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Angiotensin II Delays The Extinction of Active Avoidance in Rats
The Role of Transmitter Receptors

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***Abstract*— The extinction of active avoidance behaviour in rats following the intracerebroventricular (ICV) injection of different doses of angiotensin II (Ang II) was studied. The influence of antagonists of different neurotransmitter receptors on the effects of Ang II was also followed. Ang II induced a delay in the extinction in a dose-dependent fashion (U-shaped curve). When the animals were pretreated with different receptor blockers in doses which themselves had no action on the extinction of active avoidance behaviour, the action of Ang II on this paradigm was completely blocked by saralasin, haloperidol, bicuculline, atropine, naloxone Phenoxybenzamine, propanolol and methysergide were ineffective. The data suggest that Ang II delays the extinction of active avoidance behaviour, and that angiotensin, dopamine, GABAa, opiate might be involved in this action.**

***Index Terms*— Active avoidance; . Angiotensin II, extinction,; ; transmitter receptors.**

I. INTRODUCTION

The octapeptide angiotensin II (Ang II) is the basic component o f the brain renin-angiotensin system. Ang II participates membrane functions, the maintenance of salt and volume homeostasis, blood pressure control, the release of pituitary hormones, and the control of behavior responses such as thirst and drinking. In addition to these well-known central action, Ang II injected intracerebroventricularly (icv) enhances exploratory behaviour and locomotor activity[1-3]/; increases the threshold of seizures, and decreases the intensity of PTZ-kindled seizures [/4,5]/ and apomorphine stereotypy [6]/; exerts a dose-dependent antinociceptive effect in the acetic acid-induced abdominal constriction test.[7] and suppresses the behavioural responses of the defensive burying paradigm in rats, suggesting anxiolytic activity [8]. Ang II mediates its function after binding to specific receptors, namely receptor subtypes, AT_1, AT_2, and AT_4, in different brain areas [9.] Ang II administered icv at doses of 0.1 to 1 µg/rat immediately after a training procedure improves the memory process in two-way active (shuttle-box) and passive (step-through) avoidance tasks [3,10-15]. There are data which suggest that the memory effect of Ang II dependent on the activities of brain dopamine, GABA and cholinerq receptors [3,10-14]. The present study provides further details of the effects of Ang II on extinction through an active avoidance paradigm.

In order to elicit whether the effects of Ang II on extinction are exerted only through Ang II receptors, but also through certain neurotransmitter receptors, rats were pretreated with Ang II receptor agonists and different receptor blockers which were earlier shown to be effective in modifying the action of a number of peptides in the same paradigm [16].

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II. MATERIALS AND METHODS

The experiments were performed on male Wistar rats weighing 160-200 g. All animals had access to commercial food and tap water *ad libitum* and were kept at constant room temperature (20-22 C^o and on a standard 12-h light and 12-h dark cycle (lights on at 6 m.Experiments were carried out daily between 9 a.m. and noon.

A. Surgical procedure

The rats were anaesthetized with pentobarbital sodium (Nembutal, 35 mg/kg intraperitoneally (IP) and a cannula was placed into the left lateral cerebral ventricle and fixed to the skull with dental cement. The following stereotaxic coordinates were used: AP: +1.0; L:1.5; V:3.0[17]. A volume of 2 µl/animal was injected throught the cannula with a 10 µ1 Hamilton syringe over 30 s. The correct positioning of the cannula was checked individuallyby injecting methylene blue after the experiments were completed.

B. Active avoidance behavior.

The extintion processes were studied by using bench - jumping conditioning methods [18]. The experimental apparatus was a bench jumping conditioning box with a plexiglass window in the front. A pleiglass bench (13x9 cm) was fixed on the side of the box,7 cm above the grid floor. The conditional signal was the light of a 45 W electric bulb. The unconditional stimulus was the electric shock of a 1.0 mA alternating current, delivered through the grid floor. Sessions consisting of 10 trials, at an interval of 60 s were performed each day for 3 consecutive days. The conditional stimulus was presented for a maximum of 15 s. If the rat jumped onto the bench during the first 10 s, the conditional signal was terminated and the animal could escape the footshock. If the animal did not jump onto the bench during this time, the

conditional stimulus in the next 5 s was associated with an unconditional stimulus (electric footshock). The intertrial period was the time the animal spent on the grid floor. The criterion of learning the conditioned avoidance response was a response of 80 % or more during consecutive days. When the animals had reached the criterion they were subjected to extinctional trials. The extinctional trials were performed 3, 6 and 24 h after the learning session, when the conditional stimulus was not followed by the unconditional stimulus. The drugs under investigation were applied immediately after the learning session.

