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INFLUENCE OF CONVERTING ENZYME INHIBITION ON THE RELEASE OF VASOPRESSIN INDUCED BY ANGIOTENSIN

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1 The effects of intravenous infusion of angiotensin I, II and (des-1-Asp) angiotensin II (angiotensin III) on the plasma vasopressin levels, with and without converting enzyme inhibition, were investigated in conscious rats by use of a specific radioimmunoassay.

2 All three peptides caused a dose-dependent increase in vasopressin release, angiotensin III infusion being less effective than angiotensin I or II.

3 The converting enzyme inhibitor, SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) (1.0 mg/kg, i.v.), had no effect on the plasma vasopressin concentrations in bilaterally nephrectomized rats, but increased them in intact or sham-operated animals.

4 SQ 20881 potentiated vasopressin release elicited by angiotensin I, leaving that elicited by angiotensin II unchanged. The receptor antagonist, saralasin, prevented the angiotensin-induced increase in plasma vasopressin concentration, even after pretreatment with SQ 20881.

5 These data support the assumption that the renin-angiotensin system may be involved in the control of vasopressin release, and indicate that in addition to angiotensin II, angiotensin I and III may also contribute, acting in concert.

Introduction

Plasma osmolality, blood volume and blood pressure govern the release of vasopressin from the posterior pituitary (see Kurtzman & Boonjarern, 1975). Another mechanism, the renin-angiotensin system, may also contribute to the regulatory process. This assumption is based on reports that intravenous infusions of angiotensin II increase the plasma levels of vasopressin in man (Uhlich, Weber, Eigler & Gröschel-Stewart, 1975) and dogs (Bonjour & Malvin, 1970; Ramsay, Keil, Sharpe & Shinsako, 1978). In an attempt to study more closely the angiotensin-induced release of vasopressin we investigated the effect of intravenous infusions of angiotensin I, II and (des-1-Asp) angiotensin II (angiotensin III) on the plasma levels of vasopressin in conscious rats and the influence of converting enzyme inhibition by SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) thereon. Angiotensin I and III were used in addition to angiotensin II, since they too have been recently shown to be biologically active in several systems (see Peach, 1977).

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Methods

Male Wistar rats (250 to 300 g) were used. They were allowed food and water *ad libitum* until the experiments began. The rats were placed in a room at a constant temperature (23°C) and humidity (55%), with light from 06 h 00 min to 18 h 00 min. Saralasin doses quoted are in terms of the salt.

Angiotensin infusion studies

For intravenous infusion, rats were restrained in cylindrical wire cages and the tail vein cannulated (PE 50). The angiotensins or the solvent (0.1 M phosphate buffer, pH 7.4) were infused for 20 min by means of an 'infusor'-pump (Braun-Melsungen) (infusion rate, 42 µl/min) and blood was collected immediately. In studies including angiotensin receptor blockade by saralasin, infusion of the antagonist began 10 min before that of angiotensin I or II and continued until the end of the experiment. 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

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saline. It has been demonstrated in previous studies that the effects of this dose of SQ 20881 last for at least 1 h (Hertting & Meyer, 1974). Five minutes after this injection the infusion began.

Experiments with nephrectomized animals

Bilateral nephrectomy was performed through a dorsal incision under light ether anaesthesia. In sham-nephrectomized rats the kidneys were exposed, but not ligated and removed. SQ 20881 was injected into a tail vein 2.5 h after the operation. Controls received saline. Blood was collected 30 min after this injection.

Determination of vasopressin in plasma

Vasopressin was determined as described elsewhere (Knepel & Meyer, 1980). Briefly, blood was collected, after decapitation, in pre-cooled centrifuge tubes, which contained 0.25 ml of a solution of disodium edetate (EDTA, 15 mg), centrifuged, and the plasma was separated. Column-chromatography with Bio Rex 70 (50–100 mesh, pH 5.0) was used for the extraction. Plasma aliquots (3 ml, pH 5.0) were poured on the resin, which was then washed twice with 2 ml distilled water, once with 2 ml ethanol (50%, pH 7.0), and eluted with 4 ml ethanol (75%, pH 1.5). The eluates were dried under vacuum. The residues were dissolved in 1.2 ml assay buffer (0.1 M tris-(hydroxymethyl)-aminomethane pH 7.4, 0.02 M EDTA, 0.1% gelatine). Aliquots (0.9 ml) were used for radioimmunoassay, which allowed the measurement of 2 to 800 pg of vasopressin. Mean recovery was 52%. The coefficient of variance for intra- as well as for interassay variability was 13%. All concentrations of vasopressin given are corrected for recovery. None of the peptides used in this study displayed any cross reaction.

