Hypotensive Effect of Clonidine during Sodium Depletion in the Rat

By Donald T. Pals

ABSTRACT

Clonidine was nonhypotensive in conscious unrestrained rats maintained on a normal sodium intake. In contradistinction, clonidine caused a dose-related hypotension in conscious unrestrained rats subjected to sodium depletion via furosemide. The plasma renin activity of normal and sodium-depleted rats was reduced after the administration of clonidine (100 μg/kg, iv) by 22.8% and 34.4%, respectively. Intravenous infusion of an angiotensin II antagonist, 1-Sar-8-Ala-angiotensin II, caused a significant reduction of arterial blood pressure in sodium-depleted rats but not in normal rats. Similarly, bilateral nephrectomy reduced arterial blood pressure and completely abolished the hypotensive effect of clonidine in sodium-depleted rats. Subcutaneous administration of chlorisondamine caused a significantly greater reduction of arterial blood pressure in sodium-depleted rats than it did in normal rats. Treatment of normal and sodium-depleted rats with 6-hydroxydopamine reduced the arterial blood pressure of both groups to approximately 85 mm Hg and completely abolished the hypotensive effect of clonidine in the sodium-depleted rats. The data presented in this paper are consistent with the conclusion that clonidine acts at some site in the sympathetic nervous system of sodium-depleted rats to inhibit renal nerve activity with a resultant suppression of renin secretion and a reduction of the angiotensin II-maintained arterial blood pressure. A similar sequence of events occurring in normal rats would not result in hypotension because their arterial blood pressure is not maintained by angiotensin II.

Clonidine is an effective antihypertensive agent, but its mechanism of action is not completely understood. The hypotension and bradycardia that characterize its effects are thought to be due predominantly to a decrease in the activity of the sympathetic nervous system resulting from an action of clonidine at a site in the central nervous system (1). Substantial evidence has accumulated indicating that clonidine's central actions are mediated via central alpha receptors (2). In addition, recent evidence suggests the involvement of these central alpha receptors in a clonidine-induced inhibition of transmitter release from central monoamine neurons (3). A few studies have shown that clonidine can also depress peripheral sympathetic nerve function directly (4, 5), and it has been proposed that this peripheral action of clonidine is due to a stimulation of presynaptic alpha receptors on adrenergic neurons (6). It appears that the action of clonidine on central monoamine neurons is analogous to its effect on peripheral neurons; in fact, one mechanism may account for both the central and the peripheral neuronal actions of clonidine (6).

Several investigators (7-9) have demonstrated that clonidine, in addition to causing a reduction of several cardiovascular parameters, also suppresses renin secretion. Reid et al. (10) have provided evidence that the suppression of renin secretion by clonidine results from a centrally mediated decrease in renal sympathetic neural tone. However, they noted that the hypotensive action of clonidine cannot be due entirely to inhibition of renin secretion since blood pressure was reduced in their experiments regardless of whether the plasma renin activity was suppressed.

The intent of the present study was threefold. First, experiments were carried out to determine if clonidine causes a greater hypotension in rats subjected to sodium depletion via a diuretic than it does in rats maintained in normal sodium balance. The premise for these experiments was derived from the clinical observation that clonidine's antihypertensive efficacy is increased approximately twofold when it is combined with a diuretic (11).

Second, experiments were carried out to test the postulate that suppression of renin secretion can be a significant aspect of clonidine's hypotensive effect under conditions in which renin via angiotensin II has an important role in the maintenance of blood pressure, a situation that exists in the sodium-depleted rat (12, 13). Finally, experiments were carried out in an attempt to partially elucidate the mechanism of clonidine's hypotensive effect in the sodium-depleted rat.
Methods

Male Sprague-Dawley rats weighing 300-400 g were utilized throughout this investigation. Rats that had received Purina Laboratory Chow and tap water ad libitum for at least 2 weeks prior to an experiment were considered to be normal. Rats that had received an intraperitoneal injection of 100 mg/kg of furosemide (Lasix, Hoechst) 24 hours prior to an experiment and had subsequently received a low-sodium diet (ICN Nutritional Biochemicals) (14) with deionized water ad libitum were considered to be sodium depleted. Rats of the latter group consistently lost 4-8% of their body weight during the initial 6 hours after the furosemide injection; weight loss during the subsequent 18 hours was negligible. The decrease in body weight following the furosemide injection coincided with the pronounced natriuresis already documented (15) as occurring in rats and was utilized as an indication of furosemide’s efficacy and hence of sodium depletion. For comparative purposes, seven 250-g rats received only a low-sodium diet and deionized water ad libitum for 5 weeks prior to study to cause a depletion of sodium which was not furosemide induced.

