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COMMUNICATIONS

Effects of subacute oral administration of captopril (SQ 14,225) on the renin-angiotensin system in spontaneously hypertensive rats

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Captopril (SQ-14,225) is an orally active inhibitor of angiotensin I (Ang I) converting enzyme (ACE) (Rubin et al 1978). It has been reported to be an effective antihypertensive agent in the spontaneously hypertensive rat (SHR) (Laffan et al 1978) and in essential, malignant and renovascular hypertensive patients (Case et al 1978: Gavras et al 1978). It is generally assumed that the mechanism by which captopril reduces blood pressure is closely related to its inhibiting ACE thereby blocking the production of angiotensin II (Ang II) (Gavras et al 1978). The results of acute studies appear to be unequivocal in that the serum ACE activity was lowered following acute captopril administration (Kokubu et al 1980; Waeber et al 1980). However, the effects of chronic treatment with captopril on serum ACE activity in hypertensive patients are equivocal: serum ACE levels have been reported to be decreased (Gavras et al 1978), increased (Larochelle et al 1979) or unchanged (Waeber et al 1980) during captopril therapy. Recently it has been reported that while the administration of a single dose of captopril lowered serum ACE activity in normotensive rats, a significant elevation of serum ACE activity was found during 30 days of captopril administration (Kokubu et al 1980).

We have investigated the effects of captopril on several parameters of the renin-angiotensin system during subacute treatment in the SHRs to determine the relationship between the antihypertensive activity and its effects on the renin-angiotensin system. We have confirmed the antihypertensive efficacy of captopril in the SHRs and extended the finding of captopril-induced increase in serum ACE in normotensive rats to the SHRs.

Male Okamoto-Aoki SHR's (Taconic Farms, Germantown, N.Y.), 250–300 g, were surgically prepared for the direct measurement of arterial blood pressure (BP) and heart rate (HR) with an indwelling catheter according to the method of Weeks & Jones (1960). At least 4 days elapsed between the installation of aortic catheters and the start of drug administration. Rats were given 0·5% methylcellulose or captopril (E. R. Squibb & Sons, Princeton, N.J.) (100 mg kg^{-1}) orally in the morning, daily for 15 days, by a rubber gavage tube. After the last dose, each rat was placed unrestrained in a cage and the cannula was connected to a Statham P23 pressure transducer fixed outside the cage approximately at the animal's heart level. Systolic BP and diastolic BP were determined at approximately 6·5 h after the dosing, since in a preliminary acute study, the time of maximum antihypertensive effect of captopril at 100 mg kg^{-1} was found to be approximately 6·5 h after oral dosing. After the BP and HR measurements, captopril and 0·5% methylcellulose treatments were continued for additional 1 and 3 days and 1 ml and 2 ml of arterial blood samples were collected from each rat approximately 6·5 h after the last dosing. Serum or plasma was separated by centrifugation, stored at −20 °C for five days and assayed for ACE or plasma renin activity (PRA), respectively. Serum ACE activity was assayed according to a modified method of Rohrback (1978) using [glycine-1-^{14}C]-hippuryl-L-histidyl-L-leucine (New England Nuclear Corp., Boston, MA) as substrate. [^{14}C]Hippuric acid formed was separated from the unreacted substrate by extraction with ethyl acetate and aliquots of the ethyl acetate extracts were counted in an ACS scintillator (Amersham) using a Packard Tri-Carb Scintillation spectrometer. PRA was assayed using a [^{125}I]angiotensin I radioimmunoassay kit (New England Nuclear Corp., Boston, MA) according to the supplier's instructions.

The results are summarized in Table 1. Mean arterial BPs were not significantly different between captopril and vehicle groups at the beginning of the experiment. After 15 days treatment, there was a significant ($P < 0·05$) fall in mean BP (14%) of the captopril-treated groups in comparison with control. No effect on heart rate was observed in control or captopril-treated animals. Both serum ACE and PRA were significantly elevated (about 2·5 and 12 times higher than control, respectively) when assayed on the 16th and 18th days, respectively, of captopril administration.

The increase in the PRA after ACE inhibition by captopril is readily explainable by the negative feedback effect of Ang II on renin. However, the increase in ACE activity after subacute treatment of captopril is not readily explainable. If captopril lowers blood pressure by inhibiting ACE, a decrease in serum ACE activity is expected after

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Table 1. Effects of subacute treatment of captopril on mean arterial blood pressure (MBP), heart rate (HR), serum angiotensin I (Ang I) converting enzyme (ACE) activity, and plasma renin activity (PRA) in male spontaneously hypertensive rats.

Treatment*	BP (mm Hg)		HR (beats min^{-1}) 15th day	ACE (nmol [^{14}C] hippuric acid formed ml^{-1} min^{-1}), 16th day	PRA (ng Ang I formed ml^{-1} h^{-1}), 18th day
	MBP before treatment	Overall maximum changes on 15th day			
0·5% Methylcellulose	165 ± 3 (7)	−6·7 ± 1·5 (6)	313 ± 14 (6)	24·3 ± 1·6 (6)	5·0 ± 8·0 (5)
Captopril (in 0·5% methylcellulose)	169 ± 4 (9)	−23·7 ± 1·1 (8)	297 ± 8 (8)	59·9 ± 2·8 (8)	60·0 ± 5·5 (8)
P-value	NS	$P < 0·05$	NS	$P < 0·001$	$P < 0·001$

* See text for detail. MBP = diastolic BP + (systolic BP − diastolic BP)/3. The number of animals is indicated in parentheses. The progressive decrease in the number of animals is due to some arterial cannulae becoming non-patent and therefore readings were no longer obtained. All data derived from captopril-treated animals were compared with those of vehicle controls by a two-tailed t-test. NS = $P > 0·05$.

captopril treatment. However, measurements of serum ACE activity after chronic captopril treatment have been the subject of some controversy. The discrepancies in results of serum ACE activity after chronic captopril treatment have been attributed to: (a) the dissociation of enzyme-inhibitor complex during the period of sample storage before they were assayed for ACE activity (Lai et al 1980; Dux et al 1981); and (b) the induction of ACE by captopril (Fyhrquist et al 1980; Kokubu et al 1980). These discrepancies have resulted in hypothesis that captopril reduces blood pressure by a mechanism other than ACE inhibition (Abe et al 1980; Waeber et al 1980). The finding in this study that serum ACE activity in captopril-treated rats was about 2·5 times higher than in the vehicle-treated rats tends to support (b) the induction of ACE but not (a) the dissociation of enzyme-inhibitor complex. The dissociation of enzyme-inhibitor complex would result in the recovery of captopril-inhibited ACE activity back to but not beyond the control level. However, since the serum samples used in this study were stored for about 5 days, the possible attribution, at least in part, of captopril-induced increase in ACE activity to the dissociation of enzyme-inhibitor complex cannot be excluded at this time. It is interesting to note that MK-421, a potent non-sulfhydryl ACE inhibitor, was found recently to double serum ACE after given orally to rats for 1 or 2 weeks at 10 mg kg^{-1} day^{-2} (Ulm & Vassil 1981).

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