

Effects of an orally active converting-enzyme inhibitor, SQ 14225, on pressor responses to angiotensin administered into the brain ventricles of spontaneously hypertensive rats

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Summary

1. Two hours after oral feeding of normotensive Wistar Kyoto rats with the converting-enzyme inhibitor SQ 14225 (1 and 10 mg/kg), an increase of angiotensin I plasma concentrations from 892 ± 113 to 1660 ± 167 and 2951 ± 405 pg/ml was observed.

2. Administration of SQ 14225 with the drinking fluid overnight suppressed the pressor responses to intravenous angiotensin I, although the blood pressure increase after intravenous angiotensin II was not altered in normotensive and spontaneously hypertensive rats. No change of pressor effects after injections of angiotensin I and II into the brain ventricles was observed after oral SQ 14225.

3. Intracerebroventricular infusions of SQ 14225 in spontaneously hypertensive rats abolished the central pressor responses to angiotensin I.

4. The results show that SQ 14225 effectively blocked the conversion of endogenous and exogenous angiotensin I into angiotensin II in plasma, and the conversion of exogenous angiotensin I in brain. Under the conditions studied, SQ 14225 does not cross the blood-cerebrospinal fluid barrier in spontaneously hypertensive rats.

Key words: angiotensin, brain, converting enzyme, hypertension, spontaneously hypertensive rats, SQ 14225.

Abbreviation: ANG, angiotensin.

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Introduction

The role of the renin-angiotensin system has been investigated by selective inhibition of the enzymes leading to the formation of the effector peptide angiotensin II (ANG II) or by blockade of ANG II receptors. The formation of angiotensin I (ANG I) from angiotensinogen by renin can be inhibited by pepstatin and by competitive substrate analogues. The conversion of ANG I into ANG II is prevented by peptides such as the nonapeptide SQ 20881 (for review see Ganten & Gross, 1977). Recently, a new converting-enzyme inhibitor, SQ 14225, has been synthesized. This compound is a proline derivative (3-mercapto-2-D-methylpropanoyl-1-proline) and is remarkable because it is the first renin-angiotensin system inhibitor that is orally effective (Ondetti, Rubin & Cushman, 1977). SQ 14225 (Captopril) has been shown to competitively inhibit purified lung converting enzyme *in vitro* (Ondetti *et al.*, 1977) and, after oral application, it antagonizes the pressor action of intravenously administered ANG I in rats (Rubin, Laffan, Kotler, O'Keefe, Demaio & Goldberg, 1978), in cats (Vollmer & Boccagno, 1977) and in man (Ferguson, Turini, Brunner, Gavras & McKinstry, 1977). However, intravenously injected SQ 14225 did not prevent the pressor effects of ANG I infused into the brain ventricles of cats (Vollmer & Boccagno, 1977).

The present experiments were undertaken to investigate the effects of oral SQ 14225 on ANG I and ANG II concentrations in plasma and on the pressor responses to intravenous ANG I and ANG II. Moreover, we wanted to test whether SQ 14225 can cross the blood-cerebrospinal fluid barrier in

hypertensive rats, because chronic elevation of arterial blood pressure may increase the permeability of the blood-cerebrospinal fluid barrier (Haebara, Dietz, Mann & Lüth, 1977). In spontaneously hypertensive rats we had previously found that central angiotensin blockade by the ANG II receptor antagonist [Sar¹, Val⁵, Ala⁸]ANG II (saralasin) induced a decrease of arterial blood pressure, whereas peripheral administration of saralasin led to an increase of blood pressure (Mann, Phillips, Dietz, Haebara & Ganten, 1978a). These results suggested a role for the brain renin-angiotensin system in the maintenance of high blood pressure in spontaneously hypertensive rats. Thus, if SQ 14225 could cross the blood-brain barrier, it might decrease ANG II concentrations in the brain and lower blood pressure in spontaneously hypertensive rats.

