Hypotensive Action of Captopril and Saralasin
in Intact and Anephric
Spontaneously Hypertensive Rats

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SUMMARY Intravenous injection of the converting enzyme inhibitor SQ14,225 (captopril, 2 mg/kg) reduced the blood pressure of anesthetized, spontaneously hypertensive rats (SHR) progressively over a 3-hour period. An indistinguishable fall in blood pressure occurred in SHR that were bilaterally nephrectomized 1 hour prior to injection of the converting enzyme inhibitor. In the nephrectomized animals, plasma renin activity (PRA) had fallen to less than 30% of its initial values at the time of injection. Injection of the vehicle alone had no effect on blood pressure in either anephric or intact SHR. The converting enzyme inhibitor produced no significant change in the blood pressure of either intact or anephric normotensive Wistar-Kyoto (NT-WK) rats. Infusions of Sar'-Ala'-angiotensin II (saralasin, 10 µg/kg/hr) similarly reduced blood pressure of both intact and anephric SHR. These results indicate that captopril and saralasin lower blood pressure in the SHR by some mechanism(s) independent of the kidneys, circulating renin, or bradykinin potentiation. It is suggested that angiotensin II, locally produced at some critical tissue site(s), is involved in the maintenance of raised blood pressure in SHR. (Hypertension 2: 119-124, 1980)

KEY WORDS • SQ14,225 (captopril) • Sar'-Ala'-angiotensin II (saralasin) • spontaneously hypertensive rat • converting enzyme inhibitor • circulating angiotensin II • plasma renin activity • angiotensin receptor blockers • bradykinin

ACUTE administration of converting enzyme inhibitors1 or of angiotensin II receptor blockers2 induces a rapid fall in blood pressure when initial plasma levels of renin3 and/or angiotensin II4 are raised. The orally active inhibitor of angiotensin converting enzyme, -D-methyl-3-mercapto-propanoyl-L-proline (SQ14,225; captopril), however, produces falls of blood pressure in hypertensive patients with low as well as in those with high levels of plasma renin. Riegger et al.6 found that infusion of saralasin or of converting enzyme inhibitor, over a period of 11 hours, produced a slow, progressive fall in blood pressure in renal hypertensive rats that was unrelated to the initial concentration of plasma renin. Captopril has been reported to lower blood pressure in spontaneously hypertensive rats (SHR).7 These animals were reported to have high, normal,8 or low9 plasma renin activity (PRA).

These observations conflict with the widely held hypothesis that converting enzyme inhibitors or angiotensin receptor blockers lower blood pressure by blocking the production or action of circulating
angiotensin II. Since renin and converting enzyme exist in many tissues including vascular wall,\textsuperscript{11, 13} and since renin substrate has been found in brain\textsuperscript{13} and kidney,\textsuperscript{14} it is probable that angiotensin II is formed locally in tissues. This paper compares the blood pressure responses of both intact and anephric SHR and normotensive rats to captopril or saralasin. Since nephrectomy rapidly reduces circulating levels of renin\textsuperscript{10} and angiotensin II,\textsuperscript{16, 17} we compared the blood pressure responses 1 hour after bilateral nephrectomy or sham operation in order to distinguish the effects of the inhibitors on the circulating, as distinct from the tissue, renin-angiotensin systems.

**Methods**

Male SHR and normotensive Wistar-Kyoto rats (NT-WK) weighing 280–360 g were anesthetized with the sodium salt of 5-ethyl-5-(1-methyl-propyl)-2-thiobarbituric acid (Inactin, 110 mg/kg i.p.). This anesthetic was chosen because it does not have the hypotensive effect of other anesthetic agents.\textsuperscript{17} Spontaneously hypertensive rats were prepared as for pressor bioassay\textsuperscript{18} to study the time course of blood pressure responses to 2.5 ng angiotensin I and 1.25 ng angiotensin II before and for 6 hours after i.v. injection of 2 mg SQ14,225/kg. Pulsatile and mean arterial blood pressures were measured continuously via Statham P23dB pressure transducers on a Grass model 7C polygraph recorder.

