Pharmacology Biochemistry and Behavior, Vol. 44, pp. 623–626, 1993
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0091-3057/93 $6.00 + .00
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Effect of Nonpeptide Angiotensin AT-1 and AT-2 Antagonists on Isoproterenol-Induced Renin Release

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Received 26 May 1992

ROWLAND, N. E. AND M. J. FREGLY. *Effect of nonpeptide angiotensin AT-1 and AT-2 antagonists on isoproterenol-induced renin release.* PHARMAC BIOCHEM BEHAV 44(3) 623–626, 1993.—The AT-1 receptor antagonist, losartan potassium, produced a large rise in plasma renin activity (PRA) after peripheral, but not intracerebroventricular (ICV), administration. Peripheral, but not ICV administration of losartan also augmented the release of renin induced by peripheral administration of the β-adrenergic agonist, isoproterenol. The increase in PRA induced by losartan plus isoproterenol was greater than the sum of the increases in PRA induced by the individual treatments. There was, however, no significant enhancement of the hypotensive action of isoproterenol by peripherally administered losartan. The AT-2 receptor antagonist, PD 123319, produced no increase in PRA after either peripheral or ICV injection. However, peripheral injection of PD 123319 slightly increased PRA after peripheral administration of isoproterenol. The data are discussed in terms of the relationship between renin-angiotensin systems and fluid intake, with special reference to the failure of peripherally administered losartan to block isoproterenol-induced water intake.

Isoproterenol Losartan PD 123319 Plasma renin activity Blood pressure Water intake AT-1 and AT-2 receptors

PERIPHERAL and central components of the renin-angiotensin system have been implicated in thirst and sodium appetite, as well as in regulation of blood pressure (6,9,12). The principal active peptide in this system is angiotensin II (ANG II), and it has recently been determined that there are at least two subtypes of the ANG II receptor (14). This determination has been made largely through the development of nonpeptide antagonists of the ANG II receptor. The AT-1 receptor, for which losartan potassium (DuP 753) is a selective antagonist, is predominant in many tissues, including blood vessels and the circumventricular organs in the brain which have been implicated in thirst (6,9,11,16). The AT-2 receptor, for which PD 123319 is an antagonist, occurs in several tissues including brain, but its function is poorly understood.

We (3) and others (14) have shown that water intake induced by peripheral (SC) administration of ANG II is blocked by either peripheral or central administration of losartan. However, water intake produced by SC injection of the β-adrenergic agonist and hypotensive agent, isoproterenol, is not attenuated by SC losartan (4). This is unexpected because it is widely believed that water intake induced by isoproterenol is either partially or completely dependent upon increased peripheral renin secretion induced by the hypotension with a resulting increase in formation of circulating ANG II (1,2, 4,7,10).

One possible explanation for this paradox is that AT-1 receptor blockade might remove the inhibitory effect of ANG II on renin release, thus causing large increases in plasma renin activity (PRA), ANG I, and ANG II that might potentially overcome the blockade. We have thus determined the effects on PRA and blood pressure of losartan alone and in combination with isoproterenol. Parallel studies for PRA were performed using PD123319 and isoproterenol. Further, because peripheally administered losartan crosses the blood-brain barrier, at least to the circumventricular areas (4,15), we also determined the effects of central administration of the AT-1 and AT-2 antagonists on the isoproterenol-induced increase in PRA.

METHOD

Animals and Housing

Male Sprague-Dawley rats (3 to 6 mo old, from Harlan Industries) were used in these studies. All were housed singly and provided with Purina Rodent Chow pellets and tap water ad lib, unless stated. Lights were on from 0700–1900 h,

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and all testing was done during the middle part of the light phase.

