

Dopamine Inhibits Angiotensin-Stimulated Aldosterone Biosynthesis in Bovine Adrenal Cells

TERENCE J. MC KENNA, DONALD P. ISLAND, WENDELL E. NICHOLSON, and
GRANT W. LIDDLE, *Vanderbilt University School of Medicine, Nashville,
Tennessee 37232*

ABSTRACT The possibility that dopamine may play a role in the *in vivo* control of aldosterone production in man was suggested to us by reports from others; (a) that bromocriptine, a dopaminergic agonist, inhibits the aldosterone response to diuresis and to the infusion of angiotensin or ACTH; and (b) that metaclopramide, a dopamine blocking agent, causes elevations in plasma aldosterone levels. To determine whether such effects were direct or indirect, we examined the action of dopamine on aldosterone biosynthesis in isolated, bovine adrenal cells. Dopamine significantly inhibits the aldosterone response to angiotensin ($P < 0.001$), but does not influence basal aldosterone biosynthesis.

It has previously been reported that angiotensin stimulates both the early and late phases of aldosterone biosynthesis. The present experiments demonstrated that the enhancing effect of angiotensin on the conversion of deoxycorticosterone to aldosterone (late phase of aldosterone biosynthesis) was almost completely inhibited by dopamine ($P < 0.001$). A significant inhibitory effect of dopamine (10 nM) was seen even when aldosterone biosynthesis was stimulated by a grossly supraphysiological concentration of angiotensin II (10 μ M). However, these studies did not demonstrate any direct effect of dopamine on the early phase of aldosterone biosynthesis (cholesterol to pregnenolone) basally or when stimulated, or on the late phase of aldosterone biosynthesis under basal conditions. These *in vitro* studies suggest a direct inhibitory role for dopamine on the late phase of aldosterone biosynthesis, which may account for the *in vivo* inhibition of the aldosterone response to angiotensin in subjects treated with a dopaminergic agent.

Dr. McKenna's present address is Consultant Endocrinologist, St. Vincent's Hospital, Elm Park, Dublin 4, Ireland.

Received for publication 7 August 1978 and in revised form 17 January 1979.

INTRODUCTION

Administration of the dopaminergic agonist, bromocriptine, to normal subjects has been reported to blunt the aldosterone responses to diuresis (1) and to infusions of angiotensin II or ACTH (2). In addition, bromocriptine suppressed aldosterone secretion in patients with primary aldosteronism caused by idiopathic adrenal hyperplasia, but not in patients with tumorous hyperaldosteronism (3). Furthermore, treatment of normal and hypophysectomized subjects with metaclopramide, an inhibitor of dopamine action, has been reported to induce a marked rise in plasma aldosterone levels with no change occurring in plasma renin activity (4). These observations have suggested to us the possibility that dopamine may modify aldosterone secretion *in vivo*. However, because these studies did not permit a distinction between a direct dopamine-related effect, as opposed to an indirect effect of dopamine (e.g., via changes in prolactin) or actions of the drugs themselves unrelated to their effects on dopamine, we have performed experiments in which dopamine was added directly to isolated suspensions of adrenal cells. Our studies demonstrate that dopamine has a direct effect on adrenal cells whereby angiotensin-stimulated aldosterone biosynthesis is consistently inhibited; however, dopamine does not influence basal aldosterone production.

METHODS

Adrenal glands were obtained from freshly slaughtered cattle. Outer 0.5-mm slices (including the capsule) were obtained from the adrenals with a Stadie-Riggs microtome. The slices were then treated with collagenase and the cells were dispersed according to the method of Fredlund (5). The entire cell suspension was then aliquoted into (17 \times 120 mm) polypropylene tubes. The adrenal enzyme inhibitors, trilostane 10 μ M (6) and aminogluthethimide, 760 μ M (7), were then added to some tubes. All the tubes containing aliquots of the cell suspension were then placed in an incubator (37°C, 95%

oxygen, 5% carbon dioxide) on an automatic shaker for 15 min. After this, dopamine hydrochloride, 1 pM–10 μ M, was added to the tubes in which its effect was to be examined. All the tubes were again placed in the incubator for a further 15 min. At the end of this time angiotensin II, 10 μ M, and deoxycorticosterone, 20 μ g in 25 μ l of ethanol, were added to appropriate tubes and the final volume in the tubes was adjusted where necessary to 1 ml by the addition of Krebs-Ringer bicarbonate buffer containing dextrose (1 g/liter) and bovine serum albumin (5 g/liter). Triplicate aliquots of the cell suspension were treated identically for each effect examined. Therefore, one set of triplicate tubes contained cells only, another, cells plus trilostane, another, cells plus angiotensin II, another, cells plus trilostane plus angiotensin II, and so on. All tubes were incubated under identical conditions for the same length of time. At the end of the final incubation the cell suspension was frozen and subsequently thawed, homogenized, and the aldosterone, pregnenolone, and cortisol concentrations in each tube of relevant sets of aliquots were measured using previously described methods of preparative chromatography and radioimmunoassay (8–10).