For the remaining experiments, the rats were anesthetized with Inactin, and the trachea, jugular vein, and carotid artery were cannulated. Body temperature was maintained at 36.5°C by means of a small electric blanket. The animals breathed room air supplemented with moist oxygen delivered to the open end of the tracheal cannula. The animals were bilaterally nephrectomized or subjected to a sham operation 1 hour later. Either 2 mg SQ14,225 or an equal volume of vehicle without the drug was injected i.v. per kg body weight 1 hour after surgery. For the saralasin experiments, either saralasin in 5% dextrose was infused at 10 μl/min or the vehicle, 5% dextrose alone, was infused at 10 μl/min. The saralasin concentration was adjusted for each rat to give 10 μg saralasin kg\textsuperscript{-1}min\textsuperscript{-1}. Blood pressure was measured as described above, for a 3-hour period. There were seven rats in each group. The groups studied were: intact SHR injected with vehicle, intact SHR injected with SQ14,225, anephric SHR injected with SQ14,225, intact SHR infused with 5% dextrose, intact SHR in-
fused with saralasin, anephric SHR infused with 5% dextrose, anephric SHR infused with saralasin, intact NT-WK injected with SQ14,225, and anephric NT-WK injected with SQ14,225. Blood pressures were expressed as mean change in mean arterial blood pressure from preinjection values.

In a separate, untreated series of rats, blood samples (800 μl) for PRA determinations were taken into heparinized plastic tubes on ice, centrifuged for 1 minute in a cold centrifuge (Hettich, Mikrolitre, Tübingen, West Germany) and the plasma frozen until assayed. The erythrocytes were resuspended in saline to a final volume of 800 μl and reinjected into each rat to minimize the effect of hemorrhage. Hourly samples were taken for 3 hours from intact SHR, anephric SHR, intact NT-WK, and anephric NT-WK (n = 7 per group). Plasma renin activity was measured by radioimmunoassay of angiotensin I generated after 4 hours of incubation of the plasma in the presence of the angiotensinase inhibitors 2,3 dimercaprol (1.25 mmol/liter), Na₂ EDTA (10 mmol/liter), and phenylmethylsulphonylfluoride (4.6 mmol/liter) at pH 6.5 and at a temperature of 37°C. Variation is expressed as one standard error from the mean. Data were subjected to analysis of variance, and variance ratios (F) calculated for the variation of blood pressure with time. Variance ratios with p ≥ 0.05 were considered to be not significant.

Results

Intravenous injection of SQ14,225 (2 mg/kg) virtually abolished the pressor response to angiotensin I by 50% of control for 6 hours (fig. 1). The pressor response to angiotensin II was slightly augmented initially but was not thereafter different from control values.

Levels of PRA were similar in the NT-WK and SHR immediately after induction of anesthesia. In the intact anesthetized NT-WK rats, PRA rose progressively, whereas it remained unchanged in the intact, anesthetized SHR. Hence, PRA after 2 and 3 hours was greater in the NT-WK than in the SHR (figs. 2 and 3). After bilateral nephrectomy, PRA fell in NT-WK to 30% of control PRA values at 1 hour,
21% at 2 hours, and 18% at 3 hours. The PRA of the nephrectomized SHR fell to 28% of control at 1 hour, 14% at 2 hours, and 17% at 3 hours (figs. 2 and 3).

In the anesthetized intact NT-WK rats, intravenous injection of SQ14,225 (2 mg/kg body weight) produced no significant change in mean blood pressure from control (fig. 2). Bilateral nephrectomy had no significant effect on mean blood pressure of NT-WK rats. There was no significant change in mean blood pressure from control after intravenous injection of SQ14,225 in these anephric NT-WK rats.

