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The Effects of Atrial Natriuretic Peptide on Active Avoidance Behavior in Rats. The Role of Transmitters

A. BIDZSERANOVA, J. GUERON, B. PENKE* AND G. TELEGDY[1]

*Department of Pathophysiology and *Department of Medical Chemistry*
"Szent-Györgyi Albert" University Medical School, Szeged, P.O. Box 531, Hungary

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BIDZSERANOVA, A., J. GUERON, B. PENKE AND G. TELEGDY. *The effects of atrial natriuretic peptide on active avoidance behavior in rats. The role of transmitters.* PHARMACOL BIOCHEM BEHAV **40**(1) 61–64, 1991.—Different doses of rat atrial natriuretic peptide ($rANP_{1-28}$) were tested as regards the extinction of active avoidance behavior following its injection into the lateral brain ventricle in rats. ANP delayed extinction of the active avoidance reflex in a dose-dependent manner. When the animals were pretreated with different receptor blockers in doses which themselves had no action on the extinction of active avoidance behavior, the action of ANP on this paradigm was completely blocked by haloperidol and atropine, whereas phenoxybenzamine/propranolol, naloxone, bicuculline and methysergide were ineffective. The data suggest that ANP delays the extinction of active avoidance behavior, and that cholinergic and dopaminergic transmitter systems might be involved in this action.

ANP Active avoidance Transmitters

REMARKABLE progress has been made in the study of atrial natriuretic peptide (ANP) since it was first discovered in mammalian hearts (4). In parallel with the investigations on the peripheral actions of the peptide, different experimental studies explore its activity in the central nervous system. Specific receptors for ANP have been found in the brain (16). The peptide is secreted and stored in a low molecular weight form in the brain (13). ANP is widespread in many brain regions, in the hypothalamus and septum, in the anterior and posterior lobes of the pituitary gland (10,13), etc. Brain ANP suppresses stimulated dipsogenesis, basal and stimulated vasopressin release and angiotensin II-stimulated pressor effects (8, 9, 17). It also interferes with brain transmitter systems (11, 12, 23). These data indicate that it would be worthwhile to follow the action of this naturally occurring peptide in behavioral studies and to specify the transmitters systems in which the peptide might interact.

In the present work, the effects of $r\text{-}ANP_{1-28}$ on active avoidance behavior were studied in rats, following its administration into the lateral brain ventricle. In order to establish whether the action of the peptide is mediated by transmitters, the rats were pretreated with different receptor blockers.

METHOD

Animals

The experiments were performed on male CFY rats, weighing 150–250 g. The animals were kept at a constant room temperature in artificial light with 12-h light (starting at 6 a.m.) and 12-h dark periods. A standard diet and tap water were given ad lib.

Surgery

The rats were anaesthetized with pentobarbital Na (Nembutal, 35 mg/kg) intraperitoneally and a 20 ga 1½'' Luer cannula was placed into the right lateral cerebroventricle and fixed to the skull with dental cement (Spofa, Cs). The stereotaxic coordinates of Fifkova and Marsala (6) were used (AP: +1.0; L: 1.5; V: 3.0). A volume of 2 μl per animal was injected through the cannula using microinjector (Mauser, BRD) and tubing. The animals were used after a recovery period of 5 days. The correct positioning of the cannula was checked individually by injecting methylene blue after the experiments were completed.

Active avoidance conditioning was performed according to the methods described in detail earlier (19,21). Briefly, the experimental apparatus was a bench-jumping conditioning box (45 × 25 × 45 cm) with a Plexiglas window in the front. A Plexiglas bench (13 × 9 cm) was fixed on one side of the box, 7 cm above the grid floor. The conditional signal was the light of a 45-W bulb. The unconditional stimulus was the electric shock of a 1.0 mA alternating current, delivered through the grid floor. The daily experimental session lasted 10 min for each animal. Each session consisted of ten trials with a mean intertrial interval of 60 s (range 50–70). 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

[1]Requests for reprints should be addressed to Prof. G. Telegdy, Department of Pathophysiology, P.O. Box 531, Szeged, Hungary.

