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Angiotensin Antagonist and Possible Release of Catecholamine (38453)

SUBHA SEN, ROBERT R. SMEBY, AND F. MERLIN BUMPUS

Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106

The role of the renin–angiotensin system in the initiation and maintenance of hypertension has been studied extensively. The most commonly used tools for evaluation have been blocking agents for renin, converting enzyme or angiotensin. Extensive studies have been reported and important results obtained with Ang II analogs which act as specific competitive antagonists. Substitution at the one and eight positions of angiotensin II have thus far produced the most important analogs. The two most extensively used blockers to study *in vivo* effect in addition to pharmacological and structure activity relationship were [Sar^1, Ala^8] ang II and [Sar^1, Ile^8]^{1}ang II. The latter was shown to be more potent, as an antagonist, while both peptides showed some transient agonistic effects. A series of compounds has been synthesized in our laboratory substituting positions 1 and 8 in the search for more potent inhibitors with less agonistic properties. The purpose of the present note is to report the *in vivo* effect of one such angiotensin blocker, namely [*N*-methyl Ile^1, Ile^8]^1 ang II which is a more potent angiotensin inhibitor *in vitro*.

Materials and Methods. Sprague–Dawley female rats, weighing approx 200 g, were used for all experiments. On the day of the experiment, rats were anesthetized with sodium amytal (6 mg/100 g) and the iliac artery and both femoral veins were cannulated with polyethylene tubing (PE 50). The iliac cannula was connected to a pressure transducer for continuous recording of blood pressure. Either saline or angiotensin II blockers in saline were infused into one femoral vein while the other vein was used to administer pressor doses of angiotensin II. Angiotensin analogs were dissolved and diluted in sterile saline to the desired concentration. Three doses of angiotensin II were selected to test for the rats' pressor sensitivity to ang II. After the pressor responses saline or angiotensin analogs were infused at the rate of 0.05 ml/min. Pressor responses to angiotensin were evaluated 15–20 min after beginning the infusion of either saline or angiotensin analogs and again 20–30 min after the termination of the infusion. Each compound was infused in three dose levels for 15–17 min (250 ng/kg/min, 500 ng/kg/min, and 1250 ng/kg/min) to compare the potencies. The compounds infused in this study were [*N*-Melle^1, Ile^8] angiotensin II and [Sar^1, Ile^8] angiotensin II.

Preparation of adrenalectomized rats. Rats were bilaterally adrenalectomized through an incision on each side, under anesthesia. Either adrenalectomy was done under light ether anesthesia and rats were allowed to recover for 6 hr and then prepared for infusion study as described earlier, or the adrenalectomy was performed right before infusion study under amytal anesthesia. No difference was observed between experimental animals handled in the two ways.

Results. When [Sar^1, Ile^8] was infused in a dose of 50 ng/min, no blocking of exogenous angiotensin was observed, but when [*N*-Melle^1, Ile^8] was infused in the same dose 75% inhibition of the pressor response of exogenous angiotensin was obtained. When the dose of [Sar^1, Ile^8] was increased to 100 ng/min, the pressor response to exogenous angiotensin was decreased 52% whereas infusion of [*N*-Melle^1, Ile^8] in the same dose (100 ng/min), 95% attenuation of the pressor response to angiotensin II was observed. This suggested that [*N*-Melle^1, Ile^8] analog is a more potent inhibitor of angiotensin II. When the dose level of [Sar^1, Ile^8] and [*N*-Melle^1, Ile^8] angiotensins were increased to 250 ng/min, both compounds blocked completely (98%) the pressor response to exogenous angiotensin II. The effects of infusion of [Sar^1,

[1] The compounds used in this study were synthesized by Dr. M. C. Khosla, Cleveland Clinic Foundation.

TABLE I.

Compound	Dose	Inhibition of exogenous ang II (%)[a]	Recovery after 30 min
Sar^1, Ile^8	50 ng/min	0	100%
N-Methyl Ile^1, Ile^8	50 ng/min	75%	80%
Sar^1, Ile^8	100 ng/min	52%	90%
N-Methyl Ile^1, Ile^8	100 ng/min	95%	85%
Sar^1, Ile^8	250 ng/min	98%	65%
N-Methyl Ile^1, Ile^8	250 ng/min	98%	90%

[a] The % of inhibition has been calculated by comparing the pressor response (mm) of highest dose (5.5 ng) of exogenous angiotensin II, before, during, and after termination of infusions of antagonists.

