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EFFECT OF CONVERTING ENZYME BLOCKADE ON ISOPRENALINE- AND ANGIOTENSIN I-INDUCED DRINKING

G. HERTTING & D.K. MEYER

Pharmakologisches Institut der Universität Freiburg i. Br., Freiburg, West Germany

1 Intravenous infusion of 0.072 μmol kg^{-1} h^{-1} (1-Asp, 5-Ile) angiotensin I, 0.116 μmol kg^{-1} h^{-1} of (1-Asp β-amid, 5-Val) angiotensin II or of (1-Asp, 5-Ile) angiotensin II, caused food-deprived and water-satiated rats to drink about 3.0 ml of water per animal. This indicates that angiotensin I has a 1.6 times stronger dipsogenic effect than the angiotensin II preparations when infused intravenously.

2 The converting-enzyme-inhibitor SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) (1.0 mg/kg i.v.) had no intrinsic dipsogenic effect.

3 SQ 20881 given in combination with angiotensin I or angiotensin II potentiated the dipsogenic effect of angiotensin I, but not that of the two angiotensin II preparations.

4 The drinking induced by isoprenaline (100 μg/kg, i.m.) was potentiated by SQ 20881 to the same extent as drinking produced by angiotensin I infusion.

5 Angiotensin I plasma levels were determined after angiotensin I infusion and isoprenaline application. Both were significantly raised by SQ 20881.

6 It is concluded that one of the mechanisms which mediate the dipsogenic effect of isoprenaline is stimulation of the renin-angiotensin system and that increased plasma levels of angiotensin I may play a substantial role in this type of drinking.

Introduction

Isoprenaline causes compulsive drinking (Lehr, Mallow & Krukowski, 1967; Peskar, Leodolter & Hertting, 1970) and a simultaneous increase in the plasma concentration of renin and angiotensin I (Peskar, Meyer, Tauchmann & Hertting, 1970; Meyer & Hertting, 1973), when given to rats intramuscularly. It has been postulated previously that isoprenaline-induced drinking is triggered off by stimulation of the renin-angiotensin system (Meyer, Peskar, Tauchmann & Hertting, 1971; Gutman, Benzakein & Livneh, 1971; Meyer & Hertting, 1973). This assumption is confirmed by the following observations:

(1) Intravenous infusion of angiotensin II causes compulsive drinking in rats (Fitzsimons & Simons, 1969).
(2) Acute nephrectomy inhibits drinking after isoprenaline (Meyer, Peskar & Hertting, 1971; Houpt & Epstein, 1971).
(3) Blockade of sympathetic β-adrenoceptors by (−)-propranolol prevents the increase in plasma renin concentration and drinking caused by isoprenaline (Meyer, Rauscher, Peskar & Hertting, 1973).

It was considered that a study of the effects of inhibition of the converting enzyme might be helpful in elucidating the role of the renin-angiotensin system in isoprenaline-induced drinking. Blockade of this enzyme prevents the conversion of angiotensin I to angiotensin II. The latter is regarded as the most active substance biologically of the renin-angiotensin system. The nonapeptide SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) has been shown to be a potent inhibitor of the converting enzyme *in vitro* (Igic, Erdös, Yeh, Sorrelss & Nakajima, 1972) and *in vivo* (Keim, Kirpen, Peterson, Murphy, Hassert & Poutsiaka, 1972).

In the present study the effect of SQ 20881 on isoprenaline-induced drinking was tested. These results were compared with the effect of SQ 20881 on the drinking caused by intravenous infusions of angiotenin I and II. Angiotensin I plasma levels were measured to check the efficiency of the converting enzyme inhibition by SQ 20881.

Methods

Male Wistar rats v re used. The animals were deprived of food for 16 h before the experiment.

Water remained accessible *ad libitum*. Animals of the same weight and age were used in individual experiments.

Drinking experiments

During these experiments the animals were kept in individual cages where they had access to burettes filled with tap water.

