Effects of Metoclopramide and Bromocriptine on the Renin-Angiotensin-Aldosterone System in Man

DOPAMINERGIC CONTROL OF ALDOSTERONE

ROBERT M. CAREY, MICHAEL O. THORNER, and ELIZABETH M. ORTT,
Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908

ABSTRACT This study was designed to investigate the possible role of dopaminergic mechanisms in the control of the renin-angiotensin-aldosterone system in normal man. Six normal male subjects in metabolic balance at 150 meq sodium, 60 meq potassium constant intake received the specific dopamine antagonist, metoclopramide, 10 mg i.v. or placebo followed by angiotensin II infusion 1 h later on 2 consecutive days. Metoclopramide increased plasma aldosterone concentration from 8.2±2.2 to 21.0±3.3 ng/100 ml ($P < 0.005$) and plasma prolactin concentration from 18.0±4.0 to 91.7±4.0 ng/ml ($P < 0.001$) within 15 min of its administration. At 1 h, plasma aldosterone and prolactin concentrations remained elevated at 16.8±2.1 ng/100 ml ($P < 0.01$) and 86.8±15.9 ng/ml ($P < 0.005$), respectively. Angiotensin II at 2, 4, and 6 pmol/kg per min further increased plasma aldosterone concentration to 27.2±3.4, 31.9±5.7, and 36.0±6.7 ng/100 ml ($P < 0.02$), respectively. Placebo did not alter plasma aldosterone or prolactin concentrations, but angiotensin II increased plasma aldosterone concentration to 13.7±2.4, 19.0±1.9, and 23.3±3.2 ng/100 ml ($P < 0.005$). The increment of plasma aldosterone concentration in response to angiotensin II was similar after metoclopramide or placebo.

The six subjects also received the dopamine agonist, bromocriptine, 2.5 mg or placebo at 6 p.m., midnight, and 6 a.m. followed by angiotensin II infusion on 2 consecutive d. Bromocriptine suppressed prolactin to <3 ng/ml. After placebo, plasma aldosterone concentration increased from 5.2±1.4 to 12.3±1.7, 17.2 ±2.2, and 21.8±3.5 ng/100 ml ($P < 0.01$) and after bromocriptine from 7.2±1.0 to 14.7±.3.0, 19.8±3.2, and 23.4±1.6 ng/100 ml ($P < 0.001$) with each respective angiotensin II dose. No difference in the response to angiotensin II after bromocriptine or placebo was observed. Plasma renin activity, free 11-hydroxycorticoid concentration, and serum potassium concentration were unchanged by metoclopramide or bromocriptine.

The results suggest that aldosterone production is under maximum tonic dopaminergic inhibition which can be overridden with stimulation by angiotensin II in normal man.

INTRODUCTION

Dopamine, a precursor of the sympathetic neurotransmitter norepinephrine, may itself be a major transmitter of the peripheral autonomic nervous system (1). The adrenergic component of this system is important in controlling renin release (2, 3), but little is known concerning the role of dopamine in the regulation of the renin-angiotensin-aldosterone system. Cuche et al. (4) have shown in man that the increase in plasma renin activity with upright posture is associated with a decrease in urinary dopamine and an increase in norepinephrine and epinephrine excretion. Alexander and colleagues (5) have reported that sodium-depleted subjects respond to dietary or intravenous sodium loading with an increase in urinary dopamine and a decrease in urinary norepinephrine. Thus, maneuvers which would be expected to alter the activity of the renin-angiotensin-aldosterone system are associated with inverse changes in urinary dopamine excretion. Recently, Edwards et al. (6) demonstrated in man that the specific long-acting dopamine receptor stimulant (agonist), bromocriptine (2-brom-α-ergocryptine), inhibits the increase in plasma aldosterone concentration induced by furosemide without altering the concomitant increase in plasma renin ac-

Dr. Carey is an Established Investigator of the American Heart Association.

Received for publication 5 September 1978 and in revised form 2 November 1978.

tivity. These observations suggest the possibility that under certain circumstances dopamine may participate directly in the regulation of aldosterone production.

The present study was designed to investigate the role of dopamine mechanisms in the control of the renin-angiotensin-aldosterone system in normal man. First, the effects of the specific dopamine-receptor blocking agent (antagonist), metoclopramide, (*N* - [diethylaminoethyl] - 2 - methoxy - 4 - amino - 5 - chlorobenzamide), on plasma renin activity and aldosterone concentration and urinary aldosterone excretion were determined. Since metoclopramide was demonstrated to increase plasma aldosterone concentration and urinary aldosterone excretion, the effects of metoclopramide on angiotensin II-induced aldosterone production also were determined. Second, the effects of bromocriptine on plasma renin activity and basal and angiotensin II-induced aldosterone production were studied. During these experiments, potassium and ACTH, factors known to stimulate aldosterone production, and prolactin, a convenient monitor of dopamine receptor effects, also were studied.

METHODS

Human subjects and study protocol. Six normal white male volunteer subjects, 24–30 yr, with normal arterial blood pressures and no history of renal disease were studied. The subjects were placed on a constant diet containing 150 meq of sodium, 60 meq of potassium, 1 g of protein/kg and 2,860 cal/day for 5 d at the Clinical Research Center before the studies. Consecutive 24 h urine samples were collected for the first 4 d of the diet and consecutive 12 h urine samples were collected for the remainder of each study and were analyzed for sodium, potassium, and creatinine. No food was given after midnight before study day 1 when the subjects assumed the supine position until completion of each study. At 6:00 a.m. on study day 1, a heparin lock for obtaining blood samples was placed in the left antecubital vein and an intravenous infusion of 5% dextrose in water at 1 ml/min was begun in the right antecubital vein. At 6:30 a.m., the subjects completed their 12 h urine collections and began a 1 h control urine collection for measurement of aldosterone excretion without arising. Also at this time, blood pressure monitoring with an Arteriosonde (Hoffmann-La Roche, Inc., Medical Electronics Div., Nutley, N. J.) was begun and was continued every 2 min until completion of the study. At 7:00 and 7:30 a.m., control blood samples were obtained for the determination of serum sodium, potassium and prolactin concentration, and plasma renin activity, cortisol and aldosterone concentration. After completion of blood sampling and the 1-h control urine collection for aldosterone excretion at 7:30 a.m., an intravenous bolus dose of 2 ml of 5% dextrose in water placebo was administered. Blood sampling then was accomplished at 15, 30, and 60 min (7:45, 8:00, and 8:30 a.m.) after the placebo injection. A second 1-h urine collection for measurement of aldosterone excretion was obtained 7:30–8:30 a.m. At 8:30 a.m., an intravenous infusion of angiotensin II at 2 pmol/kg per min begun. Thereafter, blood sampling was done at 30-min intervals until completion of the study at 10:00 a.m. The octapeptide was infused at three successively increasing dose levels for 30 min. The total duration of each angiotensin II infusion was 90 min.