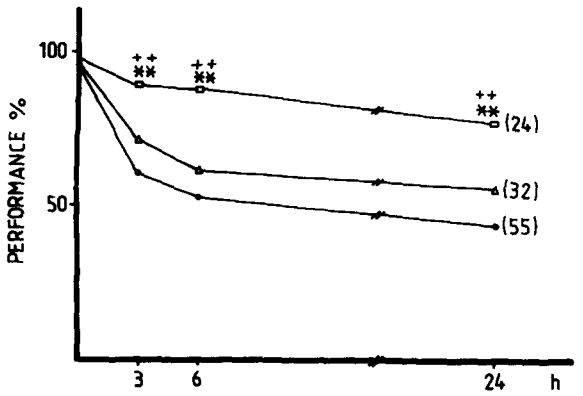

FIG. 1. Effect of different doses of ANP on extinction of active avoidance behavior. Symbols: ● control, △ ANP 200 ng/rat ICV, □ ANP 500 ng/rat ICV, $**p<0.01$ vs. control (MW), $++p<0.01$ vs. control (KW). Number in parentheses represents the number of animals used.

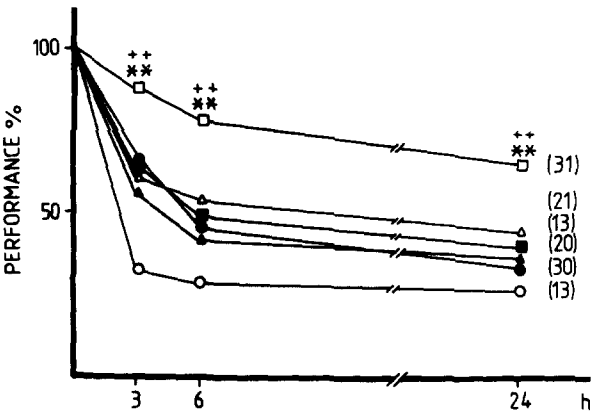

FIG. 3. Effect of ANP and two doses of atropine on the extinction of active avoidance behavior. Symbols: ● control, ▲ atropine (2 mg/kg IP), ○ atropine (3 mg/kg IP), □ ANP 500 ng/rat ICV, △ ANP + 2 mg/kg atropine, ■ ANP + 3 mg/kg atropine. $++p<0.001$ vs. control (KW). Number in parentheses represents the number of animals used. $**p<0.001$ (MW).

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 three consecutive days. When the animals had reached the criterion they were subjected to extinctional trials. During these trials on the 3rd, 6th and 24th hour after application of the peptide, the conditional stimulus was not followed by the unconditional stimulus (electric footshock).

Experimental Groups

In the first series of experiments the animals were divided into three groups: a control group (which received 2 μl saline ICV) and two dose groups, which received 200 or 500 ng ANP per rat, in a volume of 2 μl ICV immediately after the last learning trial.

In the following experiments the animals were allocated into four groups.

1) Control group: saline pretreatment (20–30 min prior to the peptide, SC or IP) plus treatment with 2 μl saline ICV immediately after the last learning trial.

2) Blocker-treated group: receptor blocker pretreatment (20–30 min prior to the peptide, SC or IP) plus treatment with 2 μl saline ICV after the last learning trial.

3) Peptide-treated group: saline pretreatment (20–30 min prior to the peptide SC or IP) plus treatment with 500 ng ANP in 2 μl volume ICV, immediately after the last learning trial.

4) Blocker plus peptide-treated group: blocker pretreatment plus treatment with ANP ICV immediately after the last learning trial.