Angiotensin infusion studies. The tail vein was cannulated for the application of drugs. SQ 20881 (1.0 mg/kg) dissolved in 0.9% w/v NaCl solution (saline) was injected intravenously in a volume of 0.1 ml per 100 g body weight. Controls received a similar volume of saline. It had been demonstrated in pilot studies that the effect of this dose of SQ 20881 lasted for at least one hour. Five min after this injection the infusion of the angiotensin preparation was begun. For constant infusion a motor pump (Braun-Melsungen) (infusion rates 0.02 ml/min) was used. Angiotensin I (0.0155 mg/ml) and the two angiotensin II preparations (0.020 mg/ml) were dissolved in 0.1 M phosphate buffer (300 mosmol) (pH 7.2). Controls received phosphate buffer. Animals of the same weight (200 g) were used in the infusion experiments. Water intake was recorded 1 h after the start of the infusion.

Isoprenaline studies. SQ 20881 was given intravenously 5 min before isoprenaline. Isoprenaline (0.2 mg/kg) was injected intramuscularly in a volume of 0.1 ml per 100 g body weight; drinking was recorded 1 h after the injection of isoprenaline. Animals of the same weight (250 g) were used in these experiments.

Statistical evaluation of the results from all drinking experiments was made with the distribution free Wilcoxon test.

Studies on plasma angiotensin concentration

For these experiments the animals were pretreated in the same way as in the drinking experiments. Animals weighing 200 g were used.

Angiotensin I infusion studies. Twenty min after the start of the angiotensin infusion (0.093 mg kg^{-1} h^{-1}) 4 ml of blood were collected from the abdominal aorta of rats lightly anaesthetized with ether. During the time of collection the infusion was continued. To prevent the breakdown of angiotensin I by the converting enzyme or angiotensinases, the blood was aspirated into a syringe containing 1.0 ml of a solution of disodium edetate (40.0 mg) and 8-hydroxyquinoline (2.0 mg). The plasma was separated immediately by centrifugation in a cooled centrifuge.

Isoprenaline studies. The animals were exsanguinated 30 min after the injection of isoprenaline (0.2 mg/kg). Angiotensin I was determined by radioimmunoassay (Haber, Koerner, Page, Kliman & Purnode, 1969). Recovery of added angiotensin I was 95%. Angiotensin II and SQ 20881 added in concentrations 10 times higher than those to be expected in the plasma samples did not show an inhibition of the binding of ^{125}J-angiotensin I to the antiserum. Student's *t*-test was used for statistical evaluation.

Experiments with nephrectomized animals

Rats weighing about 300 g were used in these experiments. They were nephrectomized under light ether anaesthesia through a dorsal incision. In sham-nephrectomized rats the operation procedure was simulated; the kidneys were prepared, but not ligated and cut out. SQ 20881 was injected into the tail vein 2.5 h after the operation. Controls received saline. Water intake was recorded for 1 h after this injection.

Drugs

The following drugs were used: SQ 20881 (Squibb); (1-Asp, 5-Ile) angiotensin I and (1-Asp, 5-Ile) angiotensin II (Schwarz-Mann); (1-Asp β-amid, 5-Val) angiotensin II (Ciba); isoprenaline hydrochloride (Boehringer, Ingelheim).

Results

Dipsogenic activity of SQ 20881

In the first experiment the dipsogenic activity of the nonapeptide SQ 20881 alone was measured. To prevent the possible interference of SQ 20881 with the renin-angiotensin system, acutely nephrectomized rats were used in addition to sham-nephrectomized ones. The effect of SQ 20881 (1.0 mg/kg) (the dose used in all experiments) is shown in Table 1. In sham-nephrectomized animals SQ 20881 increased drinking significantly ($\alpha = 0.01$). In rats deprived of the endogenous renin-angiotensin system the compound had no effect on water intake.

Effect of SQ 20881 on the drinking elicited by the infusion of (1-Asp, 5-Ile) angiotensin I, (1-Asp, 5-Ile) angiotensin II and (1-Asp β-amid, 5-Val) angiotensin II

The results of this experiment are shown in Table 2. Angiotensin I and the two angiotensin II preparations significantly increased drinking.

Identical amounts of water (~3.0 ml per animal) were drunk during the infusion of 0.120 mg/kg of the two different angiotensin II preparations (0.116 μmol) and of 0.093 mg/kg angiotensin I (0.072 μmol) for 1 hour.

Application of the converting-enzyme-inhibitor SQ 20881 significantly potentiated the effect of angiotensin I on drinking whereas the dipsogenic effect of both angiotensin II peptides was unchanged by SQ 20881.