Plasma Renin Activity

In the first study, a total of 78 rats was used. Each received two SC injections 20 min apart, and blood was sampled either 20 or 50 min after the second injection. The first injection was either vehicle (0.15M NaCl, 1 ml/kg) or losartan (10 mg/kg). The second injection was either vehicle or isoproterenol at a dose of either 5 or 25 μg/kg. There were thus 12 groups in a 2 (time) × 2 (drug 1) × 3 (drug 2) design. Blood (0.2 ml) was taken by cardiac puncture after brief inhalation anesthesia with methoxyflurane. [Most anesthetics increase PRA. A 5-min exposure to methoxyflurane results in a twofold elevation in basal PRA in rats (8). Our rats were exposed for about 1 min, so the possible effects of anesthesia should be negligible relative to the treatment effects we report.]

The blood sample was placed in a cold EDTA-coated vial, and the plasma was separated by centrifugation and stored at −60°C for later determination of PRA by a modification of a commercial ANG I radioimmunoassay (DuPont, Wilmington, DE). Duplicated 10 μl samples of plasma were used in the generation phase (with 100 μl buffer and inhibitor cocktail), and the immunoassay reagents were then added to the same tubes. This modification to smaller volumes and a tenfold dilution has proven suitable for rat studies, allowing readings of PRA from the standard curve in the range 1–50 ng ANG I/ml plasma/h at 37°C.

In the second study, using 20 additional rats divided into four equal groups ($n = 5$), either PD 123319 (20 mg/kg SC) or saline was injected SC 20 min before either isoproterenol (25 μg/kg SC) or saline, and 20 min later blood was taken after rapid decapitation and processed for PRA assay as above.

Blood Pressure

Systolic blood pressure was determined by the indirect tail-cuff method (Narco Biosystems, Houston, TX; electrosphygmomanometer and Physiograph) in 12 rats that were adapted to the restrainers and warmed from an incandescent lamp. Rats were placed in the restraint tubes for only the time necessary to obtain a reliable reading, and then were removed and placed in a group cage under the lamp until their next scheduled recording. Pressures were recorded before and 5, 20, 35, 50, 65, and 80 min after administration of either isoproterenol alone (25 or 100 μg/kg SC), losartan (10 mg/kg SC), or their combination. Losartan was administered 20 min before isoproterenol. Rats were tested repeatedly in various conditions at 2–5 day intervals.

Intracerebroventricular (ICV) Cannulation and Testing

Another 24 rats were equipped with an indwelling stainless steel (23-gauge) cannula aimed at the lateral cerebral ventricle. These were implanted stereotaxically under general anesthesia (ketamine + xylazine, 50 + 5 mg/kg), with flat skull coordinate 1.5 mm lateral to the midline 0.5-mm posterior to bregma and 3.5-mm ventral from skull, at least 1 week before the studies. During this recovery period, the rats were handled and accustomed to manipulation of the obturator in the cannula and were screened for drinking following ICV injection of ANG II (10 ng). Rats that drank <2 ml water were dropped from the study. On the day of the main experiment, an ICV injection of 5 μl of either the saline vehicle or losartan (50 μg) was given at the same time as SC injection of either saline or isoproterenol (25 μg/kg). Blood samples were taken by heart puncture 20 min later, as described above, for assay of PRA. Two weeks later, the same rats were tested similarly, but using ICV PD 123319 (100 μg) and isoproterenol (25 μg/kg SC), or their saline vehicles.

Statistics

Data were analyzed by either one- or two-way analyses of variance (ANOVA) using SAS (PC version), and the significance level for post hoc Duncan and *t*-tests was set at 0.05.

RESULTS

Blood Pressure

The results of the blood pressure studies are shown in Table 1. Losartan did not significantly exascerbate the hypotensive effect of isoproterenol at any dose or time after administration. Thus, at each dose of isoproterenol, two-way ANOVAs gave significant effects of isoproterenol ($p < 0.001$), but no significant effects of losartan or interactions between losartan and isoproterenol.

