Role of Dopamine in the Inhibition of Vasopressin Secretion by L-Dopa in Carbidopa-Treated Dogs

IAN A. REID, LANCE CHOU, DING CHANG, AND LANNY C. KEIL

SUMMARY Elevation of brain catecholamine levels by systemic administration of L-dopa in dogs pretreated with the dopa decarboxylase inhibitor carbidopa inhibits the secretion of vasopressin and adrenocorticotropic hormone (ACTH) and decreases arterial blood pressure. The aim of the present study was to determine 1) whether the inhibition of vasopressin secretion is mediated by dopamine or norepinephrine, both of which have been implicated in the control of vasopressin secretion, and 2) whether the decrease in vasopressin secretion contributes to the suppression of ACTH secretion and fall in blood pressure produced by L-dopa. This was accomplished by comparing the effects of dopamine and α-adrenergic receptor antagonists on vasopressin, ACTH, and blood pressure responses to L-dopa. The effect of a specific antagonist of the vasoconstrictor action of vasopressin also was studied. Injection of L-dopa (20 mg/kg i.v.) in dogs pretreated with carbidopa (20 mg/kg i.v.) caused reductions in plasma vasopressin concentration (from 16.0 ± 4.8 to 3.8 ± 0.9 pg/ml; p<0.05), plasma ACTH concentration (from 96.0 ± 20.4 to 49.2 ± 10.0 pg/ml; p<0.05), and mean arterial pressure (from 121 ± 6 to 78 ± 5 mm Hg; p<0.05). Pretreatment with pimozide (1 mg/kg i.p.) completely blocked the inhibition of vasopressin secretion by L-dopa but failed to block the suppression of ACTH secretion (57.6 ± 11.8 to 34.0 ± 5.1 pg/ml; p<0.05) or the decrease in mean arterial pressure (126 ± 5 to 93 ± 7 mm Hg; p<0.05). The antihypertensive effect of L-dopa was reduced by prazosin (97 ± 3 to 82 ± 6 mm Hg; p<0.05) and blocked by yohimbine, but neither drug blocked the suppression of plasma vasopressin or ACTH. Administration of a vasopressin antagonist did not decrease arterial pressure and caused only a small, delayed reduction in plasma ACTH concentration. These studies provide evidence that the inhibition of vasopressin secretion by L-dopa is mediated by dopamine rather than by norepinephrine. The failure of a vasopressin antagonist to significantly decrease blood pressure or ACTH secretion and the dissociation of the effects of L-dopa on vasopressin release, blood pressure, and ACTH secretion argue against a major role for vasopressin in the blood pressure and ACTH responses to L-dopa. (Hypertension 8: 890–896, 1986)

KEY WORDS • catecholamines • vasopressin release • dopamine • brain • adrenocorticotropic hormone secretion • blood pressure • vasopressin antagonist • pimozide • prazosin • yohimbine

It is now generally accepted that brain catecholamines are involved in the regulation of vasopressin secretion. The supraoptic and paraventricular nuclei are innervated by adrenergic neurons, and depletion of catecholamines with 6-hydroxydopamine impairs the release of vasopressin in response to a variety of stimuli.

From the Department of Physiology (I. A. Reid and L. Chou), University of California, San Francisco, Peninsula Laboratories (D. Chang), Belmont, and the Ames Research Center (L. C. Keil), Moffett Field, California.

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Address for reprints: Dr. Ian A. Reid, Department of Physiology, S-762, University of California, San Francisco, CA 94143.

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cholamine precursor l-dopa, which can cross the blood-brain barrier, in dogs in which peripheral but not central dopa decarboxylase had been inhibited by intravenous administration of carbidopa suppressed vasopressin release and caused a diuresis.\(^9\) This inhibition of vasopressin release was particularly impressive since it occurred in association with a marked fall in arterial pressure, which would normally stimulate vasopressin secretion.\(^9\) These studies provided additional evidence for a potent inhibitory effect of brain catecholamines on the release of vasopressin. However, no attempt was made to determine if the mediator was dopamine or norepinephrine, both of which have been implicated in the control of vasopressin secretion. The major aim of the present investigation was to distinguish between these two possibilities by studying the effects of dopamine and \(\alpha\)-adrenergic receptor blocking drugs on the vasopressin response to l-dopa.

