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Possible evidence of angiotensin II and endogenous opioid modulation of novelty-induced rearing in the rat

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Summary

Rats treated with captopril (CAP, 10mg/kg i.p) naltrexone (Nalx 0.1mg/kg i.p) and saralasine (100ug/kg i.p) displayed significantly less novelty-induced rearing (NIR) compared to saline injected animals. Naltrexone potentiated the inhibitory effect of CAP on NIR. Pretreatment with NALX did not alter SARA-induced decrease in NIR. It is suggested that the endogenous release of AII and/or opioids somehow modulate basal rearing activity.

Résumé

Les rats traités avec le captopril (CAP, 10mg/kg i.p.), le naltrexone (Nalx 0.1mg/kg i.p.) et le saralasine (100ug/kg i.p.) ont montré, d'une facon significative, moins de nouveauté déclenché d'élevage (NDE), comparés aux animaux injectés de saline. Le naltrexone a potentialisé l'effet inhibiteur du CAP sur le NDE. Un prétraitement avec NALX n'a pas changé le rabaissement du SARA-provoqué dans le NDE. Il a été suggéré la libération endogène de l'AII et/ou de l'opioids module en quelque sorte l'activité basal/fondamentale de l'élevage.

Introduction

The presence of angiotensin II (AII), the precursors and the enzymes necessary for its synthesis and degradation have been demonstrated in several brain areas[1,2]. The receptors for this octapeptide have been localised in the subfornical, subthalamic, hypothalamic nuclei, the superior colliculus and other brain stem nuclei[3,4,5,6]. Distribution of AII receptors in the brain is discrete. The highest concentration of AII receptors was demonstrated in the medulla, hypothalamus, septum, thalamus etc., where AII could potentially be involved in neuro-endocrine and behavioural regulation[7,8,9]. For example intracerebro- ventricular administration of AII stimulated thirst and sodium appetite[10] and enhanced vertical motor activities and learning[7] in experimental animals.

This study was undertaken to further clarify the behavioural effects of endogenous (synaptic) AII following the intraperitoneal (i.p) administration of captopril, an angiotensin converting enzyme (ACE) inhibitor or saralasin, and AII receptor antagonist. We have also studied the interaction between the opiod receptor antagonist, naltrexone and the AII system. This is because preliminary clinical observation indicate an interaction between captopril and naloxone[11,12]; and that captopril or AII released opioid peptides and potentiated enkephalin-induced analgesia[13,14,15].

Materials and methods

The experiments were carried out in male albino rats (Vom strain), weighing 150-200g and housed in groups of 5 rats per cage (40 x 20 x 20 cm). All animals were housed in a quiet laboratory room under natural lighting conditions and ambient temperature of 26 ± 1°C. Food and drinking water were available *ad libitum*.

Assessment of novelty-induced behaviour

Observation and scoring of novelty induced rearing, was carried out in a standard laboratory room with a temperature of 26 ± 1°C. Behavioural changes induced by novelty were assessed by placing the animals directly from home cages into a transparent plexiglass cage (45 x 25 x 25 cm) containing

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saw-dust. Each cage was illuminated by a soft 60 watt white bulb. All rats were observed singly after drug or saline administration.

Rearing: Consisted of vertical posture with animals standing on hind limbs and fore paws against the side of the cage or in the air. Novelty-induced rearing were quantified for a period of 30 minutes by trained observers. Total rearing counts were summed up every 10 minutes. Each rat was used once.

Drugs: The following drugs were used: captopril (Squibb & Sons) naltrexone hydrochloride (Research Biochemical Inc.), saralasin (Sarenin, Rohmpharma), angiotensin II (Hypertensin, Ciba) were administered dissolved in physiological saline.

Statistics: All results are expressed as mean ± standard error of the mean (S.E.M.). The significance of differences between behavioural counts obtained after the different treatments was evaluated by one way analysis of variance (ANOVA). Once this showed a significant overall effect, a *post hoc* unpaired t-test was undertaken to detect within group differences. Statistical significance was accepted at $P < 0.05$.

