchicine directly on dispersed decapsulated rat adrenal cells was examined [8]: colchicine could maximally stimulate steroidogenesis to the same degree as ACTH. To observe a stimulation however, cells had to be incubated in the presence of colchicine for 24 h; a 2 h-incubation did not produce any stimulation of steroid synthesis. A 6–9 h lag period was also noted

[6] before colchicine could stimulate steroidogenesis production in Y-1 adrenal tumour cell cultures. So, it seems that the short-term effect of colchicine on adrenal steroidogenesis might be different from its long-term effect. Using a short-term incubation period (90 min) colchicine was found to prevent the full corticosteroidogenesis effect of ACTH on decapsulated rat adrenal cell suspensions [14]. In [18], monolayer cultures of rat adrenal zona fasciculata-reticularis cells failed to respond to short term treatment of colchicine. It is difficult to compare our results with the above since our experimental approach was different: (i) we used glomerulosa cells instead of decapsulated adrenal; (ii) we injected colchicine to animals 6 h before sacrifice. Colchicine interacts with microtubules, binding to the tubulin protomer, and so preventing polymerization of microtubules [19]. Also, microtubules might be involved in the transport of zona fasciculata [20,21] steroids. The absence of microtubule formation due to colchicine might facilitate the access of cholesterol to mitochondria and consequently enhance steroid synthesis [6]. These results are consistent with the concept that microtubules can influence the process of steroid output of the zona glomerulosa since we have shown that colchicine affects the formation of both corticosterone and aldosterone in vivo (table 1). However, it is too soon to decide whether colchicine affects the transport or the synthesis, or both, at the glomerulosa cell level.

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