Plasma Renin Activity

The results from the study with SC losartan are shown in Fig. 1. Mean PRAs in vehicle-treated rats were 1.8 and 2.3 ng ANG I/ml/h after 20 and 50 min, respectively. These basal values are similar, so are shown as an average horizontal line

TABLE 1

CHANGE IN MEAN BLOOD PRESSURE AT VARIOUS TIMES AFTER ADMINISTRATION OF LOSARTAN (10 mg/kg SC) AND ISOPROTERENOL (ISO)

Treatment	Initial Pressure	Mean pressure (mmHg) change after					
		5	20	35	50	65	80 min
Isoproterenol 100 μg/kg SC							
Vehicle + ISO	146 ± 6	−41 ± 4	−50 ± 4	−47 ± 11	−41 ± 9	−36 ± 7	−22 ± 11
Losartan + ISO	139 ± 14	−48 ± 5	−55 ± 5	−51 ± 7	−50 ± 5	−42 ± 6	−33 ± 5
Isoproterenol 25 μg/kg SC							
Vehicle + ISO	141 ± 3	−48 ± 5	−39 ± 7	−25 ± 7	−17 ± 4	−7 ± 6	−8 ± 3
Losartan + ISO	132 ± 5	−53 ± 6	−53 ± 5	−46 ± 4	−31 ± 5	−18 ± 6	−8 ± 6

Means (±SEM) for 6 rats/group. All values in mmHg.

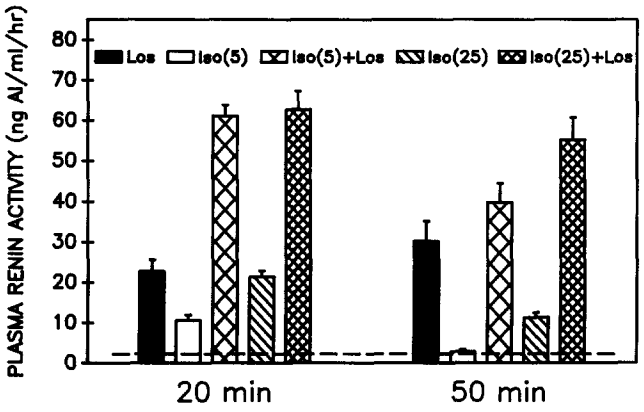

FIG. 1. Effect of isoproterenol (Iso), at SC dosages of either 5 or 25 μg/kg, and losartan potassium (Los, 10 mg/kg, SC) on plasma renin activity (PRA, in ng ANG I formed/ml plasma/h at 37°C) either 20 min (left set) or 50 min (right set) after isoproterenol. Shown are M ± SE for groups of six rats. The mean ± SE PRA of untreated controls (basal level) was 2.0 ± 0.7 ng/ml/h, was similar at both times (see text) and, for ease of comparison, the mean is shown as a horizontal dashed line. PRA values in all treatment conditions were significantly ($p < 0.01$) elevated above basal.

in Fig. 1; the values are also in the normal basal range for rats, indicating only a minimal effect of methoxyflurane sedation (cf. 8). Losartan alone produced a large and significant ($p < 0.001$) increase in PRA above basal, and this was similar at both times corresponding to 40 and 70 min after injection. Isoproterenol produced the expected dose- and time-related changes in PRA, with a large ($p < 0.001$) increase above basal after 20 min that was still significant ($p < 0.01$) after 50 min. The combination of SC losartan followed 20 min later by isoproterenol produced increases in PRA that exceeded the sum of those produced by the independent treatments. Thus, separate ANOVAs at each time point showed significant main effects of both treatments and an interaction between the treatments ($p < 0.001$).

In the second study, SC administration of PD 123319 alone did not increase PRA (vehicle-vehicle: 0.2 ± 0.2, PD-vehicle: 1.3 ± 0.3 ng AI/ml/h), but it reliably increased isoproterenol-stimulated PRA (vehicle-isoproterenol: 8.6 ± 1.0, PD-isoproterenol: 139 ± 1.3 ng ANG I/ml/h; $p < 0.05$).

ICV administration of either losartan or PD 123319 had no significant effect on PRA either alone or in combination with peripheral isoproterenol (Fig. 2).