Ile^8] and [*N*-Melle^1, Ile^8] analogs are summarized in Table I.

The details of synthesis and pharmacological effect of [*N*-methyl Ile^1, Ile^8] angiotensin II have been reported by Khosla *et al.* (1). When [*N*-Melle^1, Ile^8] was infused in rats, a persistent rise in blood pressure was observed instead of a transient rise as in the case of [Sar^1, Ile^8] angiotensin II. Rats pretreated with phenoxybenzamine prevent the rise in blood pressure due to infusion with [*N*-Melle^1, Ile^8]. Also when phenoxybenzamine was given intravenously during infusion of [*N*-Melle^1, Ile^8] an immediate drop in blood pressure was observed. A detailed report on the study with phenoxybenzamine will be reported elsewhere. However, when a higher dose of [Sar^1, Ile^8] angiotensin was infused, the blood pressure of rats did not return to the base line in all cases.

When the [*N*-Melle^1, Ile^8] compound was infused in adrenalectomized rats, no persistent rise in blood pressure was observed (Fig. 1). However, infusion of a higher dose (250 ng/min) caused a transient rise in blood pressure.

Discussion. The involvement of this renin–angiotensin system in experimental renal hypertension has been investigated extensively. The most important experimental approaches have utilized antibody or inhibitors of renin or angiotensin. The discovery of angiotensin antagonists provided a tool to evaluate the role of renin or angiotensin in experimental hypertension. The two analogs used most extensively for *in vivo* studies are [Sar^1, Ile^8] angiotensin II, and

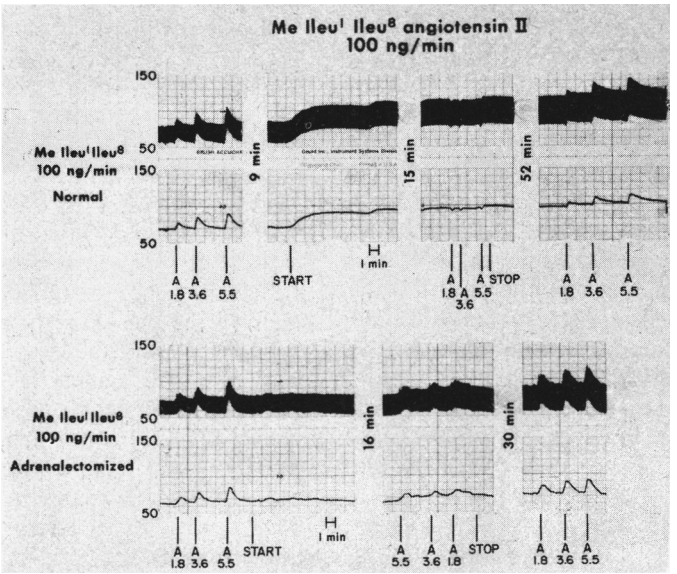

FIG. 1. Blood pressure change during infusion of *N*-Melle^1, Ile^8 in normal and adrenalectomized rats. The ordinate represents (top) arterial pressure mm Hg; (bottom) mean arterial pressure.

[Sar[1], Ala[8]] angiotensin II. The findings published so far could be summarized as follows: (a) that increased activity of the renin–angiotensin system occurs during acute renal artery constriction and is involved in the pathogenesis of both acute one-kidney (in rats and dogs) and acute two-kidney (in rats) hypertension (2, 5); (b) that increased activity of the renin–angiotensin system helps to maintain a high level of arterial pressure in chronic two-kidney hypertension in rats (3), although this has been contradicted by other investigators (2, 4, 5).

The present experiments have shown that another aspect of the antagonists that deserves consideration is the release of catecholamine from adrenal medulla. A transient pressure rise with a small dose of [Sar[1], Ile[8]] analog while a persistent rise in blood pressure was observed with a small dose of [*N*-methyl Ile[1], Ile[8]] angiotensin. The rise in pressure could be due to two reasons: (a) a small direct agonistic effect of the peptide on smooth muscle and as the receptors become tachyphylactic, the pressure returns to base line, or (b) due to a release of catecholamine from adrenal medulla.

Our experiment suggested that the [*N*-methyl Ile[1], Ile[8]] derivative is a more potent antagonist but induced a release of catecholamine which caused an immediate rise in blood pressure that was abolished by adrenalectomy. It is highly possible that both mechanisms, *i.e.*, direct agonistic effect, and release of catecholamine occur simultaneously. These data indicate the need for further study of *in vivo* effect of angiotensin antagonists.

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