Effect of Urapidil, Clonidine, and Prazosin on Sympathetic Tone in Conscious Rats

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SUMMARY To test the hypothesis that the hypotensive action of urapidil is in part related to a direct action on the brain, the central (intracerebroventricular) and peripheral (intravenous) effects of urapidil were studied and compared with those obtained with clonidine and prazosin. All studies were conducted in conscious, chronically instrumented stroke-prone spontaneously hypertensive rats (SHRSP). Efferent sympathetic nervous system activity was estimated by means of a bipolar electrode implanted on the splanchnic nerve. Only clonidine, administered intracerebroventricularly and intravenously, decreased sympathetic nerve activity. Urapidil and prazosin either did not affect sympathetic nerve activity after central administration or increased it after peripheral administration at low and high doses, respectively. Centrally administered urapidil and prazosin lowered blood pressure but also blocked the response to intravenously administered phenylephrine; this result suggests a peripheral effect. Centrally administered urapidil decreased heart rate. Urapidil given either intracerebroventricularly or into the cisterna magna had no influence on baroreceptor responses. Intravenous infusions of urapidil and prazosin in sufficient doses to lower blood pressure in spontaneously hypertensive rats by 50 mm Hg completely blocked the actions of phenylephrine. These data suggest that in conscious SHRSP urapidil lowers blood pressure through peripheral blockade of α₁-adrenergic receptors rather than by means of central sympathetic suppression. In this regard urapidil resembles prazosin rather than clonidine; however, the effect of urapidil on heart rate is consistent with a central mode of action. (Hypertension 8: 303-311, 1986)

KEY WORDS • urapidil • sympathetic nervous system • adrenergic receptors • prazosin • antihypertensive drug • baroreceptor reflex • clonidine • stroke-prone spontaneously hypertensive rats

Urapidil, 6-[[3-[4-(o-methoxyphenyl)-1-piperazinyl]-propyl]amino]-1,3-dimethyluracil, is a new antihypertensive drug that lowers blood pressure by reducing total peripheral resistance without a compensatory increase in heart rate (HR).

Urapidil’s postjunctural α₁-adrenergic receptor blocking activity resembles that of prazosin. In addition, urapidil has been reported to depress noradrenergic transmission by a presynaptic action.

Further, systemic administration in anesthetized animals reduces blood pressure and sympathetic discharge from both splanchnic and cervical sources. Thus, urapidil’s mode of action appears to include both peripheral and central sympatholytic effects.

Most studies examining the mechanisms by which urapidil exerts antihypertensive effects have been performed in anesthetized animals; however, anesthetic agents may introduce confounding variables that complicate the interpretation of results. For example, the failure to exert a centrally mediated antihypertensive action in an anesthetized preparation does not rule out the possibility that the agent might be active in a preparation examined in the conscious state. Conversely, the presence of a centrally mediated antihypertensive effect might be peculiar to the anesthetized preparation in which reflex adjustments for a decrease in blood pressure are minimal. To avoid such difficul-
ties, we established the means to measure sympathetic nerve activity in resting, conscious rats by using chronically implanted bipolar electrodes. We employed this method, and other techniques, to address the hypothesis that the antihypertensive actions of urapidil in spontaneously hypertensive rats stem at least in part from centrally mediated depression of sympathetic nervous system activity. For comparison, the antihypertensive agents clonidine and prazosin were included.

Materials and Methods

All experiments were performed in conscious male stroke-prone spontaneously hypertensive rats (SHRSP) bred in Heidelberg since 1975. The rats were 6 to 8 months old and weighed 250 to 300 g. Animals were kept under controlled temperature, humidity, and light periodicity. All procedures were performed in accordance with institutional guidelines. For intracerebroventricular (i.c.v.) injections, chronic cannulas (PP20, Portex, St. Louis, MO, USA) were implanted into the lateral brain ventricle 1 week before the short-term experiments. The placement of lateral cerebroventricular cannulas in all experiments was verified by eliciting the typical drinking response following 100 ng of angiotensin II several days before the experiments began. Arterial and venous catheters (PP10 and PP50, and PP25) were inserted into the femoral artery and vein 1 or 2 days before the time of study. The operative procedures have been described in detail elsewhere. Measurements of arterial pressure (MAP) and HR were obtained through the arterial catheter and passed to a ratemeter with a time constant of 5 seconds. Mean rectified splanchnic nerve activity (SpNA), MAP, and HR were displayed on the Gould Brush recorder. The analog signal was followed continuously with a monitor and an audio amplifier. The signal to noise ratio was expressed as the measured amplitude compared with the activity 30 minutes after death. The animals were challenged with i.v. injections of 1 to 2 μg of phenylephrine and 12 μg of sodium nitroprusside, and only those showing the appropriate sympathetic responses were used in the experiments. The animals were placed in their cages and allowed 24 to 36 hours to recover from their operations before the experiments began. At that time, the rats had resumed their regular eating, drinking, and grooming habits and did not exhibit signs of stress or pain.