Drugs

$rANP_{1-28}$ was dissolved in 0.9 percent saline and administered ICV in a volume of 2 μl in two doses, 200 or 500 ng per rat. The following receptor blockers were used 30 min before the last trial: haloperidol (G. Richter, Budapest) (5 and 10 μg/

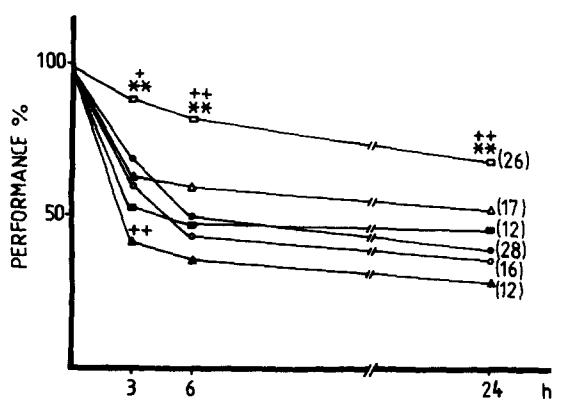

FIG. 2. Effect of ANP and two doses of haloperidol on the extinction of active avoidance behavior. Symbols: ● control, ○ haloperidol (5 μg/kg IP), ▲ haloperidol (10 μg/kg IP) □ ANP 500 ng/rat ICV, △ ANP + 5 μg/kg haloperidol, ■ ANP + 10 μg/kg haloperidol. $**p<0.01$ vs. control (MW), $+p<0.05$ vs. control (KW), $++p<0.01$ vs. control (KW). Number in parentheses represents the number of animals used.

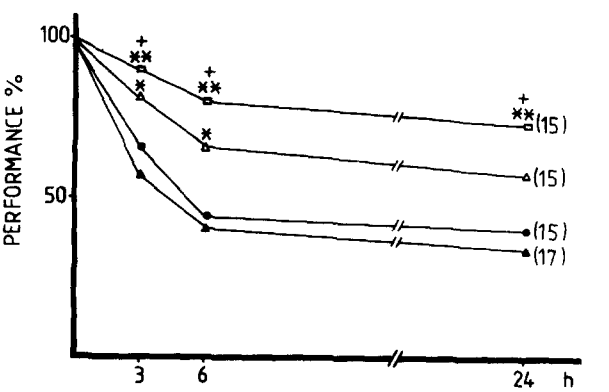

FIG. 4. Effect of ANP and phenoxybenzamine on extinction of active avoidance behavior. Symbols: ● control, ▲ phenoxybenzamine (2 mg/kg IP), □ ANP 500 ng/rat ICV, △ ANP + phenoxybenzamine. $+p<0.05$ vs. control (KW), $*p<0.05$ vs. control (MW), $**p<0.01$ vs. control (MW). Number in parentheses represents the number of animals used.

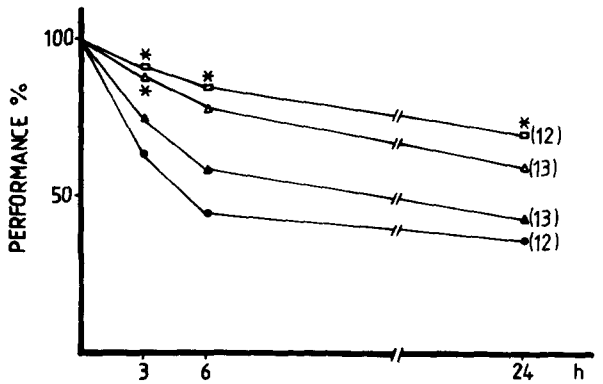

FIG. 5. Effect of ANP and propranolol on extinction of active avoidance behavior. Symbols: ● control, ▲ propranolol (10 mg/kg IP), □ ANP 500 ng/rat ICV, △ ANP + propranolol. *$p<0.05$ vs. control (MW). Number in parentheses represents the number of animals used.