Results

The effects of captopril, naltrexone, angiotensin II and saralasin on novelty induced rearing

Captopril (CAP 10mg/kg i.p. 20 min prior to novelty exposure) or naltrexone (0.1mg/kg i.p. 25 mins prior to novelty exposure) significantly decreased novelty-induced rearing compared with controls (Saline, SAL 1cc/kg i.p. 20 min prior) (Fig. 1, $P < 0.05$). The administration of naltrexone (0.1mg/kg i.p. 5 mins prior CAP) potentiated the inhibitory action of CAP (10mg/kg i.p. 20 mins prior novelty exposure) on rearing (Fig. 1, $P < 0.01$).

Administration of AII receptor antagonist, saralasin (SARA 1µg - 100µg/kg i.p. 5 mins prior exposure to novelty) induced a dose dependent decrease in rearing compared to controls (Fig. 2, $P < 0.01$). Naltrexone (0.1mg/kg i.p. 5 mins prior) did not alter the inhibitory action of SARA on rearing.

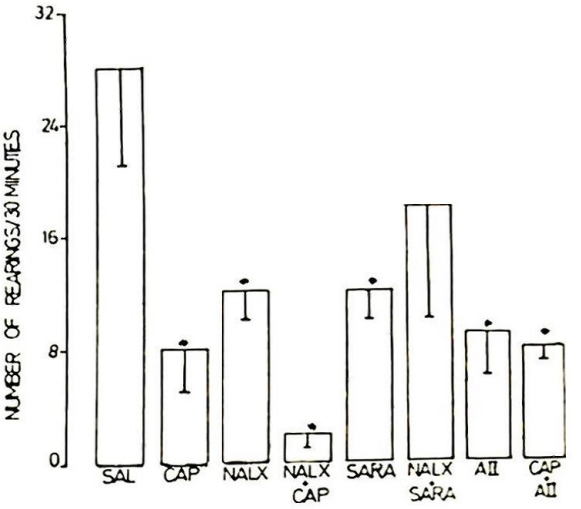

Fig. 1: The effects of captopril (CAP), naltrexone (NALX), saralasin (SARA) and angiotensin II (AII) on novelty- induced rearing (NIR). Each bar is mean ± S.E.M. for 6 animals. Note the following: (A) that captopril (CAP 10mg/kg i.p.), and SARA (100µg/kg i.p.) significantly decreased NIR (B) that Nalx (100µg/kg i.p.) potentiated the inhibitory action of CAP on NIR but did not alter the effect of SARA in NIR; (C) Paradoxically AII (500ng/kg i.p.) inhibited NIR and did not alter the CAP-induced decrease in NIR. (*) indicate significant difference from saline control group.

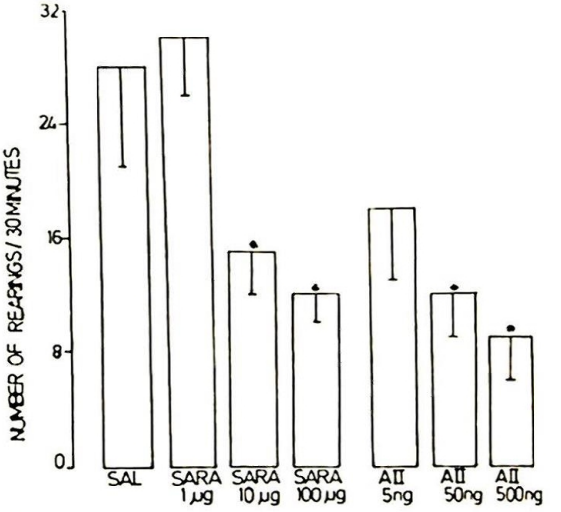

Fig. 2: The effects of saralasin (SARA) and angiotensin II (AII) on novelty-induced rearing (NIR). Each bar is mean ± S.E.M. for 6 animals. Note that both SARA (10 - 100µg, i.p.) and AII (50-500ng/kg, i.p.) significantly decreased NIR. (*) indicate significant different from saline control group.