DISCUSSION

The present studies were designed to determine whether combined treatment with losartan and isoproterenol produces physiological effects that might explain the failure of peripheral administration of losartan to attenuate isoproterenol-induced drinking (4). The fact that nephrectomized rats are capable of drinking in response to low doses of isoproterenol has been interpreted as a role for baroreceptor mediation (5); in that study, the hypotensive action of isoproterenol was much greater in nephrectomized rats that cannot generate circulating ANG II as a counterregulatory mechanism to attenuate the reduction in blood pressure. We thus anticipated, but did not find, that blockade of the pressor effect of ANG II with losartan would greatly increase isoproterenol-induced hypotension. This may indicate that sufficient circulating ANG II is generated by the elevated plasma renin activity to offset the effect of the receptor blockade by losartan.

The interaction of losartan and isoproterenol on PRA, and its implication for thirst, is a more complex issue. The increase in PRA induced by losartan alone presumably is a result of removal of end product (ANG II) inhibition of renal renin release. The reason that high levels of PRA, and presumably ANG II, do not induce water intake in losartan-treated rats most likely reflects the fact that drinking requires AT-1 receptor occupancy. However, we have found that rats drink slightly more to a combination of a lower dose (3 mg/kg SC) of losartan in the presence of isoproterenol (25 μg/kg) than to isoproterenol alone (4). Thus, for blood pressure and thirst, the relationship among losartan, isoproterenol, and PRA is not a simple one and will require additional study. The results are not inconsistent with the hypothesis that very high levels of ANG I and/or ANG II may penetrate some brain regions preferentially relative to losartan, and so overcome the effective antagonism.

Neither the AT-1 nor the AT-2 antagonist, administered into the cerebral ventricles, affected isoproterenol-stimulated PRA. We reported (4) that ICV administration of 100 μg losartan blocked the water intake induced by SC injection of isoproterenol. However, in subsequent studies we have not consistently reproduced this effect. We have likewise found that ICV injection of PD 123319 usually, but not always blocks water intake induced by peripheral administration of isoproterenol. We currently believe that the discrepancies can be explained by the loss of efficacy of the antagonists after 1-2 administrations, but further study is required.

In summary, the traditional view that isporoterenol induces drinking by generation of circulating ANG II, which then stimulates receptors in sites with no blood-brain barrier (circumventricular organs) seems untenable because losartan, which has access to these parts of the brain, does not reduce isoproterenol-induced drinking. We cannot rule out the possibilities that either losartan or ANG II has differential access to other regions of the brain or that isoproterenol-induced drinking is mediated by ANG II of cerebral origin. In the latter case we must accept the unlikely hypothesis that ICV-

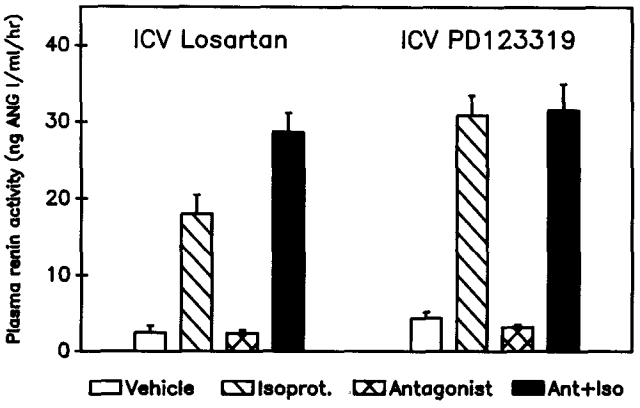

FIG. 2. Effect of intracerebroventricular (ICV) injection of either the AT-1 antagonist, losartan potassium (50 μg), or the AT-2 antagonist, PD 123319 (100 μg), on PRA values 20 min following either isoproterenol (25 μg/kg, SC) or vehicle. Shown are M ± SE for groups of six rats. Isoproterenol-treated groups had elevated PRA ($p < 0.01$) relative to vehicle- or antagonist-only groups.

administered losartan does not reach these sites. Another possibility is that AT-1 receptors are not involved, and it is AT-2 receptors that are important for water intake after isoproterenol. Further studies will be needed to test these possibilities.

ACKNOWLEDGEMENTS

This work was supported by a grant-in-aid from the American Heart Association, FL affiliate. The authors thank Gloria Smith and Anita Del Bianco for assistance.

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