The early phase of aldosterone biosynthesis, i.e., up to and including pregnenolone production, was measured as previously described from this laboratory (6). Trilostane, an inhibitor of the 3β -ol-dehydrogenase, Δ^4 - Δ^5 -isomerase enzyme system (6), inhibits the conversion of pregnenolone to progesterone and, therefore, to aldosterone. Accumulation of pregnenolone in trilostane-treated cell suspensions provides an index of activity in the early phase of steroidogenesis. However, pregnenolone is a precursor of both cortisol and aldosterone, and angiotensin II stimulates production of both steroids in bovine adrenals (11). To attribute changes in pregnenolone accumulation to alterations occurring only in the aldosterone biosynthetic pathway, it is necessary to demonstrate that the cortisol concentration does not change in response to angiotensin II in the cell suspensions used.

The late phase of aldosterone biosynthesis was also studied as previously described (6). Aminoglutethimide was used to inhibit aldosterone biosynthesis by interrupting the conversion of cholesterol to pregnenolone. Activity in the late phase of aldosterone biosynthesis may be assessed by measuring the conversion of exogenous deoxycorticosterone to aldosterone in aminoglutethimide-treated cell suspensions under a variety of conditions. Because endogenous aldosterone biosynthesis was blocked by aminoglutethimide, changes in aldosterone production must reflect changes occurring only in steps involved in the conversion of deoxycorticosterone to aldosterone, i.e., steps exclusive to the late phase of aldosterone biosynthesis.

RESULTS

The effect of dopamine (10 μ M) on total aldosterone production. Aldosterone production under basal conditions was not consistently affected by the addition of dopamine (10 μ M) (Fig. 1). Aldosterone production was significantly stimulated in the presence of angiotensin II ($P < 0.001$). However, the aldosterone response to angiotensin was significantly diminished by the addition of dopamine, $P < 0.001$, (Fig. 1). The data has been normalized so that the total amount of aldosterone present under basal conditions and when stimulated by angiotensin was taken as 100%. The percentage of change in aldosterone accumulation in each experiment induced by the presence of dopamine could then be

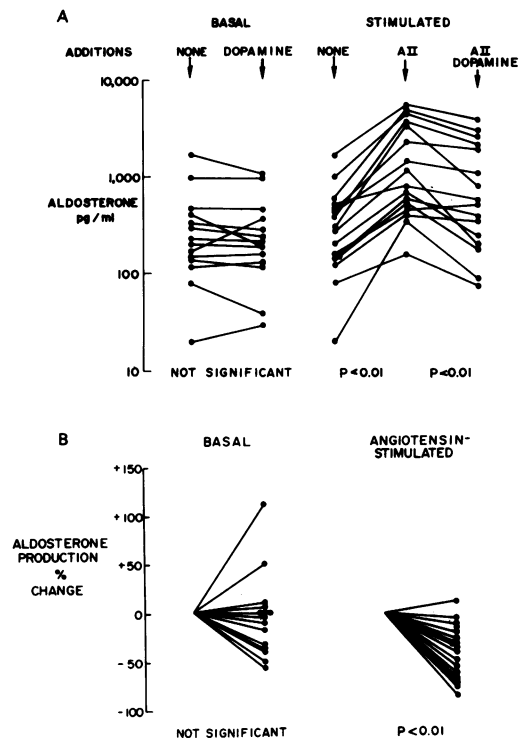


FIGURE 1 The effect of dopamine on net aldosterone production. (A) aldosterone levels in bovine adrenal cell suspensions without further addition and with additions of dopamine alone, angiotensin (A II) alone, and dopamine plus angiotensin; (B) percent change induced in each experiment by dopamine on the amount of aldosterone produced under basal conditions and when stimulated with angiotensin. Dopamine inhibited angiotensin-stimulated aldosterone biosynthesis but did not significantly affect basal aldosterone biosynthesis.

calculated and is shown in Fig. 1B. Under basal conditions the percentage of change induced by dopamine on aldosterone production was increased in four, unchanged in three, and decreased in seven experiments. In contrast angiotensin-stimulated aldosterone production was decreased in the presence of dopamine in all but one experiment.

