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Comparative Hypotensive Activity of REV 6207 and Enalapril in the Conscious Furosemide-Treated Monkey

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ABSTRACT

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The cardiovascular effects of ascending doses (0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg i.v.) of two angiotensin I-converting enzyme (ACE) inhibitors, REV 6207 and enalapril, were assessed in conscious furosemide-treated (3 mg/kg s.c.) monkeys. Both ACE inhibitors produced a dose-related inhibition of the pressor response to angiotensin I (0.66 μg/kg i.v.) with concomitant decreases in mean arterial pressure and no change in heart rate. The calculated ED_{50} values for REV 6207 (0.316 mg/kg) and enalapril (0.275 mg/kg) were similar and both abolished the pressor response to angiotensin I at a dose of 3 mg/kg. The results of the study show that REV 6207 is a potent nonsulfhydryl-containing ACE-inhibitor with blood-pressure-lowering activity comparable to enalapril in the conscious monkey with high renin activity.

Key words: converting enzyme inhibitor, diuretic, antihypertensive, plasma renin activity

INTRODUCTION

REV 6207, N-,B-,1-(ethoxycarbonyl)-3-phenylpropyl.-L-alanyl,-N-pyrrolidinylglycine hydrochloride (Fig. 1), has been shown to be a potent angiotensin I-converting enzyme (ACE)

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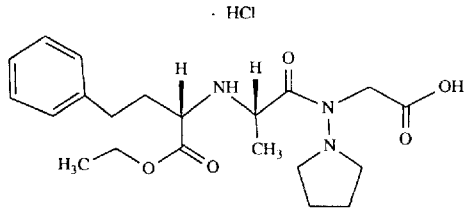

Fig. 1. Chemical structure of REV 6207.

inhibitor in vitro (Schwab and Macerta, unpublished observations) with an IC_{50} value of 0.7 μM against ACE activity from rabbit lung preparations.

Previous studies [Keeton and Campbell, 1980; Wilkes, 1984] have demonstrated that pharmacological and/or dietary-induced sodium depletion in experimental animals serves as a potent stimulus for activation of the renin-angiotensin system. This technique for increasing plasma renin activity (PRA) has proven to be useful for assessing the vasodepressor activity of both ACE inhibitors and renin inhibitors in vivo [Blaine et al., 1985; Burton et al., 1980; Laubie et al., 1984]. Consequently, the purpose of this study was to compare the ACE inhibition and vasodepressor activity of REV 6207 with enalapril in conscious sodium-depleted monkeys.

MATERIALS AND METHODS

Subjects

Female cynomologus monkeys *(Macaca fascicularis)* weighing 3–5 kg (N = 5) were maintained on a diet of Purina monkey chow, fresh fruit, and water.

Surgical Preparation

Each monkey was anesthetized with ketamine HCl (10 mg/kg s.c.) as described by Hartley et al. [1984]. Using sterile surgical technique, a polyvinyl chloride catheter (0.64 mm i.d.) was introduced into the abdominal aorta via the right internal iliac artery. The catheter was then passed subcutaneously up to the midscapular region and the incision was sutured closed. Each monkey then received an injection of antibiotic and was returned to its home cage. All monkeys were given a 1 week recovery period prior to experimental treatment during which time temperatures were monitored and antibiotics administered as needed. The care of the monkeys was in accordance with the National Institute of Health Guidelines for the Use of Laboratory Animals (NIH, Department of Health and Human Services publication No. 85-23, revised 1985).

Sodium Depletion

Sodium depletion was accomplished by 3 consecutive days of diuretic treatment with furosemide (3 mg/kg s.c.). Arterial blood samples (2 ml) were drawn from the arterial catheter before diuretic administration and prior to the experiment (3 days following treatment) for the determination of hematocrit (Hct) and plasma renin activity (PRA).

Assessment of Plasma Renin Activity

The blood samples were drawn into ice cold polypropylene tubes containing 20 μl of an EGTA-glutathione solution, pH 7.4. The blood samples were centrifuged for 10 min (1,000*g* at 4°C) and the plasma was stored at −80°C until the assays were performed.

Plasma renin activity (PRA) was determined by radioimmunoassay as previously described [Hubbard et al., 1986].

