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The Effects of Receptor Blockers on Atrial Natriuretic Peptide-Induced Action on Passive Avoidance Behavior in Rats

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BIDZSERANOVA, A., G. TOTH AND G. TELEGDY. *The effects of receptor blockers on atrial natriuretic peptide-induced action on passive avoidance behavior in rats.* PHARMACOL BIOCHEM BEHAV **40**(2) 237–239, 1991.—In previous experiments, it was shown that rat atrial natriuretic peptide ($rANP_{1-28}$) is able to increase the passive avoidance latency in a dose-dependent manner in the learning and consolidation phase (3). In order to clarify whether ANP has a direct action on this behavioral paradigm, or whether the action is mediated by neurotransmitters, rats were pretreated with different receptor blockers. The selected doses of the different receptor blockers could themselves not influence the behavioral paradigms. Haloperidol or atropine blocked the action of ANP on the consolidation of the passive avoidance response. Phenoxybenzamine, propranolol, methysergide, bicuculline and naloxone were ineffective. The data suggest that dopaminergic and cholinergic mediations are involved in the action of ANP on the passive avoidance response.

Atrial natriuretic peptide Receptor blockers Avoidance behavior

THE extract of the rat atria possesses natriuretic, diuretic and vasorelaxant activity (4). This discovery led to the isolation of atrial natriuretic peptide from both rats and humans (5,8). ANP has been demonstrated to be present independently in the periphery and in the brain (9, 12, 18). Specific ANP receptors have been found in the central nervous system (14).

Centrally administered ANP is able to increase the blood pressure (17). However, angiotensin II-induced hypertension is attenuated by centrally administered ANP (16). The exaggerated salt appetite in spontaneous hypertensive rats is blunted by centrally administered ANP (7). Central administration of ANP causes diuresis and natriuresis (6), diminishes vasopressin release (15) and has antidipsogenic action (2).

In previous experiments we have shown that ANP administered into the lateral brain ventricle is able to facilitate the passive avoidance response in a one-trial learning paradigm, when the peptide is given before or immediately after the learning trial. It was less effective, or even ineffective, when it was given before the retention testing (3).

In order to elicit the possible involvement of neurotransmitters in the ANP-induced action, animals were pretreated with different receptor blockers which were effective in modifying the action of a number of peptides in the same paradigm (20).

METHOD

CFY adult male rats weighing 150–250 g were used. All animals had access to commercial food and tap water ad lib and were kept at a constant room temperature (20–22°C) and on a standard 12-h light-12-h dark cycle (lights on at 6 a.m.). Experiments were carried out daily between 9 a.m. and noon.

The animals were anesthetized with pentobarbital (35 mg/kg, IP), and a cannula was placed into the lateral cerebroventricle and fixed to the skull with dental cement. The animals were used 7 days following operation. The correct positioning of the cannula was checked individually by the injection of methylene blue after the experiments had been completed. Animals with incorrect placement of the cannula were discarded and excluded from the statistical evaluations.

The experimental apparatus consisted of an illuminated platform (30 × 7 cm) attached to a large, dark compartment (40 × 30 × 30 cm) with a grid floor. In this apparatus, one-trial learning step-through passive avoidance behavior was measured according to Ader and De Wied (1). Rats were placed on the platform and allowed to enter the dark compartment. Since rats prefer dark to light, they normally entered within 15 s. Two additional trials were given on the following day. After the second one, unavoidable electric footshocks (0.75 mA, 2 s) were delivered through the grid floor. The animal could not escape the footshock. After this single learning trial, the rats were immediately removed from the apparatus. Passive avoidance behavior was tested 24 h after the learning trial: the rats were placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 s. Animals were treated with