Lack of effect of dopamine (10 μ M) on the early phase of aldosterone biosynthesis. Pregnenolone accumulation was not affected by the addition of dopamine to trilostane-treated cells, either under basal conditions or when stimulated with angiotensin II (Fig. 2). Aldosterone production in trilostane-treated cell suspensions was 0.15 ± 0.02 ng/ml (mean \pm SE) under basal conditions and 0.21 ± 0.05 ng/ml when angiotensin II was added. The data has been normalized so that the amount of pregnenolone accumulating under basal conditions and when stimulated with angiotensin II was taken as 100%. It was then possible to calculate the percent change in pregnenolone accumulation induced by dopamine in each experiment. It can be seen in the

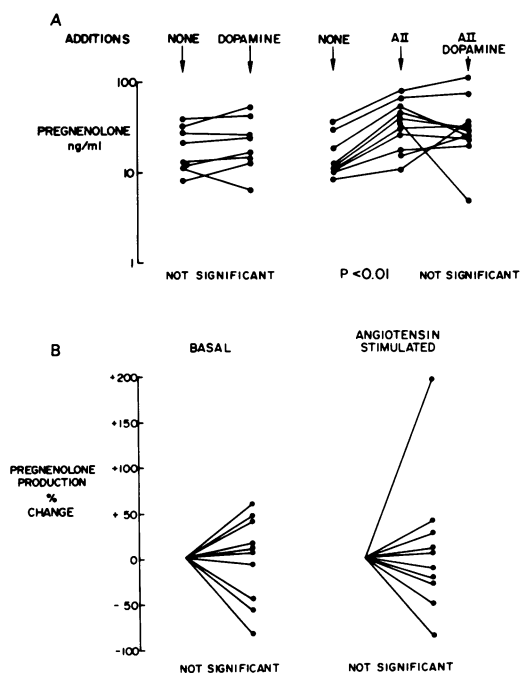


FIGURE 2 The effect of dopamine on the early phase of aldosterone biosynthesis. (A) pregnenolone levels in trilostane-treated bovine adrenal cell suspensions (an index of activity in the early phase of aldosterone biosynthesis) without further addition and with the additions of dopamine alone, angiotensin (A II) alone, and dopamine plus angiotensin; (B) percent change induced in each experiment by dopamine on the amount of pregnenolone accumulating under basal conditions and when stimulated with angiotensin. Dopamine did not significantly affect either basal or angiotensin-stimulated activity in the early phase of aldosterone biosynthesis.

lower panel of Fig. 2 that the effect of dopamine appears randomly distributed about the 0% change points.

Additional aliquots for the same cell suspensions used in the experiments examining the early phase of aldosterone biosynthesis were not treated with trilostane. The aldosterone and cortisol values in the untreated aliquots under control conditions and when stimulated with angiotensin II are shown in Fig. 3. In the absence of trilostane, angiotensin II stimulated a significant increase in aldosterone production, $P < 0.01$. The cortisol concentration in the same cell suspensions did not respond significantly (Fig. 3).

The effect of dopamine (10 μ M) on the late phase of aldosterone biosynthesis. Our experiments failed to demonstrate a significant effect of dopamine on the conversion of deoxycorticosterone to aldosterone under basal conditions (Fig. 4). Angiotensin II significantly enhanced the conversion of deoxycorticosterone to aldosterone, $P < 0.002$, but this effect was almost completely inhibited by the presence of dopamine, $P < 0.005$. Aldosterone production in aminoglutethimide-treated cell suspensions under basal conditions was

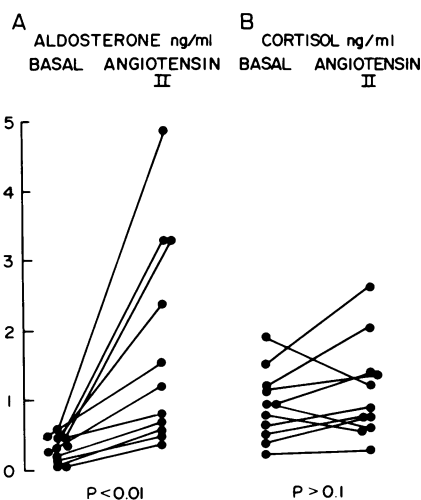


FIGURE 3 The effect of angiotensin (A II) on aldosterone and cortisol production. Aliquots obtained from the cell suspension used in the experiments depicted in Fig. 2 were not treated with trilostane to permit assessment of the potential of these cell suspensions to produce aldosterone and cortisol in response to angiotensin. Although angiotensin II significantly stimulated aldosterone biosynthesis (A), cortisol biosynthesis (B) was not significantly affected.