The animals were divided into four groups:

1. Control group: pretrement with 0.1 ml/100 g b.w.,saline 0,1m1/100 g b.w. subcutaneously (SC) or 2 µl/rat icv, 15, 20, or 30 min prior to saline (icv) immediately after the last learning trial.
2. Blocker-treated group: receptor blocker pretreatment (IP, SC or icv) 15, 20 or 30 min prior to saline (icv) immediately after the last learning trial.
3. Peptide-treated group: pretreatment with saline (IP, SC or icv 15, 20 or 30 min before treatment with Ang II (icv,immediately after the last learning trial.
4. Blocker plus peptide-treated group: blockers pretreatment. (IP, SC, icv) 15, 20 or 30 min prior to treatment with Ang II (icv) immediately after the last learning trial.

C. DRUGS

Ang II (hypertensin, Ciba-Geigy) was dissolved in 0.9 % saline and administered ICV in a volume of 2 µl/rat in three doses 0.1., O.5 or 1 µg/rat.

The following receptor blockers were used: saralasin (Sar_1 $A1a^8$/ Ang II, Sigma Chemical Co.), administered icv in a volume of 2 µl/rat 15 min before saline or Ang II. Bicuculline methiodide (Serva) at a dose of 1 mg/kg SC, and naloxone hydrochloride (Narcan, Winthrop) at 0.3 mg/kg SC were administered 20 min before saline or Ang II. Atropine sulphate (EGYT, Budapest) at a dose of 1 mg/kg IP, haloperidol (G.Richter, Hungary) at 0.01 mg/kg IP, were administered 30 min before saline or Ang II.

III. STATISTICAL ANALYSIS

Statistical evaluation of the active avoidance data was performed by multifactor analysis of variance (ANOVA). A probability level of 0.05 or less was accepted as a significant difference.

IV. RESULTS

The effects of the three doses (0.1, 0.5 or 1 µg/rat icv, of Ang II on the extinction of active avoidance behaviour in rats are shown in Fig.l. Ang II at a dose of 0.1 µg/rat significantly delayed the extinction only at 3 [$F_{1,19)}$=18.45]; p<0.0004) and 6 h [$F_{1,19}$,=21.25]; p<0.0002, vs. the control group. Ang II injected at a dose of 0.5 µg/rat did not change the extinction significantly. Ang II at a dose of 1 µg/rat significantly delayed the extinction at.3^h [$F_{1,19]}$=12.89]; p<0.002, 6^h [$F_{1,19}$=17.31]; p<0 0006 and 24^h [$F_{1,19}$=23.96] ;p<0.001, after the learning session. The receptor blockers saralasin at a dose of 1 µg/rat icv (Fig.2) haloperidol at 0.01 mg/kg IP (Fig. 3), bicuculline at 1 mg/kg SC (Fig.4), atropine at 1 mg/kg IP (Fig.5), naloxone at 0.3.mg/kg SC (Fig.6) did not change the extinction significantly vs. the control group. Saralasin completely blocked the Ang II (1 µg/rat-induced delay in extinction at 3 [F_{1-9}=6.08]; p<0.02, 6 [F_{1-19}]=4.64], p<0,04, and 24^h [$F_{1,-19}$],=9.58; p<0.006), with a significant difference vs. the group treated with Ang II (Fig.2). Haloperidol completely blocked the Ang II (1 µg/rat)-induced delay in extinction at 3 [$F_{1,19}$ =7.87, p<0.01), 6 [$F_{1,19}$ =17.28]; p<0.005) , and 24^h [$F_{1,19}$ =33.32]; p<0.0001, with a significant difference vs. the group treated with Ang II (Fig.3) . Bicuculline completely blocked the Ang II (1 µg/rat-induced delay in extinction at 3 [$F_{1,19}$ =5. 02]; p<03 , 6 [$F_{1,19}$=3. 94]; p<O. 05 and 24h [$F_{1,-19}$ =7.94]; p<0.01, hour with a significant difference vs. the group treated with Ang II (Fig.4) Atropine completely blocked the Ang II (1 µg/rat)-induced delay in extinction at 3 [$F_{1,19}$ =9.13]; p<0.007} , 6^h [$F_{1,19}$,=8.93], p<0.007) and 24^h [$F_{1-19]}$=21.03], p<0.0002), hour with a significant difference vs. the group treated with Ang II (Fig.5). Naloxone completely blocked the AngII (1 µg/rat)-induced delay in extinction at 3 [$F_{1,19}$ =6.08,; p<0.02), 6 [$F_{1,19}$=5.04]; p<0.03) and 24^h [$F_{1,19}$ =8.15]; p<0.01), with a significant difference vs. the group treated with Ang II (Fig.6).