Experimental Protocol

All experiments were performed on conscious, chair-restrained monkeys. On the morning of the experiment, approximately 8:00 A.M., each monkey was lightly anesthetized with ketamine HCl (1 mg/kg im) and transferred from its home cage to the monkey chair. While the monkey was anesthetized, a catheter (Abbocath-T, 20 G) was introduced into the saphenous vein via a percutaneous puncture. A small incision was then made at the base of the neck to externalize the arterial catheter. The area was cleaned, infiltrated with lidocaine and sutured closed.

The catheter was attached to a Statham pressure transducer and pulsatile and mean arterial pressure (MAP) were displayed on a Gould oscillographic recorder (Gould 2800S). Heart rate (HR) was obtained from a cardiotachometer, triggered from the pulsatile arterial pressure signal and displayed on a third channel of the recorder. Arterial pressure and HR were monitored for 4 to 5 hr while the monkeys recovered from the anesthesia.

The experimental procedure was initiated when the monkey displayed alertness, absence of nystagmus, and orientation to the investigator. All drugs were prepared immediately prior to the experiment and are expressed as mg of free base per kg of body weight. REV 6207 and enalapril (Merck, West Point, PA) were dissolved in normal saline and administered in ascending doses of 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg i.v., in volumes of 1–1.5 ml. Angiotensin I, (AI) was also dissolved in normal saline and administered in a dose of 0.66 μg/kg i.v., in a volume of 0.3 ml.

Baseline cardiovascular measurements were obtained while the monkeys remained quiet in the chair. At the end of this pretreatment control period, a bolus injection of AI (0.66 μg/kg i.v.) was administered and the pressor response recorded. The average of two pressor responses was taken as the baseline response. The monkey was then allowed to sit for 10 min prior to the first dose of test compound. Either enalapril or REV 6207 was then administered in an ascending dose range to the monkey. Ten minutes following administration of each ACE inhibitor, AI was again given as a bolus injection with a 10 min "washout" prior to the next dose of ACE inhibitor. During this time, MAP returned to the pre-AI level and remained there for at least 5 min before another injection was given. Consequently, an AI injection followed each dose of ACE inhibitor with a 20 min period between the administration of the next dose of compound.

Statistical Analysis

Each monkey served as its own control. These data are presented as the absolute mean or mean change ± S.E.M. The antagonistic effects of ACE inhibition on the pressor response to AI are expressed as mean percent change ± S.E.M. The PRA and Hct changes following furosemide treatment were analyzed with a paired Student's t-test. The changes in MAP and HR following ACE inhibition were also analyzed with a paired Student's t-test [Tallarida and Murray, 1981]. A linear regression analysis was used to calculate ED_{50} values with 95% confidence limits for REV 6207 and enalapril inhibition of the AI pressor response. A $P < 0.05$ (one-tailed) was considered to be statistically significant.

RESULTS AND DISCUSSION

Three consecutive days of diuretic treatment in the conscious monkey caused a significant 14% increase in Hct (from 41.0 ± 0.6 to 46.7 ± 0.7) and a significant 3.4-fold increase in PRA (from 1.6 ± 0.5 to 6.1 ± 1.2 ng AI/ml/hr). Table 1 shows that both REV 6207 and enalapril caused progressive and significant ($P < 0.05$) reductions in MAP with peak decreases of 16 ± mmHg and 11 ± 4 mmHg, respectively, at the 3 mg/kg i.v. dose. In contrast, neither REV 6207 or enalapril caused any significant change in HR (Table 1). Vehicle treatment (2 ml of normal saline, i.v.) given to randomly selected monkeys produced no significant changes in MAP or HR during the experimental period (data not shown).

TABLE 1. The Changes in Mean Arterial Pressure and Heart Rate Following Administration of Ascending Doses of REV 6207 or Enalapril in Furosemide-Treated (3 mg/kg s.c.) Conscious Monkeys†

			Dose (mg/kg,i.v.)				
Variable	Treatment[a]	Control	0.03	0.1	0.3	1.0	3.0
Mean arterial pressure (mmHg)	6207	120 ± 19	−6 ± 1**	−8 ± 3**	−8 ± 3**	−12 ± 2*	−16 ± 6*
	Enalapril	100 ± 3	−4 ± 1**	−1 ± 3	−7 ± 2*	−8 ± 3*	−11 ± 4*
Heart rate (bpm)	6207	226 ± 6	5 ± 4	−2 ± 2	3 ± 9	−8 ± 4	−16 ± 7
	Enalapril	210 ± 13	−8 ± 4	−5 ± 8	−2 ± 10	5 ± 7	6 ± 4

†The data are presented as means ± S.E.M.
[a]N = 4 per treatment.
*P <0.05 compared to pretreatment control.
**P <0.025 compared to pretreatment control.