Bilateral nephrectomy was performed under ether anesthesia in some of the normal and sodium-depleted rats (6 hours after the furosemide injection) 18 hours prior to an experiment. During the postoperative period, the rats were deprived of both food and water. Some normal and sodium-depleted rats were given (6 hours after the furosemide injection) a single intracardiac injection of 100 mg/kg of 6-hydroxydopamine (2,4,5-trihydroxyphenethylamine hydrochloride, Calbiochem) under ether anesthesia 18 hours prior to an experiment. The 6-hydroxydopamine was prepared in 5% dextrose containing 1 mg/ml of ascorbic acid. The effects of this 6-hydroxydopamine treatment regimen have been intensively studied and described (16, 17).

At the time of the experiments, the rats were anesthetized with ether. A median ventral neck skin incision was made, and the left carotid artery and the right jugular vein were isolated. The two blood vessels were cannulated with polyethylene tubing (PE-50), and the tubes were routed subcutaneously to the back of the neck where they were exteriorized through a small skin puncture. The ventral neck skin incision was closed with wound clips. Each rat was placed in a large glass battery jar and allowed to recover from the anesthesia for at least 1 hour before the initiation of an experiment. The cannulas were loosely draped over the neck of the glass jar and were long enough so that the rat’s movements were unrestrained. The carotid artery cannula, previously filled with heparin solution (1000 units/ml), was connected to a Statham P23D transducer fixed outside the glass jar at the rat’s heart level. The jugular vein cannula, previously filled with 5% dextrose solution, allowed the intravenous injection or infusion of clonidine (Catapres, Boehringer Ingelheim) or 1-Sar-8-Ala-angiotensin II (Bachem). Chlorisondamine (Ecolid, Ciba-Geigy) dissolved in 5% dextrose solution was administered by subcutaneous injection.

Results

Intravenous administration of clonidine to conscious unrestrained normal rats caused a dose-dependent hypertensive effect and a dose-dependent bradycardia (Fig. 1). Clonidine at 10 μg/kg caused a small transient hypotensive effect. Higher doses of clonidine did not cause detectable hypertension. Clonidine at 3 μg/kg was essentially without effect either on blood pressure or heart rate, i.e., an effect, if present, was concealed by the normal variability encountered in experiments with conscious unrestrained animals.

Following intravenous administration of clonidine, conscious sodium-depleted rats exhibited not only a dose-dependent bradycardia and a dose-dependent hypertensive effect (33- and 100-μg/kg doses only) but also a dose-dependent hypotension that was evident as far as both the magnitude and the duration of the effect were concerned (Fig. 2). Clonidine at 3 μg/kg was essentially without effect on heart rate but did cause a hypotensive effect that was smaller in magnitude and shorter

![Figure 1](https://example.com/figure1.png)  
*Mean arterial blood pressure and heart rate as a function of time in conscious normal rats. Each symbol represents the results (mean ± SE) from 6-7 rats.*

Circulation Research, Vol. 37, December 1975
in duration than the effect shown in Figure 2 for the 10-μg/kg dose.

The blood pressures of the sodium-depleted rats during the control period, as a group, did not differ significantly from those of the normal group (118.5 ± 0.69 vs. 118.3 ± 0.76 mm Hg, P > 0.05). As a group, the heart rates of the sodium-depleted rats during the control period (381 ± 4.4 beats/min) were significantly (P < 0.05) greater than those of their normal counterparts (347 ± 3.7 beats/min). Except for one set of determinations (5 minutes after the 10-μg/kg dose of clonidine), the heart rates of the sodium-depleted rats following clonidine administration did not differ significantly (P > 0.05) from the corresponding heart rates of normal rats following the same dose of clonidine (compare Figs. 1 and 2).

Conscious unrestrained rats maintained on a low-sodium diet and demineralized water for 5 weeks exhibited blood pressures and heart rates during the control period that were not significantly different from those of the sodium-depleted rats. Intravenous administration of clonidine at 100 μg/kg to these diet-sodium-depleted rats resulted in transient hypertensive and prolonged negative chronotropic effects which were also not significantly different from those observed in the rats that were sodium depleted via furosemide injection. The hypotensive effects of clonidine at 100 μg/kg in the diet-sodium-depleted rats were however only about 60% as great as those shown in Figure 2 for the rats that were sodium depleted via furosemide injection.

The control plasma renin activity of normal rats was 20.0 ± 1.16 ng/ml hour⁻¹ (N = 6), and the control plasma renin activity of sodium-depleted rats was 56.4 ± 5.72 ng/ml hour⁻¹ (N = 6). At 105 minutes postclonidine (100 μg/kg, iv) when an apparent steady-state hypotensive effect had been attained in the sodium-depleted rats, the plasma renin activity of these animals was significantly (P < 0.05) reduced by 34.4% to 37.0 ± 4.25 ng/ml hour⁻¹ (N = 6). Similarly, during the same time interval postclonidine (100 μg/kg, iv), the plasma renin activity of normal rats was significantly (P < 0.05) reduced by 22.8% to 15.4 ± 0.58 ng/ml hour⁻¹ (N = 6).