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TABLE 1

EFFECTS OF ATROPINE AND HALOPERIDOL ON ANP-INDUCED ACTION ON PASSIVE AVOIDANCE BEHAVIOR

Blocker		Avoidance Response (s) Mean ± SEM	Significance
Haloperidol			
Control	(27)	106.6 ± 12.6	
ANP 200 ng	(27)	200.7 ± 16.8	$p<0.002$ vs. contr.
Halop. 2.5 μg/kg	(9)	96.6 ± 9.3	
Halop. 5 μg/kg	(9)	104.9 ± 12.3	
Halop. 10 μg/kg	(18)	106.7 ± 19.9	
Halop. 2.5 μg/kg+ANP	(9)	169.3 ± 10.7	$p<0.01$ vs. contr.
Halop. 5 μg/kg+ANP	(9)	139.3 ± 14.7	
Halop. 10 μg/kg+ANP	(18)	89.6 ± 13.0	$p<0.01$ vs. ANP
Atropine			
Control	(26)	102.0 ± 10.5	
ANP 200 ng	(27)	205.4 ± 12.7	$p<0.001$ vs. contr.
Atropine 0.5 mg/kg	(8)	106.6 ± 9.2	
Atropine 1 mg/kg	(8)	100.8 ± 8.3	
Atropine 2 mg/kg	(18)	114.2 ± 13.0	
Atr. 0.5 mg/kg+ANP	(8)	191.5 ± 18.0	$p<0.001$ vs. contr.
Atr. 1 mg/kg+ANP	(8)	171.5 ± 11.6	$p<0.001$ vs. contr.
Atr. 2 mg/kg+ANP	(19)	99.8 ± 15.2	$p<0.001$ vs. ANP

Numbers in parentheses represent the number of the animals used.

TABLE 2

EFFECTS OF NALOXONE, BICUCULLINE, PHENOXYBENZAMINE, PROPRANOLOL AND METHYSERGIDE ON ANP-INDUCED ACTION ON PASSIVE AVOIDANCE BEHAVIOR

Blocker		Avoidance Response (s) Mean ± SEM	Significance
Naloxone (0.3 μg/kg, IP)			
Control	(18)	84.8 ± 13.5	
ANP 200 ng	(16)	200.6 ± 20.3	$p<0.01$ vs. contr.
Naloxone	(12)	113.8 ± 27.9	
ANP + Nalox.	(12)	195.3 ± 38.1	
Bicuculline (1 mg/kg, SC)			
Control	(18)	105.9 ± 10.9	
ANP 200 ng	(17)	202.5 ± 18.2	$p<0.01$ vs. contr.
Bicuculline	(11)	102.8 ± 31.2	
ANP + Bicucul.	(10)	229.2 ± 35.3	
Phenoxybenzamine (2 mg/kg, IP)			
Control	(6)	92.0 ± 13.5	
ANP 200 ng	(6)	195.8 ± 30.1	$p<0.05$ vs. contr.
Phenox.	(6)	120.0 ± 18.6	
ANP + Phenox.	(6)	163.6 ± 11.2	
Propranolol (10 mg/kg, IP)			
Control	(18)	100.5 ± 21.9	
ANP 200 ng	(19)	202.0 ± 18.9	$p<0.03$ vs. contr.
Propranolol	(19)	131.1 ± 18.4	
ANP + Propran.	(19)	154.4 ± 21.9	
Methysergide (5 mg/kg, IP)			
Control	(24)	101.5 ± 13.5	
ANP 200 ng	(22)	204.3 ± 19.2	$p<0.001$ vs. contr.
Methysergide	(23)	102.1 ± 15.2	
ANP + Methyser.	(24)	167.1 ± 17.3	

Numbers in parentheses represent the number of the animals used.

the peptide immediately after the learning trial.

Rat atrial natriuretic peptide (1–28) was purchased from Bachem (Torrance, CA) and also synthesized by one of us (G. Toth) by solid phase technique, utilizing ^{t}Boc (tert. butyloxycarbonyl) chemistry. The product was compared to authentic sample of rat ANP (American Peptide Co. Inc., Santa Clara, CA). Peptide purity was above 97% (HPLC). The peptide (200 ng) was dissolved in 0.9% saline and administered in a volume of 2 μl to freely moving animals. The control animals received the same amount of 0.9% saline.