After completion of the infusion at 10:00 a.m., the subjects continued on the constant diet and 12-h urine collections. On study day 2, an identical protocol as for study day 1 was followed except that instead of placebo, a 2-ml bolus injection of metoclopramide (10 mg) was substituted at 7:30 a.m. The bolus injections were given on a single-blind basis, so that the subjects were unaware of whether they were receiving a placebo or metoclopramide injection. The diet was discontinued, and the subjects were discharged from the Clinical Research Center after completion of the final 12-h urine collection at 6:30 p.m. on study day 2. The total volume of blood obtained from each subject during the two study days was 504 ml.

After a 3–4-wk interval on an ad lib. diet, the same subjects were placed again on the same 150-meq sodium, 60-meq potassium constant diet at the Clinical Research Center for 5 d before study. Urine collections for 24 and 12 h were obtained as before. Placebo capsules were administered at 6:00 p.m. and midnight on study day 1 and at 6:00 a.m. on study day 2. No food was given after midnight before study day 1 when the subjects assumed the supine position until completion of the study. At 6:30 a.m. on study day 1, the subjects again completed their 12-h urine collection without arising. Indwelling catheters for control 5% dextrose in water infusion and heparin lock for blood withdrawal were inserted and blood pressure monitoring was accomplished beginning at 6:30 a.m. as previously described. At 7:00 and 7:30 a.m. blood samples were obtained for determination of the parameters measured previously, after which an intravenous infusion of angiotensin II at 2 pmol/kg per min was begun. Thereafter, blood samples were obtained at 30-min intervals until completion of the study at 9:00 a.m. Angiotensin II again was infused in successively increasing doses of 2, 4, and 6 pmol/kg per min each dose for 30 min. The total duration of the infusion was 90 min.

After completion of the infusion at 9:00 a.m., the subjects continued on the constant diet and 12-h urine collections. On study day 2, an identical protocol as for study day 1 was followed except that the angiotensin II infusion was preceeded by three doses of bromocriptine orally. 2.5 mg bromocriptine was administered on study day 1 at 6:00 p.m. and midnight and on study day 2 at 6:00 a.m. The bromocriptine was given for 12 h before study to assure dopamine receptor stimulation would be present during the study period. On study day 2, the subjects remained supine from the end of the angiotensin II infusion at 9:00 a.m. until noontime to prevent postural hypotension as a result of the bromocriptine. Blood pressure was monitored every 30 min by means of mercury sphygmomanometer. The diet was discontinued and the subjects were discharged from the Clinical Research Center after completion of the final urine collection at 6:30 p.m. on study day 2. The total volume of blood obtained from each subject during these two study days was 310 ml.

Before and during the angiotensin II infusions, blood pressure was monitored with an Arteriosonde automatic ultrasonic blood pressure recorder. A blood pressure cuff (with a width approximately two-thirds of the width of the arm and a length such that the bladder completely encircled the arm) was wrapped snugly around the left arm. The Arteriosonde was calibrated daily against a random-zero mercury sphygmomanometer. Control blood samples are expressed as the mean of 15 readings between −60 and −30 min (6:30–7:00 a.m. = −30 control value) and between −30 and 0 min (7:00–7:30 a.m. = zero control value) before administration of any intravenous pharmacologic agents. After the con-

trol period, blood pressure is expressed as the mean of 15 readings for each 30-min period during the angiotensin II infusions, or after metoclopramide or its placebo as the mean of 7 readings for each 15-min period or the mean of 15 readings for each 30-min period as applicable. The change in blood pressure is the difference between the zero control value and the value during each 15- or 30-min study period. Written informed consent for these studies was obtained from all subjects. If the mean blood pressure increase during any peptide infusion was 20 mm Hg, the infusion was terminated; however, this did not occur during these studies.

Synthetic [Asn[1], Val[5]]-angiotensin II (Hypertensin) was purchased from CIBA-Geigy Corp., Pharmaceutical Div. Summit, N. J. Metoclopramide was provided by A. H. Robbins and Co., Richmond, Va. Bromocriptine (CB154) was provided by Sandoz Pharmaceuticals, East Hanover, N. J.

Analytical methods. All blood samples were collected on ice, centrifuged immediately, and the plasma was separated and frozen until time for assay. Samples for plasma renin activity and aldosterone used EDTA as the anticoagulant; heparin was used in the samples for cortisol.

Serum sodium and potassium were measured by flame photometry (model 143, Instrumentation Laboratory, Inc., Lexington, Mass.). Plasma aldosterone was measured by the radioimmunoassay method of Bühler et al. (7). The normal range of plasma aldosterone concentration in ambulatory subjects on a 150-meq sodium intake/day is 5–17 ng/100 ml. Urinary aldosterone excretion was measured by radioimmunoassay of the acid-labile conjugate of aldosterone (8). After incubation, plasma renin activity was determined by radioimmunoassay of angiotensin I generated as described by Sealey et al. (9). Plasma-free 11-hydroxycorticosteroids (11-OHCS)[1] were measured by the fluorometric method of Mattingly (10). The normal range of plasma 11-OHCS concentration in ambulatory subjects is 7–21 μg/100 ml. Serum prolactin was measured by the previously described radioimmunoassay method of Sinha et al. (11).