AII (5ng - 500ng/kg i.p., 5 mins prior to novelty) significantly decreased rearing compared to control (Fig. 2, $P < 0.05$). There was no clear dose relationship in the inhibitory action of AII on rearing (Fig. 2, $P < 0.05$). There was no significant difference in the frequency of rearing in rats receiving combined treatment with CAP and AII and those treated with CAP or AII alone (Fig. 2, $P < 0.05$).

Discussion

It is of interest to note that novelty-induced rearing (exploratory behaviour) was inhibited by captopril. The mechanism by which captopril antagonised novelty-induced exploratory behavior is not clear. It is known that ACE-inhibition is associated with increased concentrations of substance P, and related peptides[16] and decreased plasma angiotensin concentrations[17,18] and brain angiotensin II receptors and angiotensin II levels[19,20]. However, alteration in brain concentrations of substance P and related peptides are not likely to be involved in the inhibitory effect of captopril on novelty- induced rearing since the icv administration of substance P enhanced locomotor activity[21]. It could, however, be suggested that the decrease in angiotensin II level associated with ACE-inhibition[19,20] may explain the inhibitory effect of captopril on novelty-induced rearing behaviours. The concept that an insufficiency of angiotensin II system could lead to a decrease in novelty-induced rearing receives further support from the fact that in this study, saralasin, an angiotensin II receptor antagonist, also inhibited novelty-induced rearing. In this context it is suggested that endogenously released angiotensin II tonically facilitates novelty-induced exploratory behaviours.

A second interesting finding is the fact that intraperitoneal administration of AII also decreased novelty-induced rearing. This observation is in contrast to the fact that ACE-inhibition or angiotensin II receptor blockade with saralasin also decreased rearing behaviours. The interpreation of these results is further compounded by the report that icv administration of angiotensin II enhanced vertical activity[22]. The reason for this discrepancy in the effect of angiotensin II on novelty-induced behaviours is not clear. It has however been reported that AII exerts both stimulant and depressant actions on locomotor activities depending on the dose used. For example icv administration of 1 and 10μg AII decreased rearing whilest 5μg increase rearing[23].

Furthermore, it is also known that the effects of angiotensin II on learning and conditioned reflex in rats are unexpectedly mimicked by (Sar^1 Ile^8) angiotensin II, a specific inhibitor of angiotensin II[10]. In this context, an action independent of angiotensin II with its known receptors has been invoked. The neurobehavioural effects of angiotensin II is complicated and a biomodal action, with low doses but not high doses being stimulatory[10].

Novelty induced arousal is well described and is mediated by enkaphalinergic[24-26] and dopaminergic pathways[7,25]. The exact biochemical pathway of angiotensin II modulation of arousal is unclear. There is evidence, however, that angiotensin II can produce specific psychomotor changes through central dopamine systems[7,27]. It is thus possible the dopaminergic NIR is affected by changes in central angiotensin II levels.

Blockade of opioid receptors with naltrexone decreased novelty- induced rearing behaviour. This finding is consistent with previous reports that activation of opioid receptors is involved in novelty-induced rearing behaviour[24,25]. However, naltrexone potentiated the captopril-induced inhibition of exploratory behaviour, in accord with our earlier clinical observation[12] of a sedative action of captopril and naloxone in man. Whereas in the present animal model, naltrexone and saralasin had a mutual antagonistic effect. This suggests that captopril and saralasin induced inhibition of exploratory behaviour is probably not mediated via the same type of receptor mechanisms.

Although these data collectively suggest an involvement of the angiotensin II system in exploratory locomotor activity, further experiments are necessary to clarify the exact role of angiotensin and its receptors in rearing behaviour.

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