Measurement of angiotensin II in plasma

Angiotensin II was determined as described elsewhere (Meyer, Eisenreich & Nutto, 1979). Briefly, under light ether anaesthesia, aortic blood was collected within 20 s in a pre-cooled syringe containing an inhibitor solution (EDTA, *o*-phenanthroline, phenylmethanesulphonylfluoride, dimercaprol), centrifuged and the plasma was separated. Plasma aliquots were immediately extracted, by column-chromatography with Dowex 1 (×8, 100 to 200 mesh, pH 8.9). The eluates were dried under vacuum. Aliquots of the dissolved residues were used for radioimmunoassay. Neither angiotensin I nor SQ 20881 display any cross reaction. This method separates angiotensin III, but it does not distinguish between angiotensin II and (des-1-Asp, des-2-Arg) angiotensin II. The ratio angiotensin II/(des-1-Asp, des-2-Arg) angiotensin II is 5:1 (Meyer *et al.*, 1979).

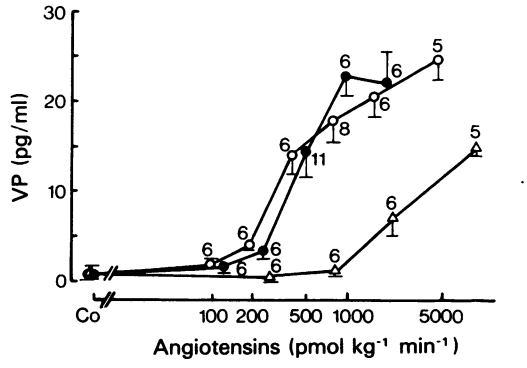

Figure 1 Dose-response curves for the effects of angiotensin I (○), angiotensin II (●), and angiotensin III (△) infusions on plasma vasopressin concentration (VP) in conscious rats. Mean values are given, vertical lines denote s.e. means, figures show number of animals used. Controls (Co) received the solvent.

Measurement of blood pressure

A cannula (PE 50) was placed in the ventral tail artery under ether anaesthesia. At least 2 h later, the cannula was connected to a transducer and a Watanabe recorder. Recordings were performed in conscious rats.

Statistical evaluation

All values are expressed as mean ± s.e. mean. The significance of the difference between mean values was evaluated by Student's *t* test.

Drugs

The following drugs were used: SQ 20881 (Squibb); (1-Asp, 5-Ile) angiotensin I and (1-Asp, 5-Ile) angiotensin II (Schwarz-Mann); (des-1-Asp) angiotensin II (Serva); saralasin acetate (Röhm Pharma).

Results

Intravenous infusion of angiotensin I, II and III

Intravenous infusions of angiotensin I, II and III caused dose-dependent increases in plasma vasopressin concentrations (Figure 1). Angiotensin I and II were of similar potency, angiotensin III infusions being less effective.

Vasopressin releasing activity of SQ 20881

The vasopressin releasing activity of the nonapeptide SQ 20881, when given alone, was evaluated in acutely nephrectomized rats and sham-nephrectomized ones. In nephrectomized rats, i.e. lacking the endogenous renin-angiotensin system, SQ 20881 (1.0 mg/kg, the dose used in all experiments) had no effect on the plasma vasopressin concentrations: 12.7 ± 2.9 pg/ml ($n = 6$) as compared to 10.8 ± 1.4 pg/ml ($n = 6$, NS) after solvent-treatment. However, in sham-nephrectomized rats, injection of SQ 20881 resulted in significantly higher vasopressin levels (7.1 ± 0.9 pg/ml, $n = 6$) than solvent injection (3.0 ± 0.4 pg/ml, $n = 6$, $P < 0.005$).