Statistical analysis. The zero values obtained at 7:30 a.m. for plasma aldosterone and 11-OHCS and serum prolactin concentrations and plasma renin activity were utilized as the control values for statistical comparison. The results are expressed as the mean±1 SE. Statistical analysis was carried out with the double-tailed Student's *t* test for paired data, and *P* values of <0.05 were considered significant.

RESULTS

Characteristics of the subjects before metoclopramide and bromocriptine studies. The characteristics of the same subjects in sodium balance on the 5th d of normal sodium intake (150 meq/day) before each set of experiments are summarized in Table I. The 24-h urinary sodium and potassium excretion closely matched intake. No differences in weight, urinary sodium and potassium excretion, serum sodium, blood pressure, plasma renin activity, or plasma aldosterone concentration were observed before the metoclopramide or bromocriptine studies.

Responses to metoclopramide and angiotensin II infusion (Fig. 1). The administration of metoclopramide was associated with a series of reproducible responses. Plasma aldosterone concentration was 11.3 ±2.5 ng/100 ml in the first 30 min of the control period and decreased to 8.2±2.2 ng/100 ml ($P < 0.005$) in the second 30 min of the control period. At 15 min after administration of metoclopramide, plasma aldosterone concentration increased to 21.0±3.3 ng/100 ml ($P < 0.005$). At 30 and 60 min after metoclopramide, plasma aldosterone concentration decreased slightly to 16.7±2.8 and 16.8±2.1 ng/100 ml, respectively, but remained significantly elevated above baseline values ($P < 0.01$). Control urinary aldosterone excretion during the hour immediately preceeding metoclopramide administration was 1.2±0.4 μg/100 mg creatinine. During the hour after metoclopramide, urinary aldosterone excretion was 2.2±0.4 μg/100 mg creatinine ($P < 0.01$). In response to angiotensin II infusion, plasma aldosterone increased significantly in stepwise fashion to 27.2±3.4, 31.9±5.7, and 36.0 ±6.7 ng/100 ml at each succeeding dose level of angiotensin II.

Serum prolactin concentration was 26.8±4.9 ng/ml at −30 min and decreased to 18.0±4.0 ng/ml ($P < 0.02$) at 0 min. In response to metoclopramide, serum prolactin increased to 91.7±4.0 ng/ml ($P < 0.001$) at 15 min and was 113.0±19.2 ng/ml ($P < 0.005$) at 30 min. At 60 min, serum prolactin decreased slightly to 86.8 ±15.9 ng/ml, but still was elevated above base line ($P < 0.005$). During angiotensin II infusion, the serum prolactin concentration continued to decrease but at the end of 90 min of the angiotensin infusion, prolactin remained elevated at 59.3±12.0 ng/ml ($P < 0.005$).

TABLE I

Characteristics of the Subjects on the 5th Day of Constant Normal Sodium Diet before Metoclopramide and Bromocriptine Studies

	Metoclopramide studies	Bromocriptine studies	*P* value
Weight, *kg*	77.2±4.4	76.9±4.0	NS
24-h Urinary sodium, *meq*	142.5±9.9	154.0±8.1	NS
24-h Urinary potassium, *meq*	62.8±5.1	57.3±4.6	NS
Serum sodium, *meq/liter*	150.3±4.0	156.5±4.3	NS
Blood pressure, *mm Hg*			
Systolic	113.4±5.7	111.6±5.8	NS
Diastolic	72.2±2.9	73.4±3.2	NS
Plasma renin activity, *ng/ml/h*	5.5±1.4	4.0±1.2	NS
Plasma aldosterone, *ng/100 ml*	6.5±2.6	5.2±1.4	NS

[1] *Abbreviation used in this paper*: 11-OHCS, 11-hydroxycorticosteroids.

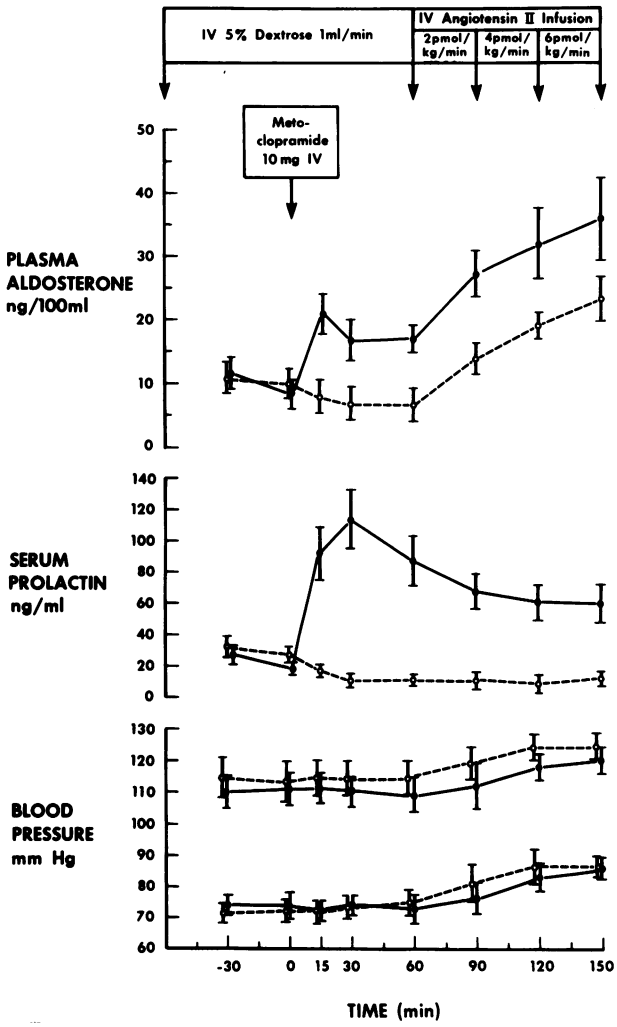

FIGURE 1 Aldosterone, prolactin, and blood pressure responses to metoclopramide (solid line) and to metoclopramide placebo (broken line) of normal subjects (n = 6). Responses to three cumulative doses of intravenous angiotensin II also are shown.