Experiment 1

To study the effects of clonidine, urapidil, and prazosin on sympathetic nerve activity, SHRSP were prepared as already described. Eight groups of six animals each were studied. In six groups the three drugs were given either centrally or peripherally in sufficient quantities to lower MAP by 30 mm Hg at the following doses: clonidine, 5 μg i.c.v. or i.v.; urapidil, 300 μg i.c.v. or i.v.; and prazosin, 2 μg i.c.v. or i.v. Two additional groups received either urapidil or prazosin at doses sufficient to normalize blood pressure in the SHRSP (i.e., 1500 or 5 μg i.v., respectively). The i.c.v. injections of active drug were preceded by 10 μl of saline vehicle. The MAP, HR, and SpNA were monitored for 45 minutes after drug administration. Peripheral α1-adrenergic receptor blockade was tested with periodic i.v. bolus injections of 1 μg of phenylephrine.

Experiment 2

To determine whether central doses of urapidil in quantities insufficient to lower systemic blood pressure by penetrating into the peripheral circulation might influence baroreceptor sensitivity, 30 μg of urapidil was injected into the lateral brain ventricle. The dose was selected on the basis of a prior dose-response experiment involving doses of 3, 10, 30, 100, and 300 μg i.c.v. The 100- and 300-μg doses lowered MAP but also diminished the response to intravenously administered phenylephrine and thus suggested leakage of urapidil into the periphery. The baroreceptor mechanism was tested by the bolus injection of 1 μg of phenylephrine and 12 μg of sodium nitroprusside. The baroreceptor response was expressed as the change in systolic time interval divided by the change in MAP. Five control sets of injections after the i.c.v. injection of vehicle were compared with five provocations of the baroreceptor reflex after the i.c.v. injection of urapidil.
Further, to examine the possibility that the central effects of urapidil were of a regional nature, we compared the effects on blood pressure and HR of i.c.v. and intracisternal injections of incremental doses of urapidil, 3, 10, 30, 100, and 300 μg. Again, the 100- and 300-μg doses yielded effects suggesting leakage into the periphery. Thereafter, the same baroreceptor experiment described with i.c.v. injection was repeated with intracisternal injection of 30 μg of urapidil, and the results were compared. Six rats per group were employed in each of these experiments. Intracisternal cannulations were verified by postmortem examinations.

Experiment 3
To test and compare the efficacy of peripheral α₁-adrenergic receptor blockade evoked by prazosin and urapidil, we infused the two drugs intravenously over 20 minutes into SHRSP (6 per group) in doses sufficient to lower MAP by 50 mm Hg. These doses proved to be 1 and 50 μg/min, respectively. Before the infusion of drug, vehicle was given during a control period of similar length. During each period, five bolus injections of phenylephrine were administered. α₁-Adrenergic receptor blockade was evaluated by comparing changes in systolic time interval and changes in MAP under these conditions.

Analysis of Data
Data were compared by paired and unpaired t tests, analysis of variance (repeated measures when indicated), analysis of covariance, and linear regression analysis. The 5% limits of probability were accepted as significant. Data are expressed as mean ± SEM.

Results
Figure 1 shows the representative recordings following administration of clonidine, 5 μg i.c.v. or i.v., and urapidil, 300 μg i.c.v. or i.v. The time axis in each
example extends from 0 to 45 minutes. Intracerebroventricular administration of clonidine produced a gradual decrease in MAP, HR, and SpNA, while intravenous administration caused a typical initial agonist response, followed by a gradual decrease in MAP, without blockade of the phenylephrine response. Intracerebroventricular administration of urapidil caused an initial short decrease followed by a brief increase in MAP, after which MAP gradually decreased by approximately 30 mm Hg. The HR demonstrated a sustained decrease, while SpNA reciprocated the initial changes in blood pressure and then gradually increased. Intravenous administration of urapidil caused a short, abrupt, substantial decrease in MAP, followed by a stabilization at approximately 30 mm Hg below the baseline value. After the initial baroreceptor-mediated responses, HR and SpNA were little changed.