Effect of SQ 20881 on vasopressin release and blood pressure response following the infusion of angiotensin I or II

The results of these experiments are shown in Table 1. As in sham-nephrectomized rats, SQ 20881 significantly increased plasma vasopressin concentrations in vehicle-infused rats without altering mean arterial blood pressure.

Angiotensin I (500 ng kg^{-1} min^{-1}) and angiotensin II (500 ng kg^{-1} min^{-1}) increased vasopressin release as well as blood pressure to the same extent in controls. The converting enzyme inhibitor, SQ 20881, did not interfere with these effects of angiotensin II but potentiated the vasopressin release elicited by angiotensin I infusion, the blood pressure increase being simultaneously diminished.

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

In controls, angiotensin I (500 ng kg^{-1} min^{-1}) increased the plasma levels of angiotensin II from 65 ± 10 pg/ml ($n = 6$) to 2,231 ± 455 pg/ml ($n = 6$); however, in the presence of SQ 20881, plasma levels were increased only to 272 ± 40 pg/ml ($n = 5$, $P < 0.001$, when compared to angiotensin I without SQ 20881).

Effect of saralasin on vasopressin release following angiotensin infusion

In doses of up to 25 μg kg^{-1} min^{-1}, saralasin changed neither plasma vasopressin concentration nor blood pressure when infused alone (data not shown). However, it completely prevented vasopressin release induced by angiotensin I or II as well as by angiotensin I after pretreatment with SQ 20881 (Table 1).

Discussion

In previous investigations into the involvement of the renin-angiotensin system in the control of vasopressin release, attention has been focused mainly on angiotensin II. The data presented here indicate that angiotensin I and even angiotensin III may also play a role in regulating vasopressin release. Angiotensin I, II and III increased the plasma levels of vasopressin, the dose-response curves of angiotensin I and II being identical in shape and position.

Table 1 Effects of angiotensin I (AI) or II (AII) alone, and in combination with SQ 20881 (SQ) and/or saralasin (Sar), on plasma vasopressin concentration (VP) and mean arterial blood pressure (MAP)

Drug	*VP (pg/ml)*	P <	*MAP (mmHg)*	P <
Controls	2.1 ± 0.3 (8)	—	114 ± 2 (10)	—
SQ 20881	4.3 ± 1.0 (6)	0.05	113 ± 3 (6)	NS
AI	11.0 ± 2.7 (9)	0.01	169 ± 3 (6)	0.001
AI + Sar	1.5 ± 0.4 (6)	0.02	—	—
AI + SQ	19.0 ± 2.0 (9)	0.05	135 ± 7 (5)	0.005
AI + SQ + Sar	1.1 ± 0.4 (6)	0.02	—	—
AII	14.3 ± 2.5 (6)	0.001	171 ± 2 (6)	0.001
AII + Sar	1.7 ± 0.6 (6)	0.001	—	—
AII + SQ	13.2 ± 1.2 (6)	NS	172 ± 2 (6)	NS

Measurements were taken 20 min after the start of angiotensin or solvent infusion. Values are expressed as mean ± s.e. mean, figures in parentheses denote number of animals used. Rats infused with angiotensin I (500 ng kg^{-1} min^{-1}) or angiotensin II (500 ng kg^{-1} min^{-1}) or SQ 20881 (1.0 mg/kg) alone were compared with solvent-infused controls. Rats infused in addition with SQ 20881 and/or saralasin (25 μg kg^{-1} min^{-1}) were compared with animals infused with angiotensin I or II alone.

The efficacy of angiotensin III was about 10 times less than that of angiotensin I or II. This low activity of angiotensin III infusion argues against the physiological importance of this metabolite in the release of vasopressin, since this peptide contributes only approximately one third to the angiotensin-like activity in rat plasma (Semple & Morton, 1976; Meyer *et al.*, 1979). However, angiotensin III has full intrinsic activity (see Regoli, Park & Rioux, 1974) and is even somewhat more potent in displacing ^{125}I-angiotensin II binding to central receptors than angiotensin II (Sirett, McLean, Bray & Hubbard, 1977; Snyder, 1978). Therefore, a physiological role for angiotensin III in vasopressin release, for instance after local conversion of angiotensin II to angiotensin III, cannot be entirely ruled out.