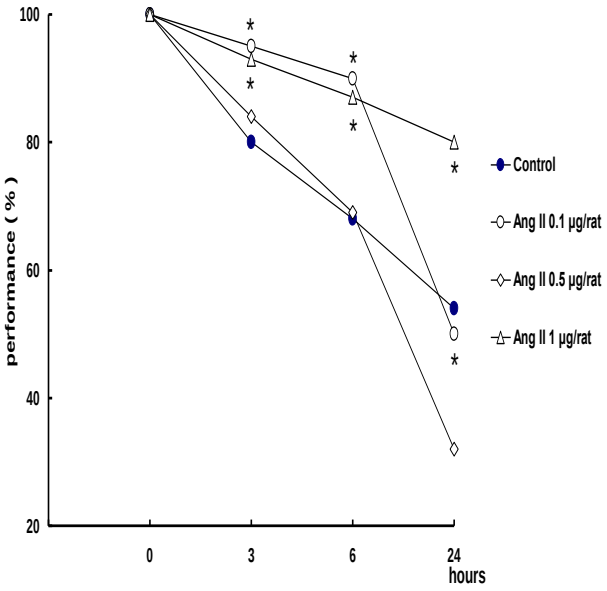

Fig.1. Effect of different doses of Ang II (ICV) on extinction of active avoidance behavior of rats. * p < 0.05 vs. control.

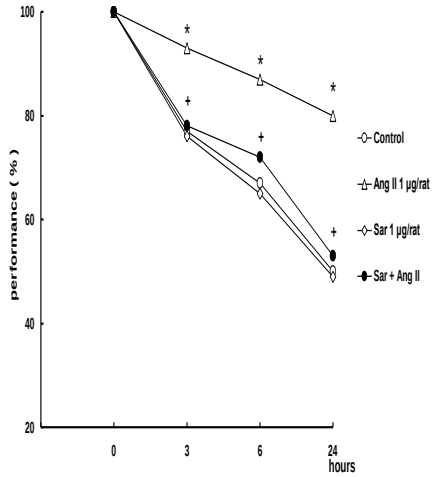

Fig. 2. Effect of Ang II (ICV), saralasin /Sar/ (ICV) and combination of both (saralasin prereated 15 min. before Ang II) on extinction of active avoidance behavior of rats. * p<0.05 vs. control; + p<0.05 vs. group with Ang II.

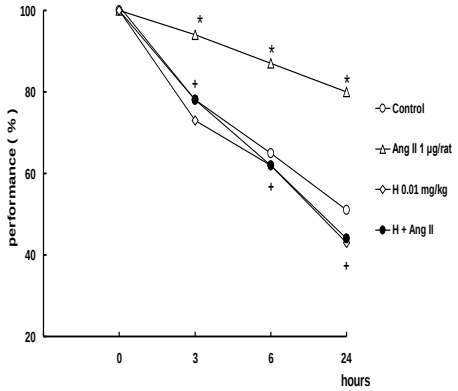

Fig.3. Effect of Ang II (ICV), haloperidol /H/ (IP) and combination of both (haloperidol pretreated 30 min. before Ang II) on extinction of active avoidance behavior of rats. * p<0.05 vs. control; + p<0.05 vs.group with Ang II.

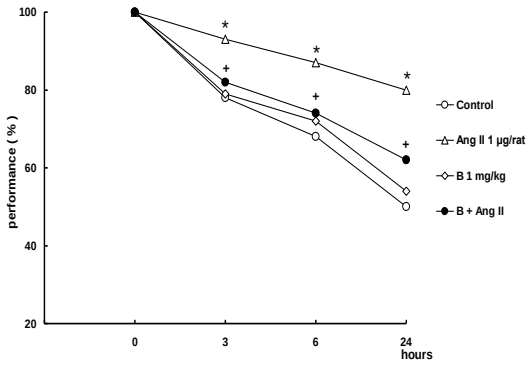

Fig .4. Effect of Ang II (ICV), bicuculline / (SC) and combination of both (bicuculline prereated 20 min.beforeAng II on extinction of active avoidance behavior of rats. * p<0.05 vs. control; + p<0.05 vs. group with Ang II.