Effect of SQ 20881 on plasma levels of angiotensin I and drinking induced by the infusion of angiotensin I

Angiotensin I infusion (0.093 mg kg^{-1} h^{-1}, i.v.) caused a significant increase in plasma angiotensin I concentration and drinking as compared to the controls (Figure 1). The administration of SQ 20881 (1.0 mg/kg, i.v.) significantly enhanced the angiotensin I plasma level as well as drinking. Given alone, SQ 20881 caused a significant increase in the angiotensin I plasma levels. The increase in water intake after SQ 20881 was not significant because of the great variation (Figure 1).

Effect of SQ 20881 on isoprenaline-induced elevation of plasma angiotensin I and drinking

The increase in water intake and plasma angiotensin I normally observed after the application of isoprenaline (200 μg/kg) was further enhanced by SQ 20881 (Figure 2).

Table 1 Water intake of rats 1 h after intravenaus SQ 20881

Drug: total amount (mg/kg)		*Number of animals*	*Water drunk/animal (ml)*	P
Controls nephr.		7	0.24	–
Controls sham.		7	0.79	–
Nephr. + SQ 20881	1.0	11	0.33	NS
Sham. + SQ 20881	1.0	11	2.9	0.01

Mean values are given. SQ 20881-treated animals were compared statistically with respective controls. Nephr., nephrectomized animals. Sham = sham-operated animals

Table 2 Effect of angiotensin I or II alone and in combinations with SQ 20881 on water intake of rats

Drug: total amount (mg/kg)		*Number of animals*	*Water drunk/animal (ml)*	P
Controls		6	1.15	–
SQ 20881 (1-Asp β-amid, 5-Val)	1.0	6	1.23	NS
At II (1-Asp β-amid, 5-Val)	0.120	6	3.00	0.025
At II + SQ (1-Asp, 5-Ile)		6	2.66	NS
At II (1-Asp, 5-Ile)	0.120	6	3.20	0.025
At II + SQ (1-Asp, 5-Ile)		6	3.09	NS
At I (1-Asp, 5-Ile)	0.093	6	2.89	0.025
At I + SQ		6	5.29	0.01

Water intake was measured 1 h after start of angiotensin or solvent infusion. Values given are means. Rats infused with angiotensin I (At I) or II (At II) or SQ 20881 alone were compared with solvent-infused controls. Rats infused with angiotensin I or II plus SQ 20881 were compared with animals infused with angiotensin I or II alone.

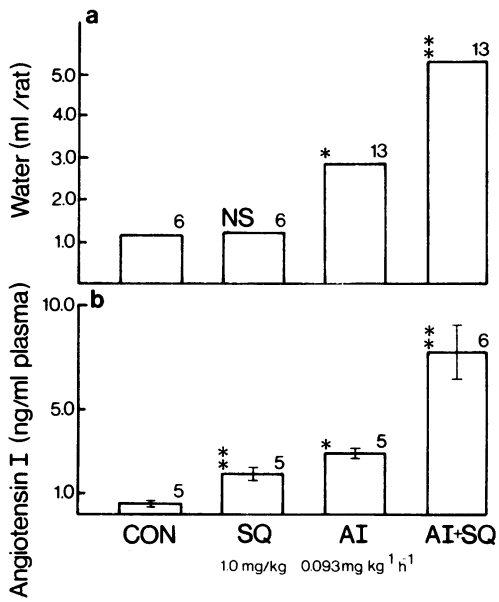

Fig. 1 (a) shows total water intake per rat after 1 h of infusion of solvent or angiotensin I. SQ 20881 or its solvent were given (i.v.) 5 min before the start of the infusion.

(b) shows angiotensin I plasma levels determined 20 min after the start of infusion (plasma levels were constant at this time). Asterisks indicate a significant difference from the next column on the left side. Means are given for the water intake (Wilcoxon test). Means with s.e. mean are given for the angiotensin I plasma levels (Student's *t*-test). *, $P < 0.05$; **, $P < 0.01$; NS, not significant.