Blood pressure was 110.1±4.8/73.7±3.1 mm Hg in the first 30 min of the control period was 111.4±4.8/72.2±3.6 mm Hg (P = NS) in the second 30 min of the control period. Blood pressure was not changed significantly by metoclopramide administration. As expected, blood pressure increased in stepwise fashion in response to increasing doses of angiotensin II to a value of 119.7±4.2/86.1±2.6 mm Hg ($P < 0.01$) at the completion of the angiotensin II infusion.

Responses to placebo for metoclopramide and angiotensin II infusion (Fig. 1). In the control period, plasma aldosterone concentration was 10.8±2.4 and 10.0±2.4 ng/100 ml (P = NS) at −30 and 0 min, respectively. At 15 min after placebo, plasma aldosterone decreased to 7.9±2.6 ng/100 ml ($P < 0.05$) and thereafter was unchanged for 45 min. Control urinary aldosterone excretion was 1.2±0.4 μg/100 mg creatinine and was unchanged in the hour after placebo administration. With angiotensin II infusion, plasma aldosterone increased significantly to 13.7±2.4, 19.0 ±1.9, and 23.3±3.2 ng/100 ml with each respective dose of angiotensin II. The increment in plasma aldosterone concentration in response to 2 pmol/kg per min of angiotensin II after metoclopramide, 10.3 ±3.2 ng/100 ml, did not differ significantly from the increment in plasma aldosterone with the same dose of angiotensin II after placebo, 7.2±2.1 ng/100 ml.

Serum prolactin concentration was 33.1±6.1 ng/ml at −30 min of the control period and decreased to 26.9±4.8 ng/ml ($P < 0.02$) at 0 min. After placebo, serum prolactin continued to decrease, 16.4±3.1 ng/ml ($P < 0.05$) at 15 min and 11.2±3.5 ng/ml ($P < 0.001$) at 30 and also at 60 min. Thereafter, with angiotensin II infusion, there was no change in serum prolactin concentration.

Blood pressure was 114.5±6.3/71.3±2.7 mm Hg during the first 30 min of the control period and was 113.4±5.7/72.2±2.9 mm Hg during the second 30 min of the control period. Placebo resulted in no significant change in blood pressure from zero control values. However, angiotensin II infusion resulted in a stepwise increase in blood pressure from 114.4±5.0/75.0±3.6 to 124.4±4.5/86.2±3.3 mm Hg ($P < 0.001$) at the final angiotensin dose of 6 pmol/kg per min.

Comparison of factors which may influence aldosterone secretion in the metoclopramide studies. A comparison of some of the factors which may alter aldosterone secretion during the metoclopramide and placebo studies are shown in Table II. Metoclopramide or placebo administration was not associated with any change in serum potassium or plasma 11-OHCS concentration or plasma renin activity. Angiotensin II reproducibly suppressed plasma renin activity to low values after metoclopramide and after placebo ($P < 0.01$). There was no difference between values of serum potassium or plasma 11-OHCS concentration or plasma renin activity in response to angiotensin II infusion after metoclopramide or placebo administration.

Responses to angiotensin II after placebo for bromocriptine (Fig. 2). Plasma aldosterone concentration was 6.9±2.4 and 5.2±1.4 ng/100 ml (P = NS) at −30 and 0 min, respectively. Angiotensin II infusion resulted in a progressive increase in plasma aldosterone concentration to 12.3±1.7, 17.2±2.2, and 21.8 ±3.5 ng/100 ml ($P < 0.01$) with 2, 4, and 6 pmol/kg per min cumulative doses, respectively.

Serum prolactin concentration was 18.7±5.2 ng/ml at −30 min and 13.8±4.9 ng/ml ($P < 0.05$) at 0 min. Serum prolactin did not change significantly in response to angiotensin II.

Blood pressure was 113.3±5.2/74.1±3.6 mm Hg dur-

TABLE II

Comparison of Factors Which May Influence Aldosterone Secretion in the Metoclopramide Studies

	Time, *min*							
						Angiotensin II infusion		
	−30	0	15	30	60	90	120	150
Serum potassium, *meq/liter*								
Placebo	3.9±0.1	3.9±0.1	3.9±0.1	3.9±0.1	3.8±0.1	3.8±0.1	4.1±0.2	4.0±0.1
Metoclopramide	4.0±0.1	4.0±0.1	4.0±0.1	4.0±0.1	4.0±0.1	4.0±0.1	4.1±0.1	4.0±0.1
P value	NS	NS	NS	NS	NS	NS	NS	NS
Plasma 11-OHCS, *μg/100 ml*								
Placebo	33.1±6.4	35.0±4.9	36.3±6.9	26.2±3.8	28.5±2.2	26.0±3.8	28.0±3.7	25.2±3.2
Metoclopramide	33.7±4.9	41.2±6.0	39.0±5.4	41.0±8.3	35.7±4.0	28.0±3.9	28.2±3.4	28.7±5.2
P value	NS	NS	NS	NS	NS	NS	NS	NS
Plasma renin activity, *ng/ml/h*								
Placebo	—	4.3±1.2	4.1±1.4	—	5.5±1.4	—	—	1.7±0.5
Metoclopramide	—	5.2±1.5	4.9±1.8	—	6.0±1.1	—	—	2.9±0.1
P value	—	NS	NS	—	NS	—	—	NS

ing the first 30 min of the control period and 111.6 ±5.8/73.4±3.2 mm Hg (P = NS) during the second 30 min of the control period. Blood pressure thereafter increased in response to angiotensin II infusion. The values were 117.8±5.6/81.3±2.7 mm Hg ($P < 0.005$) at 2 pmol/kg per min, 122.2±5.9/86.1±2.8 mm Hg ($P < 0.001$) at 4 pmol/kg per min and 126.3±6.2/89.0±3.6 mm Hg ($P < 0.001$) at 6 pmol/kg per min of angiotensin II.