Infusions of 1-Sar-8-Ala-angiotensin II, an angiotensin II antagonist (19), significantly (P < 0.05) reduced the blood pressure of sodium-depleted rats but not that of normal rats, as shown in Figure 3. The infusion rate of 1-Sar-8-Ala-angiotensin II utilized (30 μg/kg min⁻¹) was adequate to inhibit the blood pressure responses to 1 μg/kg of angiotensin II administered intravenously by 98% in both groups of rats. Figure 3 again illustrates the pronounced tachycardia observed in sodium-depleted rats compared with normal rats. It should be noted that the tachycardia of the sodium-depleted rats was not greatly affected by the infusion of 1-Sar-8-Ala-angiotensin II even though the blood pressures of these rats were reduced during the infusion interval. On termination of the infusion, however, when the blood pressures of the sodium-depleted rats were returning to the control level, a rather abrupt bradycardia occurred. This bradycardia was not apparent in the normal rats. Infusions of 1-Sar-8-Ala-angiotensin II (30 μg/kg min⁻¹, iv) to

![Figure 2](image_url)

**FIGURE 2**

*Mean arterial blood pressure and heart rate as a function of time in conscious sodium-depleted rats. Each symbol represents the results (mean ± SE) from 6-7 rats.*
five additional sodium-depleted rats reduced their blood pressures from a control value of 121.0 ± 1.70 mm Hg to 92.0 ± 7.20 mm Hg at 45 minutes. Subsequent administration of clonidine (100 µg/kg, iv) while the angiotensin II-antagonist infusion was being continued caused only a transient hypertensive phase. There was no apparent hypotensive phase; 90 minutes postclonidine blood pressure averaged 87.6 ± 5.68 mm Hg, and 180 minutes postclonidine it averaged 89.2 ± 3.20 mm Hg. Neither of these postclonidine blood pressure values was significantly different from the preclonidine value obtained during the 1-Sar-8-Ala-angiotensin II infusion.

The control blood pressures of sodium-depleted rats were substantially reduced following bilateral nephrectomy, an effect that did not occur to any great extent in normal rats (Fig. 4). The heart rates of the two groups of rats did not differ significantly (P > 0.05) during the control period. Intravenous administration of clonidine at 100 µg/kg caused a prolonged hypertensive response without any evidence of a hypotensive effect occurring in either group. The bradycardia following clonidine treatment was significantly (P < 0.05) less in the sodium-depleted rats than it was in the normal rats at all but two of the sampling intervals shown in Figure 4.

Subcutaneous administration of the ganglionic blocker, chlorisondamine, caused a significantly greater hypotensive effect in sodium-depleted rats than it did in normal rats (Fig. 5). Paradoxically, the tachycardia which occurred after chlorisondamine administration was better maintained in the normal rats than it was in the sodium-depleted rats, which subsequently actually presented a slight bradycardia. In this particular series of experiments, the control heart rates of the sodium-depleted rats were not significantly greater than those of the normal rats.

Figure 6 shows the effect of clonidine (100 µg/kg, iv) on the blood pressure and heart rate of normal and sodium-depleted rats that had been treated with 6-hydroxydopamine. Neither the blood pressures nor the heart rates of the two groups differed significantly from each other during the control period or, for that matter, throughout most of the experimental period following the administration of clonidine.

**Discussion**

The evaluation of clonidine in conscious unrestrained rats maintained on a normal sodium intake indicated that intravenously administered clonidine was relatively ineffective as a hypotensive agent. Under these experimental conditions, clonidine did cause transient dose-dependent hypertensive responses and prolonged dose-dependent negative chronotropic effects. These results are at variance with those of previous studies (20-22) which have shown that intravenously administered clonidine in the same range of doses as those utilized in the present study causes not only transient hypertensive responses and negative chronotropic effects but also pronounced hypotension in normotensive rats. The latter investigations were all carried out in anesthetized rats. Thus, although the type of anesthetic utilized did not appear to affect the final results obtained in those
Effect of 100 µg/kg of clonidine administered intravenously on mean arterial blood pressure and heart rate of conscious 6-hydroxydopamine-treated normal (solid circles) and sodium-depleted (open circles) rats. Each symbol represents the results (mean ± SE) from 6-8 rats. The crosses denote a significant difference (P < 0.05) between the corresponding values at each time interval.

studies, it does appear that anesthesia in general exerts a profound influence on the blood pressure response of the rat to clonidine.

Conscious unrestrained sodium-depleted (via furosemide) rats responded to the intravenous administration of clonidine with a dose-dependent hypotension and bradycardia. These results indicate that the apparent efficacy of clonidine can indeed be augmented when clonidine and diuretic therapy are combined under laboratory conditions as well as in clinical situations.