Figure 2 A and B shows the effects of prazosin, 2 μg i.c.v. and i.v. After i.c.v. administration, MAP gradually declined; however, the response to intravenously injected phenylephrine was blunted as well. In addition to the decreases in MAP, HR and SpNA increased slightly after i.v. administration. Figure 2 C and D shows the effects of larger i.v. doses of urapidil and prazosin. Substantially greater decreases in MAP were observed, while HR and SpNA were increased.

Table 1 shows the initial and final values for MAP and HR following the eight experimental regimens as well as the maximal response and its time relationship. The initial values for MAP and HR were not different among the groups. All regimens reduced blood pressure significantly, the first six by approximately 30 mm Hg, as designed, and the last two by at least 50 mm Hg, also as intended. The first six regimens did not differ with respect to blood pressure decrease, nor

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**Figure 2.** A and B. Effects of prazosin, 2 μg intracerebroventricularly (i.c.v.) or i.v. Splanchnic tone was not influenced. C and D. Effects of prazosin, 5 μg i.v., and urapidil, 1500 μg i.v. Splanchnic nerve activity (SpNA) increased. See Figure 1 for key to abbreviations.
did the last two regimens differ from each other. Both i.c.v. and i.v. administration of clonidine decreased HR significantly, as did i.c.v. administration of urapidil. Although prazosin, 5 μg i.v., increased HR, an equivalent blood pressure-lowering dose of urapidil had no significant effect.

The SpNA decreased significantly following i.c.v. or i.v. administration of clonidine (see Table 1). Significant increases were observed following i.v., but not i.c.v., administration of urapidil and prazosin at either dose.

Table 1 also shows the effects of the regimens on responses to the blood pressure-raising action of phenylephrine. The i.c.v. doses of prazosin and urapidil influenced the response, as did the i.v. doses. Figure 3 illustrates the baroreceptor reflex responses under control conditions and after 30 μg of urapidil either i.c.v. or intracisternally. No effects on systemic MAP or HR were observed following this dose of urapidil into these two central nervous system sites. No significant changes were demonstrable between the regimens following the administration of either phenylephrine or sodium nitroprusside under either experimental condition.

Figure 4 demonstrates the results of phenylephrine bolus injection superimposed on the infusion of either
urapidil or prazosin at doses sufficient to lower blood pressure by at least 50 mm Hg. The increase in blood pressure following phenylephrine injection under control conditions was slightly greater in rats subsequently given urapidil than in those subsequently given prazosin. Nevertheless, changes in both systolic time interval and blood pressure were completely abolished in the presence of either urapidil or prazosin, which indicates complete \(\alpha_1\)-adrenergic receptor blockade with both regimens.

**Discussion**

This study compared the central and peripheral actions of three sympatholytic antihypertensive agents selected because of their divergent mechanisms of action. The mechanisms of all three drugs are subjects of controversy. The centrally acting antihypertensive agent clonidine produces its effects either by acting as an \(\alpha_2\)-adrenergic receptor presynaptic agonist or by mimicking the effect of norepinephrine at postsynaptic \(\alpha_2\)-adrenergic receptor sites.\(^{18,19}\) In any event, the central blood pressure-lowering actions of clonidine are not disputed since the peripheral administration of the drug raises blood pressure by stimulating \(\alpha_2\)-adrenergic receptors. Prazosin, an \(\alpha_1\)-adrenergic receptor antagonist, produces its antihypertensive action by peripheral adrenergic receptor blockade.\(^{20}\) Nevertheless, a central action of that drug has been postulated as well, on the basis of measurements of sympathetic nervous system discharge in anesthetized cats following i.v. administration.\(^{21}\) This possibility received indirect support from a report that intravenously administered prazosin, but not equipotent doses of hydralazine, induced bradycardia in spontaneously hypertensive rats.\(^{22}\) However, other investigators were unable to identify a central action of prazosin.\(^{23,24}\)