Responses to angiotensin II after bromocriptine administration (Fig. 2). Bromocriptine administration did not alter the basal plasma aldosterone concentrations, which were 8.5±3.3 and 7.2±1.0 ng/100 ml (P = NS) at −30 and 0 min, respectively. In response to angiotensin II, plasma aldosterone increased in stepwise fashion to 14.7±3.0 ng/100 ml (P = NS) at 2 pmol/kg per min, 19.8±3.2 ng/100 ml ($P < 0.02$) at 4 pmol/kg per min, 23.4±1.6 ng/100 ml ($P < 0.001$) at 6 pmol/kg per min. There was no significant difference in the increase of plasma aldosterone concentration in response to angiotensin II infusion, whether the subjects previously had received bromocriptine or placebo.

Basal serum prolactin concentrations were decreased significantly in response to bromocriptine ($P < 0.001$). Serum prolactin after bromocriptine administration was <3.0 ng/ml at −30 min and also at 0 min. Serum prolactin was unchanged from the zero control value in response to angiotensin II infusion.

Bromocriptine decreased basal supine blood pressure ($P < 0.001$) compared to the control day. Control blood pressure values after bromocriptine administration were 106.2±6.5/64.4±4.4 mm Hg during the first 30 min and 106.1±6.1/67.8±3.9 mm Hg (P = NS) during the second 30 min. In response to angiotensin II, blood pressure increased progressively to 118.8±5.8/84.1±2.2 mm Hg ($P < 0.01$) at the 6-pmol/kg per min dose. There was no significant difference in the magnitude of the blood pressure increase in response to angiotensin II, whether or not the subjects had received bromocriptine.

Comparison of factors which may influence aldosterone secretion in the bromocriptine studies. In Table III, a comparison of factors which may influence aldosterone in these studies is presented. Serum potassium and plasma 11-OHCS concentrations and plasma renin activity were not altered by bromocriptine or placebo. Angiotensin II infusion was not associated with any change in serum potassium or plasma 11-OHCS concentrations. Angiotensin II reproducibility suppressed plasma renin activity to low levels, but the decrement in renin in response to angiotensin was not influenced significantly by bromocriptine.

DISCUSSION

The present studies were designed to determine the effects of stimulation or blockade of dopamine receptors on the control of aldosterone secretion. Dopamine itself can be administered only by intravenous infusion and further stimulates not only dopamine receptors, but also alpha and beta adrenoreceptors. Dopamine probably also serves as a precursor of

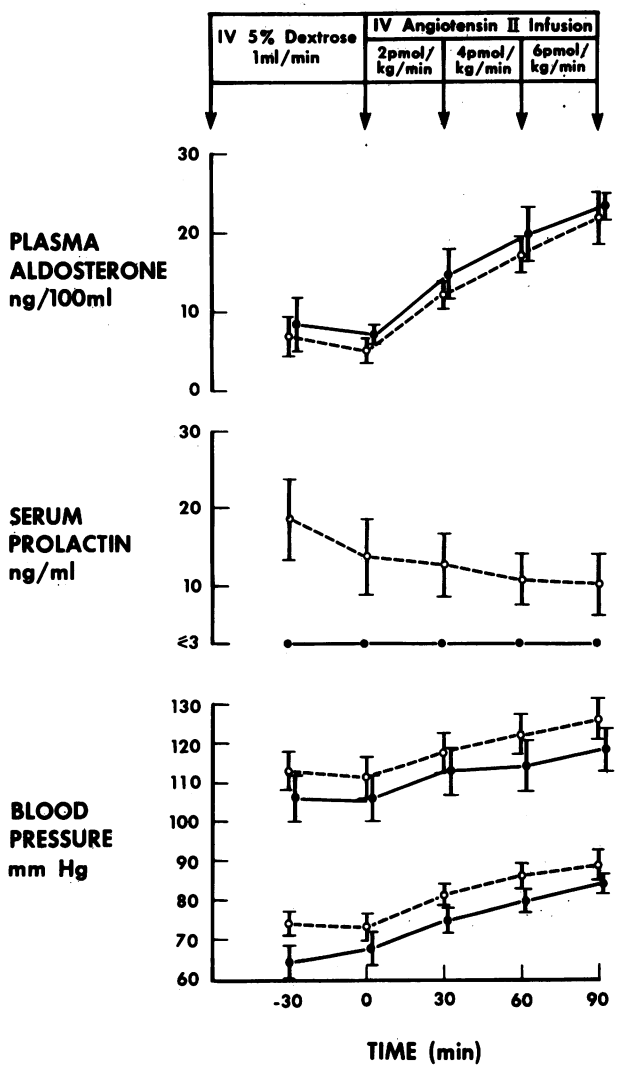

FIGURE 2 Aldosterone, prolactin, and blood pressure responses of normal subjects ($n = 6$) to three cumulative doses of angiotensin II infused during bromocriptine (solid line) and bromocriptine placebo (broken line).

norepinephrine and epinephrine biosynthesis. For these reasons, pharmacological agents were selected which we consider to be relatively specific, with the recognition that no drug is entirely specific.

Bromocriptine was selected as the dopamine receptor stimulant (agonist). It was developed originally as a specific inhibitor of prolactin secretion (12), and only later it was recognized to be a dopamine agonist (13, 14). Bromocriptine has been demonstrated to act as a dopamine agonist in the central nervous system (12, 13), at the pituitary to inhibit prolactin release in vivo and in vitro (15–17) and on peripheral vascular dopamine receptors to produce vasodilitation of renal and mesenteric arteries (18). Furthermore, bromocriptine has been proven to interact directly with dopamine receptors by means of receptor assays in membranes from brain tissue using tritiated dopamine or tritiated haloperidol as ligands (19). Although bromocriptine is recognized to have weak effects on alpha adrenoreceptors and serotonin receptors (14), it is unlikely that these effects would be observed at the dose employed in the present studies.

Metoclopramide, a procaineamide derivative, was selected as the dopamine receptor blocking agent (antagonist) since it appears to be more specific than the other available compounds. Furthermore, it does not produce adverse effects at the dose level employed in this study. Metoclopramide is effective in blocking dopamine receptors in the central nervous system (20), gut (21), and cardiovascular system (22). It also is a potent stimulator of prolactin release in vivo (23, 24) and acts in vitro to antagonize the inhibitory effect of dopamine on prolactin release (17).