In addition to decreasing vasopressin secretion, l-dopa produces marked reductions in arterial blood pressure\(^21-23\) and adrenocorticotropic hormone (ACTH) secretion.\(^24\) The reduction in blood pressure has been attributed to an increase in vagal tone and a decrease in sympathetic neural activity.\(^22,23\) but to our knowledge, the possibility that the decrease in vasopressin release also contributes has not been investigated. This is an attractive possibility because it is now known that vasopressin is a potent vasoconstrictor that plays an important role in blood pressure regulation.\(^25,28\) Moreover, it has recently been reported that part of the antihypertensive effect of clonidine is due to inhibition of vasopressin release.\(^27\) To test the possibility that the hypotensive action of l-dopa results in part from inhibition of vasopressin release, the effect on blood pressure of a vasopressin antagonist, which is known to block the cardiovascular actions of vasopressin, was compared with that of l-dopa. This antagonist was also used to determine if the inhibition of ACTH secretion by l-dopa also results from the inhibition of vasopressin release.

Materials and Methods

The experiments were performed in a total of 25 male and female mongrel dogs weighing 14 to 30 kg. The animals were fed a diet that provided approximately 70 mEq sodium per day and received their last food on the day before the experiment. They were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and allowed to breathe spontaneously. A femoral artery and vein were cannulated to allow measurement of arterial pressure and heart rate, collection of arterial blood samples, and administration of drugs. Experiments were started approximately 60 minutes after completion of the operation when blood pressure and heart rate had stabilized.

The experiments were performed according to the following five protocols.

Effects of l-Dopa and Carbidopa

A sample of arterial blood was collected in five dogs, and then carbidopa, 20 mg/kg, was infused intravenously over 1 minute. Twenty minutes later, another blood sample was collected and then l-dopa, 20 mg/kg, was infused intravenously over 1 minute. Blood samples were collected every 20 minutes for the next 140 minutes.

Effects of l-Dopa and Carbidopa Following Prazosin

Immediately following induction of anesthesia in five dogs, the dopamine antagonist pimozide was injected intraperitoneally in a dose of 2 mg/kg. Carbidopa and l-dopa subsequently were administered as described in Protocol 1.

Effects of l-Dopa and Carbidopa Following Yohimbine

Following induction of anesthesia in five dogs, the \(\alpha\)-adrenergic receptor antagonist yohimbine was injected intraperitoneally in a dose of 2 mg/kg. Carbidopa and l-dopa subsequently were administered as described in Protocol 1.

Effects of l-Dopa and Carbidopa Following Prazosin

Following induction of anesthesia in five dogs, the \(\alpha\)-adrenergic receptor antagonist prazosin was injected intraperitoneally in a dose of 2 mg/kg. Carbidopa and l-dopa subsequently were administered as described in Protocol 1.

Effects of Vasopressin Blockade

Sixty minutes after operation in five dogs, the vasopressin V\(_1\), receptor antagonist d(CH\(_2\)_3)Tyr(Me) arginine vasopressin was injected intravenously in a dose of 10 \(\mu\)g/kg. This dose of the antagonist blocks both the cardiovascular\(^29\) and the ACTH-releasing\(^29,30\) actions of vasopressin.

Measurements and Drugs

Blood pressure and heart rate were monitored continuously using a Statham transducer (Oxnard, CA, USA) and Grass polygraph (Quincy, MA, USA). In addition, 10-ml samples of arterial blood were collected and replaced with an equal volume of isotonic saline. The blood was centrifuged and the plasma frozen until analysis. Plasma vasopressin concentration was measured by an established radioimmunoassay procedure.\(^31\) Plasma ACTH concentration was measured using a radioimmunoassay kit (Immunonuclear, Stillwater, MN, USA). Plasma cortisol concentration was measured by radioimmunoassay\(^22\) and used as an additional index of changes in ACTH secretion. In the experiments with the vasopressin antagonist, plasma renin activity was measured by radioimmunoassay as described previously.\(^33\) Plasma renin activity is expressed as nanograms of angiotensin I formed per milliliter of plasma during a 3-hour incubation. Plasma osmolality was measured by freezing point depression using unfrozen plasma.

The l-dopa was obtained from Sigma (St. Louis, MO, USA), and carbidopa was generously provided by Merck Sharp & Dohme (Rahway, NJ, USA). Both compounds were dissolved in 1 N HCl (100 mg/ml) and diluted in 15 ml of isotonic saline before injection.
Pimozide was obtained from Janssen Pharmaceutical (Beerse, Belgium) and dissolved in 3 to 4 ml of 1 N tartaric acid. Yohimbine (Sigma) was dissolved in 2 ml of ethanol, and prazosin (Pfizer, Brooklyn, NY, USA) was dissolved in 1 ml of ethanol. The blocking drugs were diluted in saline before injection.

Statistics

All results are presented as the mean ± SE. Statistical evaluation of the data was performed using one-way analysis of variance for repeated measures. Comparisons between values obtained at specific time points and the control values were made using the Newman-Keuls test.