Urapidil is an N-substituted phenylpiperazine derivative possessing antihypertensive activity putatively on the basis of both central and peripheral actions.\(^{2,8}\) Evidence for a central effect of urapidil was enhanced by the observation that urapidil inhibited the blood pressure-raising response following bilateral occlusion of the carotid arteries.\(^{2}\) Direct administration of urapidil into the fourth ventricle of cats caused a clear decrease in blood pressure without evidence of peripheral effects.\(^{25}\) Intracisternal administration of urapidil in anesthetized dogs decreased MAP and HR. The expected reflex tachycardia in response to bradykinin administration was attenuated by urapidil.\(^{26}\) Further, urapidil lowered MAP, reduced sympathetic impulses, and increased vagal tone in cats and rats.\(^{10}\) With the exception of a recent report showing suppression of reflex tachycardia following \(\alpha\)-adrenergic receptor blockade in conscious dogs,\(^{27}\) data clearly indicating a

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**Figure 3.** Baroreceptor responses following administration of either vehicle or urapidil, 30 \(\mu\)g intracerebroventricularly (A) or intracisternally (B). Differences were not significant (NS) \(\Delta STI =\) change in systolic time interval; \(\Delta BP =\) change in mean arterial blood pressure.

**Figure 4.** Effect of urapidil 50 \(\mu\)g/min, and prazosin, 1 \(\mu\)g/min, infusion on the response to i.v. bolus injection of phenylephrine. With a 50 mm Hg decrease in blood pressure the response to phenylephrine was abolished with both drugs. See Figure 3 for key to abbreviations.
central effect of urapidil have stemmed from observations in anesthetized animals. Anesthesia provides study conditions under which certain vasoregulatory mechanisms can be observed that would be masked by the activity characteristic of the conscious state. Moreover, anesthesia avoids multiple technical problems. However, anesthesia may also produce confounding effects related to the suppression of higher centers that participate in blood pressure regulation.11-13

Our data clearly attest to the central action of clonidine, which lowered blood pressure when given either centrally or peripherally, albeit in the latter instance following an initial agonist response. The HR decreased with both modes of administration, as previously described.28 A blood pressure-lowering effect of clonidine in conscious rats following central administration is not invariable. For instance, in normotensive rats, central administration of clonidine did not lower blood pressure in intact animals, whereas a clear hypotensive response was observed in animals with sinoaortic denervation.23 Similar effects were observed with the \( \alpha_2 \)-agonist guanabenz.13 Thus, intact baroreceptor reflexes may quantitatively alter the blood pressure response to centrally acting agents; however, at least with clonidine, such does not appear to be the case in spontaneously hypertensive rats, which have abnormal baroreceptor responses.29 Clonidine, given intracerebroventricularly, decreased SpNA as previously described in conscious and SHRSP.14

The selective \( \alpha_2 \)-adrenergic receptor blocking agent prazosin decreased blood pressure at relatively modest doses without influencing sympathetic discharge in conscious rats. With central administration of prazosin, blockade of the phenylephrine response was observed concomitantly with the decrease in blood pressure. This effect suggests that the hypotensive response following central administration was the result of prazosin’s penetration into the peripheral circulation. When given in an i.v. dose sufficient to lower blood pressure to control values, prazosin increased SpNA. These data are in relative agreement with observations obtained in cats under a variety of experimental conditions20,31 but are at variance with a report indicating that both the \( \alpha_2 \)-adrenergic receptor antagonist WB-4101 and prazosin have direct central effects.21 In that study, decreased SpNA following i.v. drug administration was observed in both baroreceptor-denervated and intact anesthetized cats. To our knowledge, the only previous study of the effects of intravenously administered prazosin on sympathetic nerve activity in conscious animals reported variable effects on HR and SpNA in the seven animals examined.22

Urapidil exerts its antihypertensive action by decreasing total peripheral resistance without substantial reflex tachycardia and with salubrious effects on renal blood flow.5,13 The primary mode of action is related to \( \alpha_2 \)-adrenergic receptor blockade.5,8 Although urapidil is also capable of demonstrating affinity for \( \alpha_2 \)-adrenergic and \( \beta \)-adrenergic receptors, ligand binding studies indicate that the \( \alpha_2 \)-adrenergic affinity is far greater than that for other receptors.25 Although a “clonidine-like” mode of action has been postulated to explain the central effects observed in anesthetized animals, no clonidine-like agonistic activity on \( \alpha_2 \)-adrenergic receptors could be demonstrated in preparations of guinea pig ileum or in dog saphenous vein.32 Urapidil’s failure to generate reflex tachycardia may be due to a central parasympathomimetic effect, since the bradycardia caused by urapidil in anesthetized rats was abolished by vagotomy.32 Direct measurement of increased vagal tone in anesthetized cats receiving urapidil supports these observations.10