The present experiments demonstrate that dopamine mechanisms may exert a tonic inhibitory influence on aldosterone production in man. The efficacy of the two agents on dopamine receptors is indicated by their effects on serum prolactin concentrations: metoclopramide increased and bromocriptine decreased serum prolactin concentrations significantly. Blockade of dopamine receptors by metoclopramide in normal men in the present study resulted in an acute increase in plasma aldosterone concentration approximately 2.5-fold above base line. In contrast, placebo administration was associated with a significant decrement in plasma aldosterone concentration, probably as a result of decreasing stress as the experiments proceeded or normal diurnal variation of aldosterone, or both (serum prolactin and plasma 11-OHCS concentrations also decreased). The rapidity of the increase in plasma aldosterone concentration and the concomitant increase in urinary aldosterone excretion after metoclopramide administration strongly suggests an increase in aldosterone secretion. However, aldosterone secretion rates or metabolic clearance studies, which were not performed because of limitations of the experimental design, would be required to exclude absolutely a decrease in aldosterone metabolism.

We infused angiotensin II during the plateau phase of aldosterone stimulation after metoclopramide to investigate whether metoclopramide would increase adrenocortical sensitivity to angiotensin. However, we were not able to demonstrate any difference in the angiotensin-aldosterone dose-response curves after metoclopramide. Thus, no increase in adrenocortical sensitivity to angiotensin nor enhancement of potency of the octapeptide could be demonstrated acutely in the presence of dopamine receptor blockade. As expected, plasma renin activity declined during angiotensin II infusion. However, the magnitude of

TABLE III

Comparison of Factors Which May Influence Aldosterone Secretion in the Bromocriptine Studies

	Time, *min*				
			Angiotensin II infusion		
	−30	0	30	60	90
Serum potassium, *meq/liter*					
Placebo	3.8±0.1	3.9±0.1	3.9±0.1	4.2±0.2	4.1±0.1
Bromocriptine	3.9±0.2	3.8±0.1	3.9±0.1	3.9±0.2	4.0±0.2
P value	NS	NS	NS	NS	NS
Plasma 11-OHCS, *μg/100 ml*					
Placebo	31.3±4.1	27.7±4.1	25.8±3.1	29.0±3.2	27.5±5.0
Bromocriptine	32.0±6.1	27.3±4.9	27.0±4.1	30.2±4.0	31.0±3.0
P value	NS	NS	NS	NS	NS
Plasma renin activity, *ng/ml/h*					
Placebo	—	4.0±1.0	—	—	2.6±0.4
Bromocriptine	—	6.4±1.9	—	—	1.8±0.3
P value		NS			NS

renin suppression by angiotensin was not influenced significantly by metoclopramide or bromocriptine. Further evidence for a direct action of dopamine at the adrenocortical cellular level is the absence of alteration of any of the known stimuli to aldosterone secretion in this studies. Specifically, metoclopramide administration was not associated with any change in serum potassium ion concentration, plasma renin activity or adrenocorticotrophic hormone, as reflected by plasma 11-OHCS concentration.

The specific dopamine agonist, bromocriptine, neither suppressed basal aldosterone levels nor inhibited the aldosterone response to angiotensin II. In fact, within the limits of allowable pressor effects in man, the full angiotensin II-aldosterone dose-response relationship was duplicated in the presence of bromocriptine. Thus, in man under conditions of normal sodium balance, if aldosterone is under dopaminergic control, it is under maximum tonic dopaminergic inhibition.

Taken together, these findings suggest that dopaminergic mechanisms in the control of aldosterone production in man in normal sodium balance are independent of the renin-angiotensin system, and that these mechanisms act directly at the level of the adrenal zona glomerulosa. Less likely, an alternative possibility is that metoclopramide may act elsewhere (in the central nervous system, for example) resulting in the cessation of release of an inhibitor which acts directly at the adrenal cortex. Denton and co-workers (25) have shown that the act of rapid satiation of salt appetite in sodium-deficient sheep causes inhibition of aldosterone hypersecretion by the autotransplanted adrenal gland without change in plasma concentrations of stimuli known to act directly on the adrenal zona glomerulosa. The authors postulated that a humoral inhibitory agent, released acutely in response to satiation, reduced aldosterone production. In addition, Abraham and colleagues (26) have demonstrated that ventriculo-cisternal perfusion of artificial cerebrospinal fluid of high sodium content resulted in a marked decrease in plasma aldosterone concentration without alteration of the factors known to influence aldosterone secretion. These studies are consistent with the hypothesis that the known factors (angiotensin II, ACTH, and potassium) do not explain all of the changes in aldosterone secretion during sodium deficiency.

Administration of metoclopramide increased the serum prolactin concentration markedly in all subjects studied, and it is possible that the associated increase in aldosterone was mediated by prolactin rather than by dopamine blockade itself. However, several lines of evidence suggest convincingly that prolactin does not stimulate aldosterone secretion acutely. In the present studies, the decrease in serum prolactin concentration in response to bromocriptine was not accompanied by any change in plasma aldosterone concentraton. Serum prolactin concentration did not decrease when aldosterone production was stimulated by angiotensin II; thus, no negative feedback of aldosterone on prolactin secretion could be demonstrated. Administration of thyrotropin-releasing hormone to normal human subjects acutely raises plasma prolactin concentration but has no effect on aldosterone secretion (27). Plasma prolactin concentration does not change with low sodium diet, saline infusion, or upright posture in normal subjects or patients with primary aldosteronism due to idiopathic adrenal hyperplasia (28). Furthermore, in a patient with coexistent prolactin-secreting pituitary micro-

adenoma and primary aldosteronism, plasma aldosterone concentration was unchanged by normalization of the serum prolactin concentration after removal of the microadenoma (28). Previous studies from this laboratory (29) in man have shown no change in plasma aldosterone concentration measured hourly for 6 h after 25–100 mg of ovine prolactin administered intramuscularly resulting in a four to eightfold increase in serum prolactin concentration, comparable to the increase in prolactin observed in the present study. Although these studies with heterologous prolactin do not necessarily exclude an effect of the homologous hormone, to the extent that this method of study is applicable, the results do not support a role for prolactin in the acute regulation of aldosterone secretion.