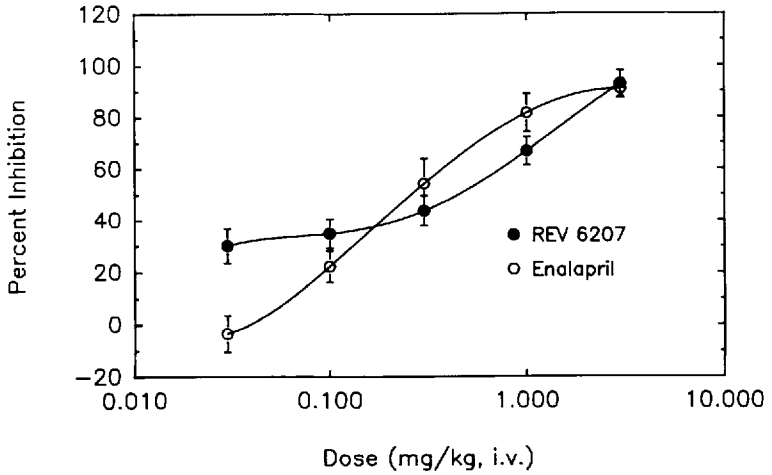

Fig. 2. Inhibitory effects of ascending doses of REV 6207 or enalapril on pressor responses to angiotensin I (0.66 μg/kg i.v.) in conscious sodium-depleted monkeys (N = 4 per drug).

Additionally, repeated injections of AI to these same monkeys produced repeatable and equivalent pressor responses (45–50 mmHg) with no indication of tachyphalaxis. Figure 2 shows that REV 6207 and enalapril caused a dose-related antagonism of the AI pressor response (an index of ACE inhibition). Complete inhibition of the pressor response was observed following the 3 mg/kg i.v. dose of each compound. The calculated ED_{50} value for REV 6207 was 0.316 (0.281–0.353) mg/kg i.v. and 0.275 (0.248–0.306) mg/kg i.v. for enalapril.

The results of this study demonstrate that significant activation of the renin-angiotensin system occurs in the conscious monkey following acute administration of furosemide. The increases in PRA and Hct following diurtetic treatment were similar in magnitude to those observed by other investigators employing combined diuretic treatment and dietary sodium depletion [Burton et al., 1980; Cody et al., 1982]. Moreover, significant hypotension was observed following ascending doses of ACE inhibitors REV 6207 or enalapril administered to conscious monkeys with elevated PRA. Hemodynamic studies have shown that sodium-depleted animals have reduced cardiac output and elevated total peripheral resistance with no change in MAP as compared to normal animals [Ferrario et al., 1981; Laubie et al., 1984].

During ACE inhibition, the vasoconstrictor effect of angiotensin II is eliminated with a concomitant fall in MAP [Laubie et al., 1984]. However, a simultaneous increase in cardiac output, as the afterload is reduced, may attenuate the dose-related hypotension during ACE inhibition. In our study, the peak fall in mean arterial pressure was observed at the same dose which caused a 90–100% inhibition of the pressor response to AI. This fall in MAP was not associated with a reflexively mediated increase in HR for either drug. Analogous observations have been made by Cody and colleagues [1982] using a primate model of high renin hypertension. Ferrario et al. [1981] have reported that enhanced cardiopulmonary receptor discharge in the sodium-depleted animal may antagonize the baroreflex-mediated tachycardia during ACE inhibition. Consequently, the cardiac sympathetic activation which normally occurs in response to unloading of the arterial baroreceptors would be offset by inhibitory afferent activity from the cardiopulmonary region.

Thus, it would appear that acute diuretic treatment in the conscious monkey is a rapid and reliable model for assessing the vasodepressor activity of ACE inhibitors in vivo. Additionally, we have shown that REV 6207 is a systemically active nonsulfhydril ACE inhibitor with a potency similar to enalapril in the conscious monkey. Further studies are in progress in order to characterize the pharmacology of this novel ACE inhibitor.

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