Material and methods

General procedure

Male spontaneously hypertensive rats of the stroke-prone strain, bred in Heidelberg for more than 3 years (body weight: 240–330 g), and male normotensive Wistar Kyoto control rats (body weight: 290–400 g) were used. Rats were housed in single cages in a room automatically lighted from 06.00 to 18.00 hours with constant temperature ($24 \pm 1^\circ\text{C}$) and humidity ($60 \pm 3\%$). Demineralized water and a standard pellet chow containing 100 mmol of sodium/kg and 210 mmol of potassium/kg were offered *ad libitum*.

Oral feeding in Wistar Kyoto rats (Expt. 1)

Wistar Kyoto rats were adapted to oral feeding by gavage for 10 days. Thereafter, SQ 14225 was administered orally in demineralized water (1 mg/kg, $n = 9$; 10 mg/kg, $n = 7$). Nine rats (controls) received demineralized water only. Two hours later, the rats were anaesthetized with ether and blood was withdrawn from the aorta in syringes containing angiotensinase inhibitors. The blood was transferred immediately into polyethylene tubes, which were placed in iced water and then centrifuged at 10 000 *g* for 10 min at 4°C . The plasma was separated from the blood cells at 4°C , quickly frozen on solid carbon dioxide and stored at -30°C until assayed for ANG I and ANG II concentrations in unextracted plasma. Cross-reactivity of ANG I antibodies with ANG II, ANG-(2-8)heptapeptide, ANG-(3-8)hexapeptide,

ANG-(4-8)pentapeptide and [Sar¹, Val⁵, Ala⁸]ANG-(1-8)octapeptide (saralasin) was less than 0.001%. Cross-reactivity of ANG II antibodies with ANG I was 1%, with saralasin 0.001%, and with ANG-hepta-, ANG-hexa- and ANG-penta-peptide fragments 100% (Oster, Hepp & Hackenthal, 1973; Ganten, Fuxe, Phillips, Mann & Ganten, 1978).

Intravenous injections of ANG I and ANG II (Expt. 2)

Wistar Kyoto rats ($n = 4$) received demineralized water *ad libitum* by graduated chemical burettes adapted with drinking spouts. Water intake during a 5 day adaptation period was 24–28 ml/night. On the day before the experiment, catheters were implanted under ether anaesthesia into the femoral vein (PP 10 tubing, Portex, Middlesex, U.K.) and into the femoral artery (PP 10 connected to PP 50) and tunnelled under the skin to exit through the scruff of the neck. The catheters were filled with heparinized NaCl solution (154 mmol/l) and sealed. The rats were returned to their home cages. During the following night, the rats were proffered SQ 14225 dissolved in the drinking water (0.1 mg/ml). Rats drank 24.3 ± 2.8 ml during that night. On the following morning at 06.00 hours, the arterial catheter was connected to a Statham P 23 Db pressure transducer for continuous recording of blood pressure via Gould blood pressure computers on a Gould-Brush model 2400 recorder (Gould Instruments Division, Cincinnati, Ohio, U.S.A.). The venous catheter was connected to a remote syringe using a 80 cm piece of PP 10 tubing. ANG I (Schwarz-Mann, Orangeburg, New York, U.S.A.) and ANG II (Hypertensin, CIBA) were injected as bolus in isomolar concentrations (47 and 94 pmol/kg) at 06.00, 07.00, 08.00, 09.00 and 11.00 hours. Injection volume was 1 ml/kg. During testing the rats were awake and freely moving in a wooden cage (12 cm \times 15 cm \times 10 cm).