Two preliminary studies showing contradictory results of the effects of metoclopramide on aldosterone in man have been published previously. Norbiato et al. (30) reported that an intravenous bolus dose (10 mg) of metoclopramide increased plasma aldosterone concentration in dexamethasone-suppressed normal subjects, patients with primary aldosteronism due to idiopathic adrenal hyperplasia and hypophysectomized subjects, without altering plasma 11-OHCS or potassium concentrations or plasma renin activity. However, no placebo control study was performed. On the other hand, Ogihara et al. (31) in a controlled study found that intravenous metoclopramide (5 mg) results in a fivefold rise in serum prolactin concentration without change in plasma aldosterone concentration. Aside from the dose of metoclopramide administered, the factors contributing to the difference between the results of these studies are obscure. Few additional investigations of possible dopaminergic control of aldosterone secretion have been performed, none involving normal subjects under conditions of metabolic balance. Olgaard et al. (32) were unable to inhibit a potassium-induced increase in plasma aldosterone concentration with bromocriptine in patients with renal failure. Similarly, Nilsson and Hökfelt (33) reported no decrease in basal urinary aldosterone excretion after bromocriptine in acromegalic patients. However, these findings are in general agreement with the results of the present study.

In conclusion, the results of these studies suggest that aldosterone production is under maximum tonic inhibition by dopamine in normal man. In this regard, it is interesting that urinary dopamine excretion is related directly to renal sodium excretion. Studies by Alexander et al. (5) have demonstrated that dietary or intravenous sodium repletion in sodium-depleted individuals was associated with a significant increase in urinary dopamine excretion. These changes in urinary dopamine occurred within minutes of extracellular volume expansion. Interpolation of this data leads to the prediction that urinary dopamine excretion should decrease during sodium depletion when aldosterone production is increasing. These studies of urinary dopamine-sodium interrelationships are consistent with the hypothesis that dopamine may play a role in the regulation of aldosterone secretion during sodium depletion. However, the relationship between circulating dopamine and urinary dopamine excretion has not been established. Decreased inhibitory control of aldosterone secretion may constitute an explanation for the failure of several investigators to account totally for the rise in aldosterone secretion during sodium depletion on the basis of increased activity of the renin-angiotensin system (25, 26, 34, 35). Further studies of the effects of dopamine agonists on aldosterone secretion in sodium-deplete man and in vitro studies to identify specific dopamine receptors in the adrenal zona glomerulosa should provide further insight concerning the physiology of dopaminergic mechanisms in the control of aldosterone secretion.

ACKNOWLEDGMENTS

The authors thank Ms. Martha Yancey for technical assistance, Mrs. Julie Mawyer for preparation of the manuscript and Dr. Michael J. Peach for his helpful criticism of the manuscript.

This work is supported by U. S. Public Health Service General Clinical Research Center grant RR-847 and in part by research grant HL-22306.

REFERENCES

1. Thorner, M. O. 1975. Dopamine is an important neurotransmitter in the autonomic nervous system. *Lancet.* **I:** 662–665.
2. Bunag, R. D., I. H. Page, and J. W. McCubbin. 1966. Neural stimulation of release of renin. *Circ. Res.* **19:** 851–858.
3. Gordon, R. D., O. Kuchel, G. W. Liddle, and D. P. Island. 1967. Role of the sympathetic nervous system in regulating renin and aldosterone production in man. *J. Clin. Invest.* **46:** 599–605.
4. Cuche, J. L., O. Kuchel, A. Barbeau, R. Baucher, and J. Genest. 1972. Relationship between the adrenergic nervous system and renin during adaptation to upright posture; a possible role for 3, 4-dihydroxy-phenylethylamine (dopamine). *Clin. Sci. (Oxf.).* **43:** 481–491.
5. Alexander, R. W., J. R. Gill, Jr., H. Yamabe, W. Lovenberg, and H. R. Keiser. 1974. Effects of dietary sodium and of acute saline infusion on the interrelationship between dopamine excretion and adrenergic activity in man. *J. Clin. Invest.* **54:** 194–200.
6. Edwards, C. R. W., P. A. Miall, J. P. Hanker, M. O. Thorner, E. A. S. Al-Dujarli, and G. M. Besser. 1975. Inhibition of the plasma aldosterone response to frusemide by bromocriptine. *Lancet.* **II:** 903–905.
7. Bühler, F. R., J. E. Sealey, and J. H. Laragh. 1974. Radioimmunoassay of plasma aldosterone. *In* Hypertension Manual. J. H. Laragh, editor. Dun-Donnelley Publishing Corp., New York. 655–672.