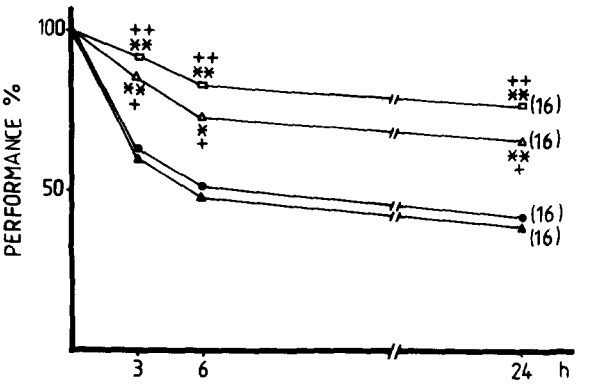

FIG. 6. Effect of ANP and naloxone on extinction of active avoidance behavior. Symbols: ● control, ▲ naloxone (0.3 mg/kg IP), □ ANP 500 ng/rat ICV, △ ANP + naloxone. *$p<0.05$ vs. control (MW), **$p<0.01$ vs. control (MW), +$p<0.05$ vs. control (KW), + +$p<0.01$ vs. control (KW). Number in parentheses represents the number of animals used.

kg, IP); atropine sulphate (EGYT, Budapest) (2 and 3 mg/kg, IP); propranolol hydrochloride (Imp. Chem. Indust. Ltd., GB) (10 mg/kg, IP); phenoxybenzamine hydrochloride (Smith, Kline and French, GB) (2 mg/kg, IP); methysergide hydrogenmaleinate; (Sandoz) (5 mg/kg, IP); bicuculline methiodide (Sigma) (1 mg/kg, SC); and naloxone hydrochloride (Endo Lab. Inc.) (0.3 mg/kg, SC) were used 20 min before the last learning trial.

Statistical Analysis

Statistical evaluation of the active avoidance data was performed by the test of Mann-Whitney (MW) and of Kruskal-Wallis (KW). A probability level of 0.05 or less was accepted as a significant difference.

RESULTS

The effects of two doses (200 and 500 ng per rat) of ANP on the extinction of active avoidance behavior in rats are shown in Fig. 1. The higher dose significantly delayed the extinction 3, 6 and 24 hours after administration of the peptide ($p<0.01$, MW and KW).

Haloperidol, in doses of 5 and 10 μg/kg IP, given 30 min before the last learning trial, completely blocked in the higher dose the inhibitory effect of ANP on the extinction of the avoidance response (Fig. 2). Atropine in doses of 2 and 3 mg/kg, IP (3), had the same effect (Fig. 3).

Phenoxybenzamine, in a dose of 2 mg/kg, IP (Fig. 4), and propranolol, in a dose of 10 mg/kg, IP (Fig. 5), both showed a tendency to block the effect of the peptide, but their action did not reach statistical significance.

The other three receptor blockers—naloxone, bicuculline and methysergide—in the doses used [selected so that they should not influence the behavioral paradigm, but were active in other experiments, see (7, 20, 22)] did not change the effect of ANP on the extinction of active avoidance behavior (Fig. 6). The results with bicuculline and methysergide are not shown.

DISCUSSION

The present study is a continuation of previous work, in which we have shown that ANP given ICV lengthens the passive avoidance response in a dose-dependent manner (2). Further, this action can be blocked completely by haloperidol and atropine, dopamine and a cholinergic receptor blockers (3). The present finding confirms the previous observation that fear-motivated learning-associated memory formation can be facilitated by ANP. In this mechanism the peptide exerts its action via dopaminergic and cholinergic transmission.

As concerns the mechanism of action, a number of possibilities can be considered. It has been reported that the diuretic and natriuretic action of ANP can be blocked by a dopaminergic receptor blocker (12, 15, 23). In frog ANP-stimulated MSH release from the neurointermediate lobes in vitro can be suppressed by dopamine, GABA and NPY (11). It seems that the learning associated action can also be mediated by dopamine and cholinergic transmitters.

There is an indication that the cholinergic system might interact with ANP, since choline acetyltransferase-like immunoreactivity coexists with ANP immunoreactivity in the lateral dorsal tegmental and pedunculopontine nuclei (18). Whether this coexistence has any functional significance as regards our findings remains to be seen.

It is interesting to note, however, that the action of ANP in the active avoidance behavioral paradigm is similar to that of the action of vasopressin and ACTH (1,5) because ANP does not stimulate ACTH secretion (14) and blocks vasopressin release (17). It is unlikely that ACTH or vasopressin mediation is involved in the mechanism described in our paper.

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