Intravenous and intraventricular administration of ANG I and ANG II in spontaneously hypertensive rats (Expt. 3)

Spontaneously hypertensive rats ($n = 12$) received demineralized water in graduated burettes as described above. Each rat had a stainless-steel cannula in the right lateral cerebral ventricle (Mann *et al.*, 1978a). One week later, catheters were implanted into the femoral vein and into the

femoral artery under ether anesthesia. After 3–4 h, blood pressure was recorded in the awake, freely moving rats. The next day, SQ 14225 (3 mg/kg) was given to the rats ($n = 6$) by gavage four times with 3 h intervals. The following night, SQ 14225 (0.1 mg/ml) was added to the drinking water. Controls ($n = 6$) received demineralized water. The experimental rats drank 26 ± 4 ml. The following morning at 06.00 hours, another dose of SQ 14225 (3 mg/kg) or demineralized water was given by gavage. Thereafter, the arterial catheter was connected to a pressure transducer for continuous recording of arterial pressure in the conscious rats. ANG I and ANG II (47 pmol/kg) were given as bolus injections intravenously. After recovery of baseline blood pressures, ANG I and ANG II (9.4 and 94 pmol/min) dissolved in NaCl solution (154 mmol/l) were infused intraventricularly at a rate of $2 \mu\text{l}/\text{min}$ for 5 min. The time interval between each dose was 20–30 min to allow for re-establishment of the original blood pressure. Two hours after the last SQ 14225 administration, ANG I and ANG II (47 pmol/kg) were again given intravenously.

Intraventricular administration of SQ 14225 in spontaneously hypertensive rats (Expt. 4)

Spontaneously hypertensive rats ($n = 6$) were each implanted with a permanent cannula in the lateral brain ventricle. One week later, catheters (PP 50) were inserted into the femoral artery under α -chloralose anaesthesia (30 mg/kg intravenously). Arterial blood pressure was recorded continuously. SQ 14225 was infused at cumulative doses of 1, 10 and 100 $\mu\text{g}/\text{min}$ for 30 min each at a rate of $2 \mu\text{l}/\text{min}$. Control saline infusions were performed in a cross-over design. Intervals between the saline control infusions and SQ 14225 were at least 1 h. Immediately after the last SQ 14225 infusion, ANG I was infused intraventricularly at 94 pmol/min for 10 min.

Data are given as means \pm SEM. Significance of differences was calculated by using Student's *t*-test for unpaired data, if not stated otherwise.

Results

The oral administration of the converting-enzyme inhibitor SQ 14225 (1 and 10 mg/kg) (Expt. 1) resulted in a dose-dependent increase of ANG I plasma concentrations from 892 ± 113 pg/ml (control) to 1660 ± 167 and 2951 ± 405 pg/ml ($P < 0.01$). ANG II plasma concentrations tended to decrease but differences were not significant

(Fig. 1). However, the ratio ANG II/ANG I, indicating conversion rate, decreased significantly from 0.099 ± 0.009 (control) to 0.053 ± 0.007 and 0.002 ± 0.001 (all differences significant, $P < 0.01$).

The effects of intravenous bolus injections of ANG I and ANG II (Expt. 2) on mean arterial blood pressure in rats that received SQ 14225 overnight are shown in Fig. 2. The first injection was administered at 06.00 hours, i.e. at the end of the dark period in the animal room. The blood pressure increase after intravenous ANG I was greatly diminished and gradually increased during the next 5 h. SQ 14225 had no influence on blood pressure increase elicited by intravenous ANG II.

In spontaneously hypertensive rats (Expt. 3), oral SQ 14225 treatment resulted in a fall in mean arterial blood pressure from 190.5 ± 13.6 mmHg to 137.5 ± 13.3 mmHg after treatment. In the control rats, mean arterial blood pressure was 184.8 ± 9.4 before, and 179.0 ± 9.9 mmHg after, sham-treatment. The decrease was significant in the experimental group when pre- and post-treatment values were compared ($P < 0.05$, paired *t*-test). This was not the case in the sham-treated group (P

