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Physiological saline diminishes central behavioural stimulation produced by angiotensin II

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Isotonic saline (NaCl 0·9%) has commonly been used as a solvent for octapeptide angiotensin II in studies requiring its intracerebroventricular (i.c.v.) administration (Cooling & Day 1975; Nahmod et al 1978; Sirett et al 1979; Sweet et al 1976). Wayner et al (1973), and later Leksell & Rundgren (1977), have pointed out that the well-known central actions of angiotensin II as a stimulant of drinking and vasopressin release may be mediated by Na^+-dependent mechanisms. Therefore it appeared reasonable to expect that the presence of Na^+ in solutions of angiotensin II injected i.c.v. might in some way be essential for the other central actions of the peptide. To test this hypothesis I have compared behavioural effects of identical doses of angiotensin II dissolved either in saline or in distilled water and given i.c.v. Rearings were chosen as an easy to measure manifestation of motor activity which has been shown to be strongly dependent on the central mesolimbic dopamine system (Kelly & Iversen 1976; Pijnenburg & van Rossum 1973). Previously we have found that angiotensin II influences stereotypy and catalepsy (Braszko & Wiśniewski 1976) i.e. the behaviour believed as reflecting a state of excitation or inhibition of central nigrostriatal dopamine system, respectively (Kelly et al 1975). A low dose of haloperidol was used in the present work to evoke motor stimulation which is thought to be due to the inhibition of dopamine autoreceptors by the drug (Carlsson 1975; Maj et al 1977; Strömbom 1977). A possible interaction between angiotensin II and haloperidol at this level was of interest since it could provide insight into the mechanism of angiotensin II-induced behavioural excitation.

Male Wistar rats, 175–200 g, were used. Under light ether anaesthesia a burr hole 0·5 mm in diameter was drilled in the skull 2·5 mm to the right of the bregma and 1 mm caudal to the coronal suture. After the animals were allowed to recover (48 h) they were injected intraperitoneally (i.p.) with haloperidol (25 $\mu g\ kg^{-1}$) or saline (1 $ml\ kg^{-1}$), the vehicle for haloperidol injections. 3 h later the animals received angiotensin II (Hypertensin Ciba, 0·5 or 5 μg per rat i.c.v.) dissolved in water or in saline. Rats not receiving angiotensin II were injected with either water or saline alone at the same time and route. The i.c.v. injections were made into the right lateral cerebral ventricle by hand with a 50 μl chromatographic Hamilton syringe the needle of which was covered by a sleeve except for the last 4·5 mm to the tip (Herman 1970). The site of injection was verified with a colour dye in every tenth rat taken randomly throughout all experiments. The i.c.v. injection volume was always 10 μl. 60 min after an i.c.v. injection the animal was placed in round glass cage (i.d. 32 cm, height 20 cm) covered with a wire mesh lid. Rearings, i.e. standing up with both forelimbs withdrawn from the bottom of cage, were counted manually in the 10 min after a 1 min habituation period. All the observations were conducted in a semi-dark room between 00·12 and 02·00 h with each group equally represented in the times of testing.

The results (Fig. 1) demonstrate that there was no difference in number of rearings between rats treated i.c.v. with water alone and those treated with saline alone. However, the animals pretreated with haloperidol i.p. and then water i.c.v. were more active ($P < 0{\cdot}001$ vs the water control group, Student's *t*-test) than those receiving saline after haloperidol ($P < 0{\cdot}05$ vs the saline control group). Angiotensin II (0·5 μg) given in water produced a greater increase in number of rearings ($P < 0{\cdot}001$ vs water control) than the peptide given in saline (not significant vs saline control, $P < 0{\cdot}01$ vs the group treated with angiotensin II dissolved in water). In the haloperidol-pretreated animals, angiotensin II (0·5 μg) in water increased the number of rearings significantly ($P < 0{\cdot}02$) but in saline it did not. The stimulation occurring in animals given both haloperidol and angiotensin II (0·5 μg) was weaker than that observed after each drug given alone. Angiotensin II at a higher dose (5 μg) applied either as a saline or water solution failed to change the number of rearings significantly in animals pretreated with haloperidol or not.