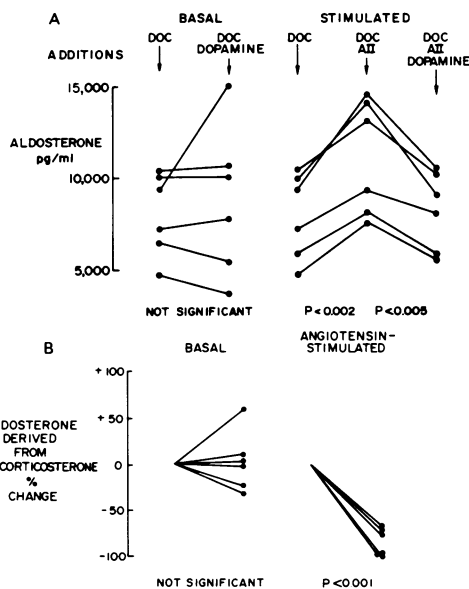


FIGURE 4 The effect of dopamine on the late phase of aldosterone biosynthesis. (A) aldosterone derived from deoxycorticosterone (DOC) added to aminoglutethimide-treated adrenal cell suspensions (an index of activity in the late phase of aldosterone biosynthesis) without further addition and with the additions of dopamine alone, angiotensin (A II) alone, and dopamine plus angiotensin; (B) bottom panel, percent change induced in each experiment by dopamine on the amount of aldosterone derived from deoxycorticosterone under basal conditions and when stimulated with angiotensin. Dopamine markedly impaired the enhancing effect of angiotensin on the activity of the late phase of aldosterone biosynthesis but had no effect under basal conditions.

0.14±0.2 and 0.18±0.05 ng/ml (mean±SE) when angiotensin II was added. The data has been normalized so that the amount of aldosterone derived from deoxycorticosterone under basal conditions and when stimulated with angiotensin II was taken as 100%. It was then possible to calculate the percent change in aldosterone derived from deoxycorticosterone induced by dopamine in each experiment. It can be seen in Fig. 4B that, whereas a consistent effect of dopamine is not seen under basal conditions, dopamine markedly inhibited the angiotensin II-stimulated conversion of deoxycorticosterone to aldosterone in each experiment.

The effect of various concentrations of dopamine on angiotensin-stimulated aldosterone biosynthesis. In a series of five experiments the effects of dopamine (in concentrations ranging from 1 pM to 10 μM) on angiotensin-stimulated aldosterone biosynthesis were measured. At each concentration studied, dopamine tended to be inhibitory (Fig. 5). This inhibitory effect achieved statistical significance ($P < 0.01$ – 0.05) in concentrations ranging from 10 μM to 10 nM but not at lower concentrations of dopamine.

DISCUSSION

In these experiments the presence of dopamine inhibited the aldosterone response to stimulation with angiotensin in bovine adrenal cell suspensions. Basal aldosterone biosynthesis was not consistently influenced by dopamine. These findings provide an explanation for the *in vivo* observations that bromocriptine blunts the rise in aldosterone secretion that occurs either in response to angiotensin (2) or in response to acute diuresis (1), an effect that is also presumably angiotensin-mediated. Taken together, all of these observations suggest a role for dopamine in the control of aldosterone secretion *in vivo*. Because dopamine does not influence basal aldosterone biosynthesis, it may

exert its influence by modifying the aldosterone response to humoral stimuli.