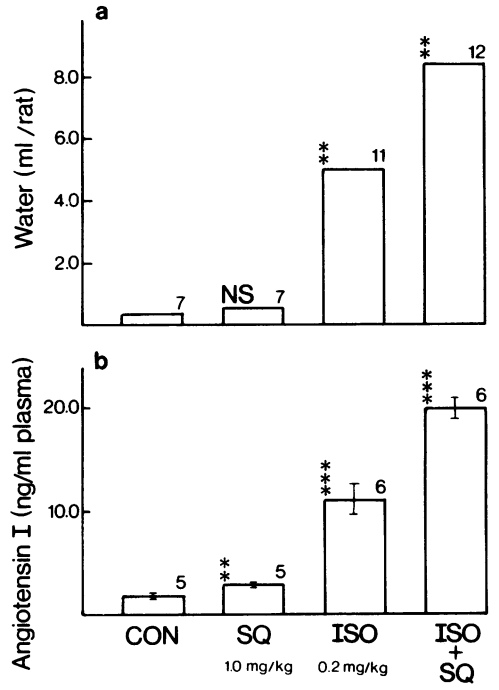

Fig. 2 (a) shows total water intake per rat 1 h after the injection of solvent or isoprenaline. SQ 20881 or its solvent were given (i.v.) 5 min before this treatment.

(b) shows plasma angiotensin I levels 30 min after the injection of isoprenaline or solvent. For further explanation see legend to Figure 1.

Discussion

The renin-angiotensin system has been firmly established as one of the factors which regulate drinking (Fitzsimons, 1969), angiotensin II receiving the greatest attention.

The data presented here indicate that angiotensin I plays an essential role in drinking. Angiotensin I does not only cause drinking but, when given peripherally, it may be an even stronger dipsogen than angiotensin II. This can be concluded from the following observations:

(1) The infusion of 0.072 μmol kg^{-1} h^{-1} of (1-Asp, 5-Ile) angiotensin I causes rats to drink about 3.0 ml of water. The animals drink the same amount, when they receive 0.112 μmol kg^{-1} h^{-1} (1-Asp, 5-Ile) angiotensin II or (1-Asp β-amid, 5-Val) angiotensin II. It should be noted that both angiotensin II preparations, though differing in their amino acid sequence, have the same potency. This result indicates that angiotensin I is about 1.6 times as active as a dipsogen as the angiotensin II preparations and confirms the results of Epstein (1972), who compared (1-Asp, 5-Ile) angiotensin I and (1-Asp β-amid, 5-Val) angiotensin II, and found angiotensin I twice as active as angiotensin II as a dipsogen.

(2) Blockade of converting enzyme by SQ 20881 in angiotensin I-infused animals leads to an accumulation of angiotensin I and consequently to a decrease of the plasma levels of angiotensin II. This is accompanied by a further enhancement of drinking.

The possibility that an intrinsic dipsogenic

activity of SQ 20881 has influenced the experiments can be excluded. It was shown that SQ 20881 does not cause drinking in nephrectomized rats, which are deprived of the renin-angiotensin system, whereas it significantly enhanced drinking in sham-operated animals. Both groups of animals were used 2.5 h after the operation. During this short period the still intact renin-angiotensin system of the sham-operated animals responded to the stress of the operation and blood loss. This response was multiplied by SQ 20881 and caused significant drinking. In the nephrectomized animals this response could not occur and so the rats did not drink. Furthermore, a possible intrinsic dipsogenic activity of SQ 20881 can be excluded because SQ 20881 potentiated the effect of angiotensin I on drinking, but not the effects of both angiotensin II preparations. The latter experiment further excludes the possibility that SQ 20881 acted by inhibition of angiotensinases, since in this instance the effect of angiotensin II should also be enhanced. The strictly parallel alteration of drinking behaviour and angiotensin I plasma levels additionally supports the assumption that SQ 20881 enhances drinking by inducing an accumulation of angiotensin I.

Severs, Summy-Long & Daniels-Severs (1973) found no effect of peripherally administered SQ 20881 on drinking caused by centrally administered angiotensin I. When both drugs were given centrally, SQ 20881 inhibited the actions of angiotensin I which, given alone, caused drinking. They concluded that peripherally administered SQ 20881 does not reach central sites. These authors did not investigate the effect of SQ 20881 on angiotensin I-induced drinking, when both drugs were given intravenously.

Isoprenaline (0.200 mg/kg) caused an increase in drinking and in the plasma level of angiotensin I. The angiotensin I plasma concentration (determined 30 min after application of the drug) was about 3-4 times higher than the plasma concentration observed during the infusion of angiotensin I (0.072 μmol kg^{-1} h^{-1}). SQ 20881 caused an increase in the isoprenaline-induced elevation of plasma levels of angiotensin I and further enhanced water intake. This supports the assumption that the increase in plasma angiotensin I produced by isoprenaline causes drinking.

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