In intact SHR, intravenous injection of SQ14,225 (2 mg/kg) produced a slow, progressive fall in blood pressure over the 3 hours of observation (fig. 3). Injection of vehicle alone had no significant effect on blood pressure of SHR (data not shown). Bilateral nephrectomy did not significantly alter the mean blood pressure of SHR, and intravenous injection of SQ14,225 (2 mg/kg) still produced a slow, progressive hypotensive response identical to that observed in the intact SHR (fig. 3).

Intravenous infusion of saralasin (10 μg/kg·min⁻¹) in intact SHR produced a slow, progressive fall in blood pressure, whereas infusion of vehicle alone did not significantly alter blood pressure (fig. 4). Similar results were observed in the anephric SHR (fig. 4), although the hypotensive response was smaller.

**Discussion**

Theoretically, converting enzyme inhibitors may lower blood pressure by either blockade of formation of the vasopressor peptide angiotensin II, or by impaired degradation of the vasodepressor peptide bradykinin. Although potentiation of the action of bradykinin has not been ruled out as a component of the response to SQ14,225, it seems unlikely to be a major cause of the fall in blood pressure because the hypotensive response observed in the present experiments was of slow onset, whereas bradykinin potentiation is rapid. Matthews and Johnston recently reported that administration of SQ14,225 to rats at doses of 1 to 3 mg/kg produced large increases of PRA and of angiotensin I but had no effect on plasma bradykinin levels unless 10-times higher doses were used. For these reasons, it seems unlikely that the
FIGURE 4. Effect of intravenous infusion of 5% dextrose solution (vehicle, 10 μl/min) or Sar1-Ala2-
anGITENSIN BLOCKADE IN SHR/Hutchinson et al. angiotensin II (saralasin, 10 μg/kg/min⁻¹) in dextrose solution on blood pressure of SHR rats. The infusions were commenced 1 hour after sham operation (intact) or nephrectomy (anephric). Infusion of vehicle had no significant effect on blood pressure of either intact (F = 0.52) or anephric (F = 1.03) SHR. Saralasin infusion significantly lowered the blood pressure of both intact (F = 3.2, p < 0.005) and anephric (F = 6.47, p < 0.005) SHR.

hypotensive effect of SQ14,225 in these experiments is due to bradykinin potentiation. It is possible that captopril lowers blood pressure by unknown actions independent of the renin-angiotensin system. The observation that saralasin produced falls of blood pressure in intact SHR of similar magnitude to those induced by captopril, however, makes this unlikely. It seems most probable that both agents are acting on the angiotensin system.

The failure of bilateral nephrectomy to abolish the hypotensive effect of captopril and saralasin in SHR suggests that these agents are interfering with angiotensin produced at an extrarenal site. At least two possible sites of extrarenal angiotensin formation need to be considered; namely, vascular smooth muscle and brain. There is considerable evidence for the existence of various components of the renin-angiotensin system at both of these sites, and it is possible that angiotensin produced at one or both of these sites could be responsible for the development and maintenance of hypertension in the SHR.

These results resemble those of Thurston and Swales who found that a converting enzyme inhibitor reduced blood pressure in two-kidney, renal hypertensive rats 1 hour after removal of both kidneys. It is possible, therefore, that the renin-angiotensin system may become activated at extrarenal sites in this experimental model. The fact that the converting enzyme inhibitor prevents the development of hypertension in two-kidney, renal hypertensive rats does not necessarily imply that renal (as distinct from extrarenal) angiotensin formation is responsible for the rise in blood pressure in this model.

In the SHR, elevated levels of angiotensin-like peptides have been found in cerebral fluid, and there is some evidence for a similar finding in man. There is also evidence that the intracranial administration of competitive angiotensin antagonists induces falls of blood pressure in various strains of hypertensive rats. Although the blood-cerebrospinal fluid barrier is thought to be impermeable to peptides such as angiotensin II, there is some evidence that saralasin might cross the barrier to antagonize central effects of angiotensin II. Captopril is thought not to penetrate the central nervous system, but this possibility has not been fully investigated. The present
study suggests that activation of the renin-angiotensin system at extrarenal sites may be an important mechanism in hypertension.

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