Results

Effects of L-Dopa and Carbidopa

The effects of administration of L-dopa following pretreatment with carbidopa are summarized in Figure 1. There was a prompt fall in mean arterial pressure from 121 ± 6 to 78 ± 5 mm Hg at 20 minutes (p<0.05). During the next 120 minutes, mean arterial pressure increased to values not significantly different from the control value. Heart rate decreased from 128 ± 11 to 103 ± 8 beats/min at 60 minutes (p<0.05) and remained low for the remainder of the experiment. Plasma vasopressin concentration decreased from 16.0 ± 4.8 to 3.8 ± 0.9 pg/ml at 40 minutes (p<0.05) and remained suppressed at approximately 5 pg/ml. Plasma ACTH concentration decreased from 96.0 ± 20.4 to 49.2 ± 10.0 pg/ml at 40 minutes (p<0.05) and remained suppressed at approximately 50 pg/ml. The decrease in plasma ACTH concentration was paralleled by a decrease in plasma cortisol concentration, the mean value decreasing from 8.0 ± 1.0 to 3.6 ± 0.6 μg/dl at 60 minutes (p<0.05).

Effects of L-Dopa and Carbidopa Following Pimozide

The effects of L-dopa and carbidopa in dogs pretreated with pimozide are shown in Figure 2. The decreases in mean arterial pressure and heart rate were similar to those in dogs that did not receive pimozide. Plasma vasopressin concentration in the pimozide-treated dogs was approximately double that in the untreated dogs. Pimozide blocked the suppression of plasma vasopressin concentration by L-dopa; indeed, plasma vasopressin concentration actually increased from 29.9 ± 6.9 to 53.5 ± 12.5 pg/ml at 40 minutes (p<0.05). Plasma vasopressin concentration then decreased to values not significantly different from the control value. Plasma ACTH concentration fell progressively following administration of L-dopa, decreasing from 57.6 ± 11.8 to a minimum value of 34.0 ± 5.1 pg/ml at 120 minutes (p<0.05). At the same time, plasma cortisol concentration decreased from 9.0 ± 0.9 to 4.8 ± 1.0 μg/dl (p<0.05).

Effects of L-Dopa and Carbidopa Following Yohimbine

The effects of yohimbine on the responses to L-dopa are summarized in Figure 3. Yohimbine blocked the hypotensive and bradycardic actions of L-dopa; indeed, in the presence of yohimbine, L-dopa caused progressive increases in mean arterial pressure and heart rate. In contrast, the suppression of plasma vasopressin and ACTH concentration by L-dopa were unaffected by yohimbine; plasma vasopressin concentration decreased from 31.0 ± 11.7 to 9.6 ± 2.8 pg/ml at 80 minutes (p<0.05), and plasma ACTH concentration decreased from 162.4 ± 28.9 to 79.9 ± 10.9 pg/ml (p<0.05). Plasma cortisol concentration decreased from 10.9 ± 1.3 to 7.8 ± 1.4 μg/dl (p<0.05).

Effects of L-Dopa and Carbidopa Following Prazosin

The effects of prazosin on the responses to L-dopa are summarized in Figure 4. Following injection of L-dopa, mean arterial pressure decreased from 97 ± 3
INHIBITION OF VASOPRESSIN SECRETION BY L-DOPA/Reid et al.

Effects of Vasopressin Blockade

The effects of the vasopressin V1 receptor antagonist are summarized in Figure 5. Mean arterial pressure decreased in two of the five dogs, but overall there was no significant change. Heart rate was elevated at all times following injection of the antagonist, the maximum increase being from 131 ± 11 to 148 ± 13 beats/min at 60 minutes (p < 0.05). There were no significant changes in plasma ACTH (see Figure 5) or cortisol concentrations. Plasma renin activity increased in three of the five dogs, but overall there was no statistically significant change. Plasma vasopressin concentration in this group of dogs averaged 24.9 ± 8.8 pg/ml. Plasma vasopressin could not be measured following injection of the antagonist because this compound cross-reacts with the antibody used in the vasopressin radioimmunoassay.

Discussion

These results confirm that administration of L-dopa in carbidopa-treated dogs causes a marked and sustained suppression of vasopressin secretion. Plasma vasopressin concentration was reduced from 16 to 4 pg/ml within 15 minutes, and since the half-life of vasopressin in the dog is 5 to 8 minutes, this indicates that there must have been almost complete inhibition of vasopressin secretion. This decrease occurred despite marked hypotension, normally a stimulus to the release of vasopressin. Thus, L-dopa must exert a very potent inhibitory effect on vasopressin secretion.

to 82 ± 6 mm Hg at 40 minutes (p < 0.05), but this decrease rapidly reversed. Prazosin prevented the bradycardic action of L-dopa but did not block the suppression of plasma vasopressin, ACTH (see Figure 4), or cortisol (from 7.5 ± 1.6 to 3.7 ± 0.6 µg/dl, p < 0.05).