We have previously reported that angiotensin directly stimulates both the early and late phases of aldosterone biosynthesis (6). To address the question of how dopamine influenced angiotensin-stimulated aldosterone biosynthesis, we examined the effect of dopamine on the isolated early and late phases of steroidogenesis. Dopamine did not affect either basal or angiotensin-stimulated pregnenolone production in trilostane-treated adrenal cell suspensions (Fig. 2). Because angiotensin significantly stimulated aldosterone but not cortisol production in aliquots of the cell suspensions not treated with trilostane (Fig. 3), the accumulation of pregnenolone can be used as an index of activity in the early phase of aldosterone biosynthesis. We conclude, therefore, that dopamine did not affect either basal or stimulated activity in the early phase of aldosterone biosynthesis. Because dopamine inhibited total aldosterone production but not the isolated early phase of aldosterone biosynthesis, it was of interest to extend the examination to the late phase. The previously demonstrated stimulatory effect of angiotensin on the conversion of deoxycorticosterone to aldosterone (6) was markedly impaired by dopamine (Fig. 4). This finding suggests that dopamine inhibits the action of angiotensin on the late phase of aldosterone biosynthesis.

It was possible either that dopamine directly inhibited enzymatic activity in the late phase of aldosterone biosynthesis, or that dopamine suppressed the stimulatory effect of angiotensin on the late steps in the aldosterone pathway. However, because dopamine did not influence the conversion of deoxycorticosterone to aldosterone under basal conditions, it is unlikely that dopamine has a direct inhibitory effect on the enzymes in the late phase (Fig. 4). Therefore, dopamine probably exerted its effect by suppressing the action of angiotensin on the late steps in aldosterone biosynthesis. In contrast, dopamine did not affect the stimulatory action of angiotensin on the early phase (Fig. 2). These observations could be explained by the presence of different receptors for the action of angiotensin on the early phase and on the late phase of aldosterone biosynthesis. Dopamine might then exclusively perturb the receptors responsible for stimulation of the late phase. Alternatively, if only a single type of angiotensin receptor exists, dopamine might selectively inhibit the postreceptor events unique to stimulation of the late phase of aldosterone biosynthesis.

Dopamine appears to selectively inhibit the action of angiotensin on the late phase of aldosterone biosynthesis by unknown mechanisms. We have previously reported that modulation of the late phase of adrenal steroidogenesis can be demonstrated in various situations. Potassium, in concentrations at least up to 6 meq/

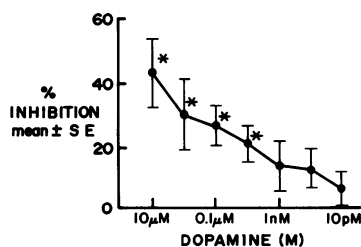


FIGURE 5 The effect of dopamine at concentrations ranging from 1 pM to 10 μM on angiotensin-stimulated aldosterone biosynthesis (the dots indicate the mean percentage of inhibitory effect of dopamine and the vertical bars through the dots indicate the standard error for five experiments). There was a concentration-dependent inhibitory effect of dopamine on angiotensin-stimulated aldosterone biosynthesis that was significant to dopamine at 10 nM (* $P < 0.01$ – $P < 0.05$).

liter, can stimulate the late phases of aldosterone biosynthesis, but when the potassium concentration increases to 12 meq/liter the late phase of aldosterone biosynthesis is inhibited (12). ACTH has enhancing effects on activity in the late phase of cortisol biosynthesis (13) that are analogous to the previously noted stimulatory effect of angiotensin on the late phase of aldosterone biosynthesis (6). It appears probable, therefore, that modulation of the late phase of adrenal steroidogenesis may play a role in the control of steroid secretion in response to a variety of stimuli.

Although some of the experiments discussed above employed a supraphysiological concentration of dopamine (10 μ M), lower, physiological concentrations of dopamine also blunted angiotensin-stimulated aldosterone biosynthesis (Fig. 5). The circulating concentration of free dopamine has been estimated to be approximately 0.2 nM (14). However, conjugated dopamine, which is present in greater abundance, might be deconjugated in several tissues including the adrenal cortex (15, 16). In addition, owing to the proximity of the adrenal medulla to the adrenal cortex and the interposition of a portal circulation, it is conceivable that adrenocortical dopamine concentrations might be higher than those in general circulation. Sympathetic innervation of the adrenal capsule may also contribute to raising the concentration of dopamine in the intimately related zona glomerulosa. However, the physiological concentration of dopamine-bathing, aldosterone-producing glomerulosa cells has not been definitively established.

In summary, it can be concluded that the *in vitro* studies reported here are compatible with a physiological role for dopamine in the *in vivo* modulation of humoral stimulators at the level of the aldosterone-producing cell.

ACKNOWLEDGMENT

This study was supported, in part, by U. S. Public Health Service grant 5-ROL-AM05318.

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