Since the furosemide treatment did not significantly alter the blood pressure of conscious rats, it was difficult to immediately ascertain whether clonidine potentiated the effects of furosemide or vice versa. The results of the experiments with clonidine in conscious rats sodium depleted via a low-sodium diet alone indicate that a direct clonidine-furosemide interaction does not occur. Rather it appears that clonidine suppresses some compensatory mechanism that maintains blood pressure at the normotensive level in the presence of sodium depletion. The most common compensatory mechanism known to be activated following both diuretic therapy and a low-sodium dietary regimen is the renin-angiotensin system. It thus seemed relevant to determine whether the suppression of renin reported to occur with clonidine could account for the hypotensive effect of clonidine in sodium-depleted rats.

Furosemide therapy with the regimen described caused approximately a threefold increase in plasma renin activity following clonidine administration. However, only the sodium-depleted rats showed a hypotensive effect after clonidine treatment. The studies with 1-Sar-8-Ala-angiotensin II demonstrated that inhibition of the vasoconstrictor effects of angiotensin II resulted in a decrease of blood pressure only in the sodium-depleted rats, which then became refractory to the hypotensive action of clonidine. These results indicate that even though clonidine suppresses plasma renin activity and presumably also angiotensin II levels in both normal and sodium-depleted rats, angiotensin II contributes to the maintenance of blood pressure only in the sodium-depleted rats. Hence, clonidine would be expected to cause hypotension only in the sodium-depleted rats via a suppression of renin secretion.

As further verification of the latter concept, elimination of the source of renin by bilateral nephrectomy in normal and sodium-depleted rats greatly reduced the control blood pressure and effectively eliminated the hypotensive effect of clonidine in the sodium-depleted rats. Both normal and sodium-depleted rats responded to clonidine with qualitatively similar hypertensive responses and negative chronotropic effects. The quantitative differences in these responses remain inexplicable at the present time.

In an effort to further elucidate the initiating site of clonidine’s hypotensive action in the sodium-depleted rat, an attempt was made to assay the degree of neurogenic participation in the maintenance of blood pressure in sodium-depleted rats in contrast to that in normal rats. The potent ganglionic blocking agent chlorisondamine was utilized for this purpose. The results of these experiments suggest that the autonomic nervous system is more active in the maintenance of blood pressure in sodium-depleted rats than it is in normal rats. However, the non-steady-state nature of chlorisondamine’s effect, even following subcutaneous administration, and the nonspecific nature of chlorisondamine’s action on both the sympathetic and the parasympathetic nervous system prevented the formulation of more definitive conclusions from these experiments.

Peripheral sympathectomy with 6-hydroxydopamine, which specifically destroys adrenergic nerve endings, caused an equivalent reduction in the blood pressures of both normal and sodium-depleted rats. This observation contrasts with the results obtained with chlorisondamine and suggests that the sympathetic nervous system at least is no more active in the sodium-depleted rats than
it is in the normal rats. However, the somewhat chronic nature of the 6-hydroxydopamine effect would have allowed compensatory mechanisms to be activated (16) which could have effectively neutralized any apparent differences between the blood pressure maintenance mechanisms operative in the normal and the sodium-depleted rats. Thus, the interpretation of these experiments has to be limited to the inference that the peripheral sympathetic nervous system plays an important role in the maintenance of blood pressure in both normal and sodium-depleted rats.

Clonidine did not cause hypotension when it was administered to conscious sodium-depleted or normal rats treated with 6-hydroxydopamine. It is known that the sympathetic nervous system plays an important role in the regulation of renin secretion (23) and that interruption of the renal sympathetic innervation inhibits the activation of the renin-angiotensin system in response to sodium depletion in dogs in some (24, 25) but not all (26, 27) laboratories. Since clonidine does not alter renin release in vitro (28), suggesting that clonidine does not affect renin secretion directly (10), the present results may be interpreted to indicate that the portion of the sympathetic nervous system responsible for the maintenance of renin secretion and hence the maintenance of blood pressure in the sodium-depleted rat had been eliminated by the 6-hydroxydopamine treatment and therefore was no longer susceptible to clonidine's action. Elimination of the neural control of renin secretion in the normal rat by 6-hydroxydopamine treatment should not greatly affect clonidine's activity, since the blood pressure of normal rats is not a function of the rate of renin secretion.

Acknowledgment

The author is deeply indebted to Mr. James Schnieders for the meticulous determinations of plasma renin activity in this study.

References

22. BENTLEY GA, Li DMF: Studies of the new hypertensive drug.


Hypotensive effect of clonidine during sodium depletion in the rat.

D T Pals

doi: 10.1161/01.RES.37.6.795

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1975 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/37/6/795

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/