A stimulatory behavioural effect of low, but not high, doses of angiotensin II appears to be compatible with our earlier observation that this peptide at the dose of 1 μg (i.c.v.) enhances amphetamine induced stereotypy (Braszko & Wiśniewski 1976). The mechanism underlying angiotensin II-induced behavioural stimulation, as well as that responsible for an inhibitory effect of saline upon it, both revealed in the present communication, awaits elucidation. It is possible that the behavioural effects of the peptide simply reflect its dipsogenic action. This seems unlikely, however, since the eliciting of drinking and ADH release by i.c.v. angiotensin II requires Na^+ and elevation of the Na^+ level in the c.s.f. enhances these effects of the peptide (Andersson 1977; Leksell & Rundgren 1977).

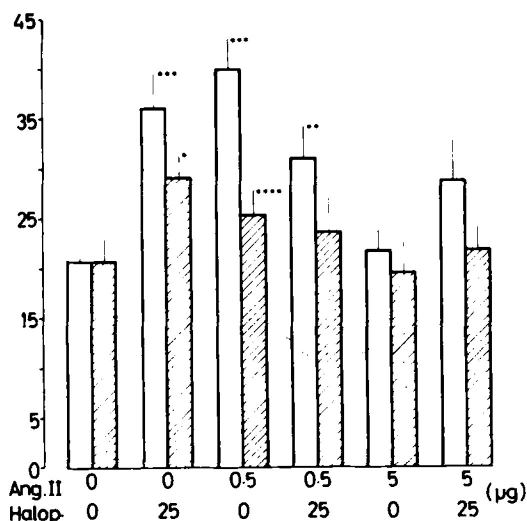

FIG. 1. Effect of angiotensin II (0·5 and 5 μg per rat i.c.v., 1 h before testing) and haloperidol (25 μg kg^{-1} i.p., 4 h before testing) on the number of rearings counted during 10 min (ordinate). The height of each column represents the mean of 10 experiments and the vertical line 1 s.e. Open columns represent animals treated with angiotensin II dissolved in water or with water alone and hatched columns those treated with angiotensin II dissolved in saline or with saline alone. The i.c.v. injection volume was 10 μl per rat. *$P < 0·05$; **$P < 0·02$; ***$P < 0·01$ compared with the appropriate control group. ****$P < 0·01$ as compared with the group treated with angiotensin II (0·5 μg) dissolved in water (Student's *t*-test).

In our case the increased behavioural activity could not be a result of increased thirst as it was more pronounced in animals receiving angiotensin II with, than without, Na^+. Angiotensin II has been described as an agent directly depolarizing cat superior cervical ganglion cells (Dun et al 1978). This effect was Na^+-dependent and decrease in the Na^+ concentration resulted in corresponding diminution of depolarization amplitude. Accordingly, in our experiments angiotensin II-induced behavioural stimulation could not be dependent on the depolarization caused by the peptide since it was much greater in the absence, than in presence, of Na^+ in the injected solutions. It may be that angiotensin II causes only some of its several central actions through membrane depolarization. In the experiments of Dun et al (1978) angiotensin II depolarized cells in the superior cervical ganglion but not in spinal or ciliary ganglions. Also in the c.n.s. only some neurons may undergo angiotensin II-depolarization.

The inhibitory influence of saline upon haloperidol excitation was not as strong as that upon angiotensin II excitation. This difference suggests different biochemical actions for each drug leading to similar behavioural effects. The stimulation caused by angiotensin II (0·5 μg) and haloperidol given together was weaker than that caused by each drug alone. Thus an interaction of both drugs at the level of dopamine autoreceptors (prejunctional receptors) requires consideration. Specifically, angiotensin II would change responsiveness of these receptors to haloperidol. Lack of any greater effect of the high dose of angiotensin II (5 μg) used in the present experiments, may be explained by an unspecific inhibitory action of the peptide masking its more specific stimulatory effects. In conclusion, the existence of an unknown kind of interaction between angiotensin II and Na^+ affecting responsiveness of central dopamine receptors is postulated.

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