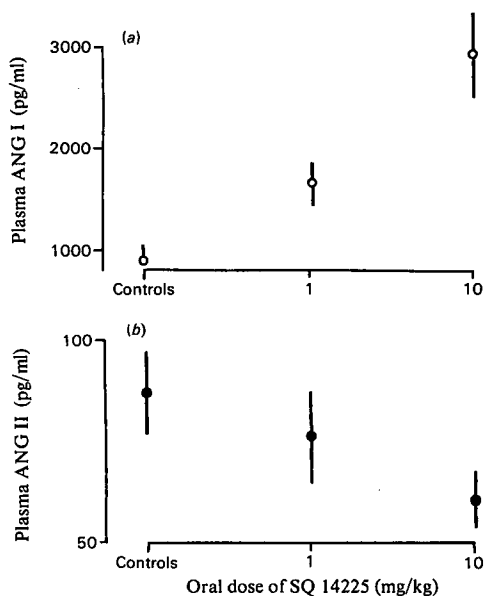


FIG. 1. Effects of oral doses of SQ 14225 in Wistar Kyoto rats on plasma concentrations of ANG I (a) and ANG II (b). Doses of SQ 14225 are given on the abscissa (controls: rats receiving water). Increases of ANG I plasma concentrations were significant at a dose of 1 mg of SQ 14225/kg ($P < 0.01$) and at a dose of 10 mg/kg ($P < 0.01$, as compared with controls and with effects of the 1 mg/kg dose).

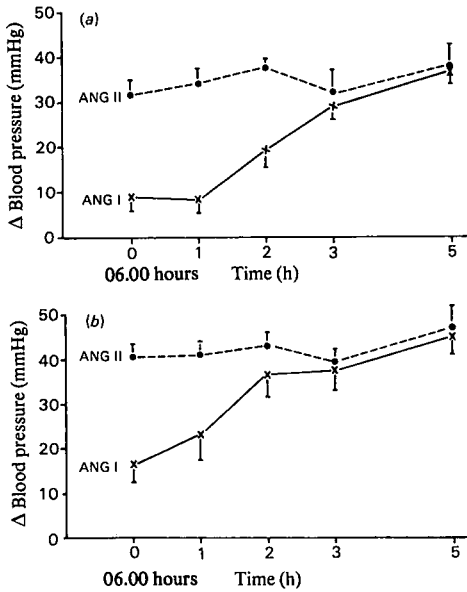


FIG. 2. Blood pressure increase to intravenous ANG I (x) and ANG II (●) in Wistar Kyoto rats after SQ 14225 was supplied overnight (18.00–06.00 hours) in the drinking fluid (4 mg/20 ml). ANG I and ANG II (a: 47 pmol/kg; b: 94 pmol/kg) were given at times indicated on the abscissa. The pressor effects of 47 pmol of ANG I/kg were reduced at 06.00, 07.00 and 08.00 hours and the effects of 94 pmol of ANG I/kg at 06.00 hours ($P < 0.01$, as compared with the effect at 11.00 hours and with the effects after ANG II).

> 0.2, paired *t*-test). Differences of mean arterial blood pressure between treated and sham-treated animals were not significant. The pressor responses after intravenous injection of ANG I were completely abolished in the SQ 14225-treated rats as compared with controls (1.5 ± 1.5 vs 18.3 ± 2.5 mmHg) and recovered 5 h later (17.0 ± 1.9 vs 18.0 ± 2.0 mmHg). There was no influence of SQ 14225 on the blood pressure effects of intravenous ANG II. When ANG I and ANG II were infused intraventricularly, mean arterial blood pressure increased in a similar fashion with both peptides (Fig. 3).

When SQ 14225 was infused intraventricularly in spontaneously hypertensive rats (Expt. 4: 190 ± 14.2 mmHg initial resting mean arterial blood pressure) the pressor responses to intraventricular ANG I were completely blocked.