Plasma osmolality in these dogs averaged 298 mosm/kg. None of the drug treatments caused significant changes in plasma osmolality.
The inhibition of vasopressin release by L-dopa was blocked by the dopamine receptor antagonist pimozide. Indeed, in the presence of pimozide, L-dopa produced a significant increase in plasma vasopressin concentration. The mechanism of this increase was not determined. It may have resulted from a stimulatory effect of central catecholamines acting on α-adrenergic or other receptor types or from the accompanying decrease in arterial pressure. Resting vasopressin levels generally were higher in the pimozide-treated dogs, possibly reflecting blockade of a tonic inhibitory effect of dopamine on vasopressin release.

Resting vasopressin levels were also higher following treatment with the α-adrenergic blocking drugs yohimbine and prazosin, possibly because blood pressure in these two groups of dogs was lower than in the untreated dogs. Neither yohimbine nor prazosin blocked the inhibition of vasopressin release by L-dopa. Taken together, these results indicate that the inhibition of vasopressin release by administration of L-dopa in carbidopa-treated dogs is mediated by dopamine rather than by norepinephrine.

Other investigators have studied the effects of dopamine on vasopressin release. Conflicting results have been obtained; different investigators have variously reported inhibition, stimulation, both or neither. These discrepancies undoubtedly result from differences in animal species and experimental design. The present results provide additional evidence that dopamine exerts an inhibitory effect on vasopressin release. The extent to which dopamine is involved in the physiological regulation of vasopressin release remains to be defined; however, previous studies have implicated dopamine in the vasopressin response to head-up tilt, angiotensin II infusion, and osmotic stimulation.

No attempt was made in the present study to determine the site at which dopamine acted to inhibit vasopressin release. Since carbidopa does not enter the brain, the site of action is presumably within the blood-brain barrier. Possible sites include supraoptic and paraventricular nuclei.
In agreement with previous reports,21-23 administration of L-dopa in carbidopa-treated dogs produced a marked decrease in blood pressure together with a bradycardia. These changes were unaffected by pre-treatment with pimozide but were reduced by prazosin and blocked by yohimbine. These results suggest that the hypotensive and bradycardic actions of L-dopa are mediated by stimulation of central α1-adrenergic receptors, and again, this conclusion is consistent with previous reports.6,52-23

The decreases in blood pressure and heart rate produced by L-dopa are thought to result from a reduction in sympathetic neural activity and an increase in cardiac vagal activity.22,23 It is also possible that the marked reduction in plasma vasopressin concentration produced by L-dopa contributes to the antihypertensive effect. This seems an attractive possibility because it has been established that vasopressin is a potent vasoconstrictor that plays an important role in the short-term regulation of arterial pressure.25,26 In addition, it was recently reported that part of the antihypertensive action of clonidine results from inhibition of vasopressin release.27 However, the hypotensive and vasopressin-lowering actions of L-dopa could be clearly dissociated in the present study. Pimozide blocked the suppression of vasopressin release but not the hypotensive action, whereas prazosin or yohimbine reduced or abolished the hypotensive action without reducing the inhibition of vasopressin release. Furthermore, injection of a specific antagonist of the vasoconstrictor action of vasopressin in another group of dogs failed to decrease arterial pressure. Interpretation of this latter observation is complicated by the fact that heart rate and plasma renin activity usually increased following injection of the antagonist, and this may have prevented blood pressure from falling. Nevertheless, taken together, the present results fail to provide evidence for a role of decreased vasopressin release in the hypotensive action of L-dopa.

In previous studies in this laboratory, it was shown that administration of L-dopa in carbidopa-treated dogs inhibited the secretion of ACTH.24 The inhibition was blocked by phenoxybenzamine but not by pimozide or phentolamine. The results of the present study generally agree well with the previous findings. The L-dopa produced marked decreases in ACTH and cortisol concentrations. These decreases were not blocked by pimozide, nor by yohimbine or prazosin. Further studies are required to characterize the receptors that mediate the suppression of ACTH secretion by L-dopa.

A possibility that has not been investigated previously is that the suppression of ACTH secretion by L-dopa results, in part, from the inhibition of vasopressin release. This possibility seemed worth considering because vasopressin is an important corticotropin releasing factor30 and because of the close parallel between the effects of L-dopa on vasopressin and ACTH release (see Figure 1). However, the effects of L-dopa on vasopressin and ACTH release were dissociated by pimozide, which blocked the inhibition of vasopressin release but not that of ACTH (see Figure 2). Moreover, injection of a vasopressin antagonist that blocks the stimulation of ACTH release by vasopressin29,30 produced only a small and delayed reduction in plasma ACTH concentration. Therefore, it appears unlikely that the suppression of ACTH secretion by L-dopa results from inhibition of vasopressin secretion.

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