Our findings in conscious rats also indicate that urapidil did not behave like clonidine when injected into the central nervous system. A brief, biphasic effect on blood pressure was followed by a gradual decrease featuring blockade of the peripheral phenylephrine response that again suggested that urapidil penetrated into the periphery. Observations in anesthetized cats suggested that the central effects of urapidil on sympathetic nerve activity are related to specific areas of the central nervous system.25 For instance, urapidil increased blood pressure when injected into the cat forebrain. Injections into the lateral cerebral ventricle had little effect, while injections into the fourth ventricle decreased blood pressure.25 Those observations prompted us to extend our studies to rats equipped with cisternal cannulas. During dose-response experiments we again found that at doses in excess of 30 \( \mu \)g, urapidil lowered blood pressure by penetrating into the periphery and inhibiting peripheral \( \alpha_2 \)-adrenergic receptors. In those studies, intracisternal administration of urapidil caused no initial biphasic effect on blood pressure. We reasoned that urapidil might alter or enhance baroreceptor sensitivity or responsiveness, an effect postulated for endogenous peptides such as arginine vasopressin as well as for antihypertensive agents.33 We purposely selected a dose insufficient to penetrate into the periphery to allow us to observe strictly central effects. Had we lowered MAP by a peripheral effect we would have influenced the baroreceptor resetting that is a feature of the SHRSP.34 However, we were unable to demonstrate an influence on baroreceptor function by urapidil injected either into the lateral cerebral ventricle or into the cisterna magna.

In conscious27 and anesthetized26 dogs, i.v. and i.c.v. administration of urapidil respectively depressed reflex tachycardia in response to drug-induced hypotension. These effects on HR are consistent with the decrease in HR we observed with central urapidil administration. The failure of our baroreceptor reflex experiment to reflect an effect on HR may be related to the use of only a single dose of nitroprusside. Further, species differences may be responsible for these apparent discrepancies. Finally, we did not test the effects of intracisternally or intracerebroventricularly administered clonidine under the same conditions. Such a comparison conceivably would have enhanced interpretation of the data.

Urapidil, 300 \( \mu \)g i.c.v., lowered HR significantly,
while urapidil, either 300 or 1500 µg i.v., did not lower HR. Prazosin, 5 µg i.v., caused a reflex tachycardia. The i.v. doses we selected were chosen to lower MAP substantially (prazosin, 2 µg; urapidil, 300 µg) or to normal levels (prazosin, 5 µg; urapidil, 1500 µg). It is possible, particularly with the higher doses, that central effects were masked by the overwhelmingly peripheral effects.

Previous observations indicate that urapidil is about 50 times less potent than prazosin in inhibiting the vasoconstrictor effect of α1-adrenergic receptor agonists in vitro and in vivo.2,7 Nevertheless, our studies in conscious rats indicate that at infused doses sufficient to normalize blood pressure in SHRSP, urapidil obliterated the phenylephrine response with a facility equal to that of prazosin.

Urapidil’s effect on HR supports a central mode of action in conscious animals. Central administration of urapidil caused a significant bradycardia that was not observed with prazosin. Bradycardia was unaccompanied by a significant effect on sympathetic tone, which is consistent with previous observations that the decreased HR is related to enhanced vagal tone.9,32

The present data indicate the efficacy of all three sympatholytic agents. Both prazosin and urapidil are potent α1-adrenergic receptor antagonists and, in conscious animals, appear to exert the important bulk of their action through this peripheral mechanism. In addition, urapidil exerts a depressant effect on HR that can be demonstrated in conscious animals when the drug is administered centrally. The present study illustrates the complexity engendered by the conscious state when interpreting the mechanisms of drug action. Decisions regarding the utility of either state of consciousness in studies examining mechanisms of drug action necessarily have arbitrary aspects. Detailed, combined studies carefully examining the role and function of higher centers are most likely to be elucidative.

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