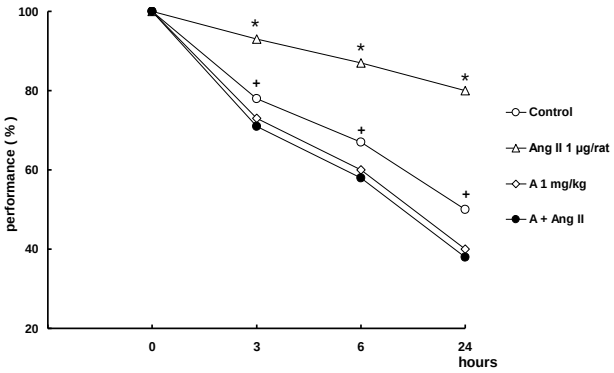

Fig.5. Effect of Ang II (ICV), atropine)A), (IP) and combination of both (A)atropine pretreated 30 min.before Ang II) on extinction of active avoidance behaviour of rats. * p<0.05 vs. control; + p<0.05 vs. group with Ang II.

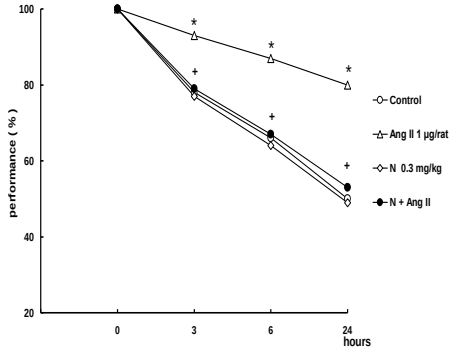

Fig.6. Effect of Ang II (ICV), naloxone /N/ (SC) and combination of both (naloxone pretreated 20 min. before Ang II) on extinction of active avoidance behaviour of rats. * p<0.05 vs. control; + p<0.05 vs. group with Ang II.

V. DISCUSSION

The results observed in this study confirm the action of Ang II in facilitating learning and improving memory in rats [3,10-15]. Ang II delayed the extinction of the active avoidance behavior according to a U-shaped dose-effect relationship, which is typical for other peptides, too [19]. The present results also show that fear-motivated learning-associated memory formation can be facilitated by Ang II. This action of Ang II was completely abolished by pretreatment with the non-selective AT_1, and AT_2, receptor antagonist peptide saralasin, the mixed dopamine receptor antagonist haloperidol, the GABA receptor antagonist bicuculline, the muscarinic cholinergic receptor antagonist atropine, the non-selective opiate receptor antagonist naloxone,

The present results point to the involvement of angiotensin receptors in the memory processes and also to the involvement of various neurotransmitter receptors in the attainment of the effect, on Ang II on memory. Thus, the observed abolition of the effects of Ang II on extinction by haloperidol, and some previous observations, suggest that Ang II requires active dopamine receptors for its facilitating cognitive effects [10,20,2l,]. The interaction of the Ang II receptor with the brain dopamine system in cognitive

and other behaviour is supported by our data on apomorphine-induced stereotypy [6]. The abolition of the effects of Ang II on extinction by bicuculline suggests that $GABA_A$ receptors are necessary for information processing in the central nervous system in general and for the attainment of Ang II action on memory processes in particular. The present observations confirm the previous observations concerning the interactions of GABAergic transmission with Ang II in memory processes [11,12,14,] in exploratory behaviour findings that Ang II requires active muscarinic cholinoreceptors for its cognitive enhancing effects [13]. It is well known that scopolamine, another muscarinic cholinoceptor antagonist, induces cognitive deficits in rodents which appear similar to those reported in patients with mild senile dementia of Alzheimer's type [24]. Thus the present findings support the notion that Ang II-interacts closely with cholinergic neurotransmission in memory process. The abolition of the Ang II [2] and in seizure susceptibility [4,5,22] It has recently been observed that a decreased GABA release is induced by Ang II in the rat hippocampus *in vitro [*23] which might provide a neurochemical mechanism for a modulatory role of the action of Ang II on the GABAergic system in behavioural studies, and in memory processes in particular. The blockade of the Ang II-induced delay in extinction of the active avoidance behaviour with atropine suggests a close interaction of Ang II with muscarinic cholinergic receptors for attainment of its effect on memory processes. These observations confirm previous induced delay in extinction of active avoidance behaviour with naloxone suggests an active contribution of opiate receptors in the Ang II facilitation of memory processes. A similar conclusion can be drawn as regards the observed blockade of the Ang II-induced delay in extinction by the alpha-adrenoblocker phenoxybenzamine. The latter confirms previous observations relating to the participation of adrenergic neurotransmission the attainment of Ang II behavioural effects [1.2]. From the present data, it seems improbable that beta-adrenoceptors and 5-HT receptors interact with Ang II receptors to cause an Ang II-induced delay in extinction of active avoidance behavior.

In conclusion, the delayed extinction of active avoidance behavior induced by Ang II in rats might be a contributory factor as concern the cognitive facilitary influence of this peptide. In achieving this action Ang II interacts closely with different neurotransmitter receptors.

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