The converting enzyme inhibitor, SQ 20881, increased the potency of angiotensin I infusions in releasing vasopressin, angiotensin I becoming now a more powerful stimulus than angiotensin II. The following three observations indicate that this enhancement by SQ 20881 was indeed due to the inhibition of converting enzyme. Firstly, at the dose used, SQ 20881 effectively inhibited the conversion of angiotensin I to angiotensin II, as demonstrated by measurement of the plasma angiotensin II concentrations following angiotensin I infusion using a specific extraction procedure and radioimmunoassay. Secondly, the effect of SQ 20881 depended on the presence of endogenous or exogenous angiotensin I, since, when given alone, SQ 20881 significantly increased the vasopressin levels in intact of sham-nephrectomized rats, but had no effect in bilaterally nephrectomized animals. An intrinsic vasopressin releasing activity of SQ 20881 could not be demonstrated. Thirdly, SQ 20881 did not alter the effect of angiotensin II on either the plasma vasopressin concentrations or the blood pressure, so that an angiotensinase-mediated or otherwise unspecific mechanism of action can be excluded.

Thus, we conclude that the effect of angiotensin I on vasopressin release is enhanced by the inhibition of its conversion to angiotensin II. A similar phenomenon has been reported before, when SQ 20881 was found to increase the thirst producing effect of angiotensin I (Hertting & Meyer, 1974).

The following considerations may reconcile this finding with our existing knowledge about vasopressin and the renin-angiotensin system:

(1) Since the effects of angiotensin I and angiotensin II on vasopressin release were antagonized by the receptor blocking agent, saralasin, both peptides might act on the same receptors. Angiotensin I might have a higher affinity and/or intrinsic activity than angiotensin II. (2) An increase in blood pressure inhibits vasopressin release (Share & Levy, 1962; Shimamoto & Miyahara, 1976). Since angiotensin I and II strongly elevate blood pressure, it might be assumed that both peptides exert a dual effect on vasopressin release: stimulate it by a direct action and diminish it indirectly by increasing the systemic blood pressure. Angiotensin II produces a stronger vasoconstrictor effect (see Regoli, Park & Rioux, 1974). Therefore, after inhibition of its conversion to angiotensin II, angiotensin I infusions were accompanied by a much less pronounced increase in blood pressure. Assuming similar efficacy of angiotensin I and II on those receptors which mediate the release of vasopressin, SQ 20881 would then reduce the inhibition of the direct action of angiotensin I by diminishing the increase in blood pressure.

However, as many actions of angiotensin I have been shown to be mediated by angiotensin II (see Peach, 1977), a note of caution is required. The angiotensins are likely to bring about vasopressin release via central receptors of the circumventricular organum vasculosum laminae terminalis and subfornical organ where the blood-brain barrier is deficient (Haack & Möhring, 1978; Johnson, Hoffmann & Buggy, 1978; Miselis, Shapiro & Hand, 1979). The susceptibility of converting enzyme to SQ 20881 may vary between different tissues, and a local generation of angiotensin II can occur without release of the peptide into the circulation in appreciable amounts (Oparil, Koerner & O'Donoghue, 1979). Therefore, our data cannot definitely rule out the possibility that angiotensin I may be converted within the circumventricular organs even after pretreatment with SQ 20881 (i.v.). Following this hypothesis, the vasopressin releasing effect of angiotensin II, locally formed near the central receptor sites during angiotensin I infusion, might be disinhibited by SQ 20881 (i.v.), because the peripheral generation of angiotensin II is prevented and the blood pressure increase thus diminished.

A direct action of angiotensin I has now been proven at least for its catecholamine-releasing potency in the adrenal medulla (Peach, Bumpus & Khairallah, 1971; Ackerly, Felger & Peach, 1976).

Taken together, with the stated limitations, our results suggest that in addition to angiotensin II, angiotensin I and III may be active components of the renin-angiotensin system in the control of vasopressin release.

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