8. Sealey, J. E., and J. H. Laragh. 1974. Measurement of urinary aldosterone excretion in man. *In* Hypertension Manual. J. H. Laragh, editor. Dun-Donnelley Publishing Corp., New York. 641–654.
9. Sealey, J. E., J. H. Laragh, J. Gertew-Banes, and R. M. Aceto. 1974. The measurement of plasma renin activity in man. *In* Hypertension Manual. J. H. Laragh, editor. Dun-Donnelley Publishing Corp., New York. 621–640.
10. Mattingly, D. 1962. A simple fluorometric method for estimation of free 11-hydroxycorticoids in human plasma. *J. Clin. Pathol. (Lond.).* **15**: 374–379.
11. Sinha, Y. N., F. W. Selby, U. J. Lewis, and W. P. Vanderlaan. 1973. A homologous radioimmunoassay for human prolactin. *J. Clin. Endocrinol. Metab.* **36**: 509–516.
12. Flückiger, E., and H. R. Wagner. 1968. 2-Br-α-Ergokryptin. Beeinflussung von Fertilitat und Laktation bei der Ratte. *Experientia.* **24**: 1130. (Abstr.)
13. Corrodi, H., K. Fuxe, T. Hökfelt, P. Lidbrink, and U. Ungerstedt. 1973. Effect of ergot drugs on central catecholamine neurons. Evidence for stimulation of central dopamine neurons. *J. Pharm. Pharmacol.* **25**: 409–411.
14. Fuxe, K., H. Corrodi, T. Hökfelt, P. Lidbrink, and U. Ungerstedt. 1974. Ergocornine and 2-br-α-ergocryptine. Evidence for prolonged dopamine receptor stimulation. *Med. Biol. (Helsinki).* **52**: 121–132.
15. Besser, G. M., L. Parke, C. R. W. Edwards, I. A. Forsyth, and A. S. McNeilly. 1972. Galactorrhoea: successful treatment with reduction of plasma prolactin levels by brom-ergocryptine. *Br. Med. J.* **3**: 669–672.
16. Flückiger, E., M. Markó, W. Doepfner, and W. Niederer. 1976. Effects of ergot alkaloids on the hypothalamo-pituitary axis. *Postgrad. Med. J.* **52**(Suppl. I): 57–61.
17. Yeo, T., M. O. Thorner, A. Jones, P. J. Lowry, and G. M. Besser. 1979. The effects of dopamine, bromocriptine, lergotrile, and metoclopramide on prolactin release from continuous perfused columns of isolated rat pituitary cells. *Clin. Endocrinol.* In press.
18. Clarke, B. J., G. Scholtysik, and E. Flückiger. 1978. Cardiovascular actions of bromocriptine. *Acta Endocrinol. Suppl. 216.* **88**: 75–81.
19. Goldstein, M., A. Lieberman, A. F. Battista, J. Y. Lew, and Y. Matsumoto. 1968. Experimental and clinical studies on bromocriptine in the Parkinsonian syndrome. *Acta Endocrinol. Suppl. 216.* **88**: 57–66.
20. Peringer, E., P. Jenner, and C. D. Marsden. 1975. Effect of metoclopramide on turnover of brain dopamine, noradrenaline and 5-hydroxytryptamine. *J. Pharm. Pharmacol.* **27**: 442–444.
21. Valenzuela, J. E. 1976. Dopamine as a possible neurotransmitter in gastric relaxation. *Gastroenterology.* **71**: 1019–1022.
22. Day, M. D., and P. R. Blower. 1975. Cardiovascular dopamine receptor stimulation antagonized by metoclopramide. *J. Pharm. Pharmacol.* **27**: 276–278.
23. McNeilly, A. S., M. O. Thorner, G. Volans, and G. M. Besser. 1974. Metoclopramide and prolactin. *Br. Med. J.* **2**: 729. (Abstr.)
24. Delitala, G., A. Masala, S. Alagna, and L. Devilla. 1976. Effect of metoclopramide on serum prolactin levels in humans. *Clin. Endocrinol.* **5**: 731–734.
25. Denton, D. A., J. R. Blair-West, J. P. Coghlan, B. A. Scoggins, and R. D. Wright. 1977. Gustatory-alimentary reflex inhibition of aldosterone hypersecretion by the autotransplanted adrenal gland of sodium deficient sheep. *Acta Endocrinol.* **84**: 119–132.
26. Abraham, S. F., J. R. Blair-West, J. P. Coghlan, D. A. Denton, D. R. Mouw, and B. A. Scoggins. 1976. Aldosterone secretion during high sodium cerebrospinal fluid perfusion of the brain ventricles. *Acta Endocrinol.* **81**: 120–132.
27. Bauman, G., and D. L. Loriaux. 1976. Failure of endogenous prolactin to alter renal salt and water excretion and adrenal function in man. *J. Clin. Endocrinol. Metab.* **43**: 643–649.
28. Holland, O. B., C. E. Gomez-Sanchez, D. C. Kem, M. H. Weinberger, N. J. Kramer, and J. R. Higgins. 1977. Evidence against prolactin stimulation of aldosterone in normal human subjects and patients with primary aldosteronism, including a patient with primary aldosteronism and a prolactin-producing pituitary microadenoma. *J. Clin. Endocrinol. Metab.* **45**: 1064–1076.
29. Carey, R. M., A. J. Johanson, and S. M. Seif. 1977. The effects of ovine prolactin on water and electrolyte excretion in man are attributable to vasopressin contamination. *J. Clin. Endocrinol. Metab.* **44**: 85–88.
30. Norbiato, G., M. Bevilacqua, U. Raggi, P. Micossi, and C. Moroni. 1977. Metoclopramide increases plasma aldosterone in man. *J. Clin. Endocrinol. Metab.* **45**: 1313–1316.
31. Ogihara, T., S. Matsumura, T. Onishi, K. Miyai, T. Uozumi, and Y. Kumahara. 1977. Effect of metoclopramide-induced prolactin on aldosterone secretion in normal subjects. *Life Sci.* **20**: 523–526.
32. Olgaard, K., C. Hagen, S. Madsen, and L. Hummer. 1977. Lack of effect of prolactin inhibition by alpha-bromocriptine (CB154) on plasma aldosterone in anephric and non-nephrectomized patients on regular hemodialysis. *Acta Endocrinol.* **85**: 587–594.
33. Nilsson, A., and B. Hökfelt. 1978. Effect of the dopamine agonist bromocriptine on blood pressure, catecholamines and renin activity in acromegalics at rest, following exercise and during insulin-induced hypoglycemia. *Acta Endocrinol. Suppl. 216.* **88**: 83–98.
34. Lowenstein, J., G. W. Boyd, A. E. Rippon, V. H. T. James, and W. S. Peart. 1972. Increased aldosterone in response to sodium deficiency in the angiotensin II-immunized rabbit. *In* Hypertension 1972. J. Genest and E. Koiw, editors. Springer-Verlag New York, Inc., New York. 481–489.
35. Burwell, L. R., W. W. David, and F. C. Bartter. 1969. Studies on the loci of action of stimuli to the biogenesis of aldosterone. *Proc. Roy. Soc. Med.* **62**: 1254–1257.