Discussion

Our results show that SQ 14225 effectively blocks the conversion of the decapeptide ANG I into the

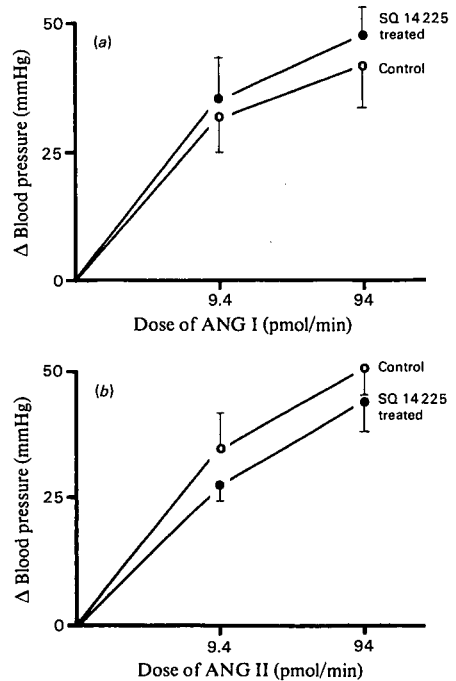


FIG. 3. Blood pressure increase in spontaneously hypertensive rats treated with SQ 14225 by gavage (●) and spontaneously hypertensive controls (○) after intraventricular infusions of ANG I (a) and ANG II (b) in isomolar concentrations (doses are given on the abscissa).

octapeptide ANG II in the blood and in the brain. Administration of the converting-enzyme inhibitor resulted in 2- to 4.5-fold increase of ANG I plasma concentrations and a decrease of the conversion of ANG I into ANG II. When the renin-angiotensin system is stimulated a significant reduction of ANG II plasma concentrations after oral SQ 14225 has been observed (Mann, Rascher, Dietz & Schömig, 1978b). The effectiveness of SQ 14225 was also demonstrated after administration of the drug overnight in the drinking fluid; intravenous ANG I pressor effects on the next morning were markedly suppressed. This shows that SQ 14225 is sufficiently stable and, at the doses used, provides effective plasma concentrations for 2 h after the last feeding. Blood pressure fell after oral SQ 14225 treatment. The extent of the pressure decrease was variable and some sham-treated rats also showed a reduction of blood pressure. This may be explained by a combination of the second test day being less 'stressful' to the rats, and drug effect.

The major portion of plasma ANG I is cleaved

to ANG II during passage through the lungs. Besides this organ, many other tissues, including arteries and the brain, have been found to contain high converting-enzyme activity (Ganten *et al.*, 1978). It is possible that SQ 14225 blocked the formation of ANG II only in the blood, leaving converting-enzyme activity in other tissues unchanged.

Our experiments show that orally applied SQ 14225 did not cross the blood-cerebrospinal fluid barrier in spontaneously hypertensive rats, since the pressor responses to intraventricular ANG I were unchanged. The effectiveness of SQ 14225 treatment was demonstrated by the abolishment of intravenous ANG I effects in the treated animals. When SQ 14225 was infused into the cerebral ventricles, it completely inhibited the central pressor responses to ANG I. This demonstrates that the brain converting enzyme can be inhibited by SQ 14225.

SQ 14225 had no effect of decreasing blood pressure in spontaneously hypertensive rats after its intraventricular administration. It had previously been reported (Mann *et al.*, 1978a; Phillips, Mann, Haebara, Hoffman, Dietz, Schelling & Ganten, 1977) that central ANG II receptor blockade by saralasin lowered blood pressure in the same strain of spontaneously hypertensive rats. Inhibition of the renin-angiotensin system at the level of the converting enzyme is much less specific, however, than by ANG II receptor blockade. Converting enzyme interferes with another peptide system: it inactivates bradykinin and SQ 14225 has been shown to potentiate the action of kinins *in vitro* and *in vivo* (Rubin *et al.*, 1978). Kinins exert a blood pressure-increasing effect when infused into the brain ventricles (Correa & Graeff, 1974). It may therefore be speculated that blood pressure decreases by inhibition of the formation of ANG II could be counteracted by the blood-pressure-increasing effects of elevated kinins in the brain. This question is presently being investigated in our laboratory. The interpretation of our results on intraventricular SQ 14225 in spontaneously hypertensive rats is limited to acute administration of the drug in α -chloralose-anaesthetized rats.