The following receptor blockers were used: atropine (atropine sulphate, EGIS, Hungary) 2, 1 and 0.5 mg/kg IP; haloperidol (G. Richter, Hungary) 10, 5 and 2.5 μg/kg IP; propranolol (ICI, Macclesfield, England) 10 mg/kg IP; phenoxybenzamine (phenoxybenzamine HCl, Smith, Kline and French) 2 mg/kg IP; methysergide (Deseryl, Sandoz) 5 mg/kg IP. All receptor blockers listed above were administered 30 min before the peptide treatment. Bicuculline (bicuculline methiodide, Serva) 2 mg/kg SC and naloxone (Narcan, Winthrop) 0.3 mg/kg SC, were administered 20 min before the peptide. The doses of the receptor blockers were selected according to our previous experience, in which the receptor blocker itself did not influence the passive avoidance paradigm but was able to block a number of neuropeptide-induced actions (20).

Statistical analysis was done by Kruskal-Wallis test.

RESULTS

The effects of haloperidol on the ANP-induced increased mean passive avoidance latency are shown in Table 1. ANP in a dose of 200 ng [selected from our previous study (3)] facilitated the avoidance response when it was given immediately after the learning trial. Haloperidol in a dose of 10 μg/kg, given 30 min before the peptide administration, blocked the action of the peptide. While haloperidol in the doses of 2.5 and 5.0 μg/kg showed a tendency to modify the action. Haloperidol alone had no effect on the passive avoidance response.

Atropine in a dose of 2 mg/kg, given IP under similar conditions, also blocked the action of ANP on the passive avoidance learning. Smaller doses (0.5 and 1.0 mg/kg) were ineffective (Table 1).

Naloxone in a dose of 0.3 mg/kg IP did not antagonize the action of ANP on the passive avoidance response (Table 2).

Bicuculline (1 mg/kg), phenoxybenzamine (2.0 mg/kg), propranolol (10 mg/kg), and methysergide (5 mg/kg) were also ineffective, although there was tendency to suppress the response of ANP, the action was not significant statistically (Table 2).

DISCUSSION

In the present experiments, we have demonstrated that in a passive avoidance paradigm ANP is able to increase the mean passive avoidance latency when it is given immediately after the learning trial, confirming our previous findings (3). In addition, it is demonstrated that, when the animals are pretreated with certain receptor blockers in a dose which itself cannot alter the passive avoidance response, these are able to modulate the action of the peptide. Haloperidol (a dopamine receptor blocker), in a dose of 10 μg/kg and atropine (a cholinergic receptor blocker), in a dose of 2 μg/kg, are each able to block the ANP response completely. Smaller doses showed a tendency to modify the action in a dose-dependent manner. Other receptor blockers such as phenoxybenzamine and propranolol, showed a tendency

to decrease the response, but the change was not significant statistically. Other receptor blockers such as methysergide, naloxone and bicuculline were ineffective in the doses used. The same doses of receptor blockers were able to block successfully the action of other peptides in the same experimental paradigm (20).

It has already been reported that ANP is able to bring about a biological action via transmitter mediation. The diuretic and natriuretic action of ANP might be mediated via central dopaminergic activation, since dopamine antagonists can prevent ANP action (11, 13, 21). In frog ANP stimulates α-MSH release from the neurointermediate lobes in vitro. Dopamine, GABA and NPY suppressed the action of ANP (10).

As regards interactions with the cholinergic system, the coexistence of ANP-immunoreactivity cells with choline acetyltransferase-like immunoreactivity has been shown in the lateral dorsal tegmental and pedunculopontine nuclei (19). Whether this morphological finding has any relevance to our observation concerning the participation of the cholinergic system in the ANP-induced action on the passive avoidance response remains to be seen.

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