We conclude that the pressor action of ANG I infused into the brain ventricles depends on its conversion into ANG II within the brain. SQ 14225 inhibited this enzymatic activity. The converting-enzyme inhibitor, however, did not cross the blood-cerebrospinal fluid barrier in spontaneously hypertensive rats and thus, when applied orally, its actions are most probably not due to blockade of

converting enzyme in the cerebrospinal fluid. The possibility that inhibition of converting enzyme in brain tissue is not detected by the procedure used in these experiments needs to be tested. It also remains to be studied whether the conclusions are valid after prolonged treatment with the converting-enzyme inhibitor.

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References

- CORREA, F.M.A. & GRAEFF, F.G. (1974) Central mechanisms of the hypertensive action of intraventricular bradykinin in the unanesthetized rat. *Neuropharmacology*, **13**, 65-75.
- FERGUSON, R.K., TURINI, G.A., BRUNNER, H.R., GAVRAS, H. & MCKINSTRY, D. (1977) A specific orally active inhibitor of angiotensin converting enzyme in man. *Lancet*, **i**, 775-778.
- GANTEN, D. & GROSS, F. (1977) Interferences with the renin-angiotensin system and their effects on high blood pressure. In: *Handbook of Experimental Pharmacology*, vol. XXXIX, pp. 517-546. Ed. Gross, F. Springer Verlag, Berlin-Heidelberg-New York.
- GANTEN, D., FUXE, K., PHILLIPS, M.I., MANN, J.F.E. & GANTEN, U. (1978) The brain isorenin-angiotensin system: Biochemistry, localization, and possible role in drinking and blood pressure regulation. In: *Frontiers in Neuroendocrinology*, pp. 61-99. Ed. Ganong, W.F. & Martini, L. Raven Press, New York.
- HAEBARA, H., DIETZ, R., MANN, J.F.E. & LÜTH, J.B. (1977) Gefäßveränderungen beim genetischen Hochdruck der Ratte. *Verhandlungsberichte der Deutschen Gesellschaft für Kreislauforschung*, **43**, 15-19.
- MANN, J.F.E., PHILLIPS, M.I., DIETZ, R., HAEBARA, H. & GANTEN, D. (1978a) Effects of central and peripheral angiotensin blockade in hypertensive rats. *American Journal of Physiology*, **234**, H629-H637.
- MANN, J.F.E., RASCHER, W., DIETZ, R. & SCHÖMIG, A. (1978b) Inhibition of the renin-angiotensin system in rats with hereditary hypothalamic diabetes insipidus. *Klinische Wochenschrift*, **56** (Suppl. 1), 67-70.
- ONDETTI, M.A., RUBIN, B. & CUSHMAN, D.W. (1977) Design of specific inhibitors of angiotensin-converting enzyme: A new class of orally active antihypertensive agents. *Science*, **196**, 441-444.
- OSTER, P., HEPP, R. & HACKENTHAL, E. (1973) Radioimmunoassay of angiotensin II in rat plasma. *Experientia*, **29**, 353-354.
- PHILLIPS, M.I., MANN, J.F.E., HAEBARA, H., HOFFMAN, W.E., DIETZ, R., SCHELLING, P. & GANTEN, D. (1977) Lowering of hypertension by central saralasin in the absence of plasma renin. *Nature (London)*, **270**, 445-447.
- RUBIN, B., LAFFAN, R.J., KOTLER, D.G., O'KEEFE, E.H., DEMAIO, D.A. & GOLDBERG, M.E. (1978) SQ 14225 (D-3-mercapto-2-methylpropanoyl-L-proline), a novel orally active inhibitor of angiotensin I-converting enzyme. *Journal of Pharmacology and Experimental Therapeutics*, **204**, 271-280.
- VOLLMER, R.R. & BOCCAGNO, J.A. (1977) Central cardiovascular effects of SQ 14225, an angiotensin I converting enzyme inhibitor in chloralose-anesthetized cats. *European Journal of Pharmacology*, **45**, 117-125.