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Effects of Atrial Natriuretic Peptide on Acute and Chronic Effects of Morphine

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AZAROV, A. V., G. SZABÓ AND G. TELEGDY. *Effects of atrial natriuretic peptide on acute and chronic effects of morphine.* PHARMACOL BIOCHEM BEHAV **43**(1) 193–197, 1992.—Atrial natriuretic peptide (ANP) is known to participate in different vegetative functions. The aim of the present study was to investigate the influence of ANP on nociception itself, pain sensitivity to morphine, and the development of acute and chronic tolerance to morphine. Morphine withdrawal signs were also evaluated by injecting naloxone. In adult, male NMRI mice, ANP administered SC or ICV did not affect pain sensitivity itself in a heat-radiant tail-flick test. Peptide treatment, however, depressed the acute nociceptive effect of a single dose of morphine (4 mg/kg, SC) after both SC (20–200 ng/animal) and ICV (5, 10, 20, or 200 ng/animal) ANP administration. ANP given SC and ICV attenuated the development of acute morphine tolerance. Acute morphine tolerance was assessed by giving a bolus injection of morphine (60 mg/kg) 24 h before the pain sensitivity to a challenge dose of morphine (4 mg/kg) was measured. ICV treatment with ANP also blocked the development of chronic morphine tolerance, but did not affect the appearance of naloxone-precipitated withdrawal syndromes. ANP seems to act differently on the development of tolerance to and dependence upon morphine.

Morphine Tolerance Dependence ANP Pain Tail-flick

ATRIAL natriuretic peptide (ANP) is involved in various physiological processes, including natriuresis, diuresis, and vasodilation [cf. (1,14)]. Besides the classical endocrine actions, ANP has been reported to influence adaptational processes, for example, learning and memory functions in rats (2). It was shown that daily injection of ANP given ICV lengthens the latency of passive avoidance response in a dose-dependent manner. The effect of a single injection of ANP could be detected for over 24-h period, suggesting a long-lasting ANP action.

In the present study, we set out to investigate another aspect of adaptation by studying the effects of ANP on narcotic tolerance and dependence. We measured the effects of ANP on pain sensitivity itself, after a challenge dose of morphine, on the development of acute and chronic tolerance to morphine, and on naloxone-precipitated withdrawal symptoms.

METHOD

Animals

Male NMRI mice (33 ± 5 g) of an inbred strain (LATI, Gödöllő, Hungary) were used. They were kept under a standard 12 L:12 D cycle (lights on between 6:00 a.m.–6:00 p.m.) with food and water available ad lib. At least a week of habituation was allowed before beginning experiments.

Surgery

For ICV cannulations, mice were anesthetized with sodium pentobarbital (Nembutal, CEVA, France; 50 mg/kg, IP), and a polyethylene cannula was inserted into the right lateral cerebral ventricle and cemented to the skull with cyanoacrylate-containing instant glue. The experiments were started 4 days after ICV cannulation. Upon conclusion of experiments, 10 μl methylene blue was injected into the ventricle of decapitated animals and the position of the cannula was inspected visually. Animals with improper cannula placement were excluded from the final statistical analysis.

Animals were lightly anesthetized with ether (Lek-Chinoin, Budapest, Hungary) and morphine pellets were implanted SC into the sacral area through a small section in the neck area.

Treatments

For SC treatment, ANP was dissolved in isotonic saline and injected in a volume of 0.2 ml. For ICV treatment, the peptide was dissolved in artificial cerebrospinal fluid and injected in a volume of 2μl. In all experiments for measuring the antinociceptive effect, morphine HCl (Alkaloida, Tiszavasvári, Hungary; 4 mg/kg, SC) was used. In acute tolerance studies, 60 mg/kg morphine HCl (SC) was used as a tolerance-inducing dose. In chronic tolerance and withdrawal

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studies, pellets containing 35 mg morphine were implanted. The precipitated withdrawal syndrome was induced with naloxone hydrochloride, 0.1 mg/kg SC (Narcanti, Du Point, Germany).

Procedures

Four major types of experiments were carried out with ANP. All experiments were started with an initial tail-flick latency measurement, pain sensitivity being measured immediately before and 30, 60, and 120 min after the test morphine challenge. The heat-radiant tail-flick method of D'Amour and Smith (3) was used. The antinociceptive effect was expressed according to the equation:

$$\text{antinociceptive effect (\%)} = \frac{TF_n - TF_0}{TF_{max} - TF_0} \times 100,$$

where TF_0 is the tail-flick latency in the preliminary test mentioned above or (in all tolerance studies) before the injection of the test dose of morphine, TF_n is the value of a repeated corresponding measure n (30, 60, or 120) min after morphine injection, and TF_{max} indicates the cutoff time (20 s). The control tail-flick latencies (TF_0) were between 1.5-2.2 s.

The following four types of experiments were conducted:

1. In the first study, the effect of the peptide itself on pain sensitivity was measured. ANP was given SC or ICV 15 min before testing of the analgesic effect.
2. In studies with ANP on the antinociceptive effect of a single dose of morphine, the peptide was given SC or ICV 45 min prior to the test dose of morphine (4 mg/kg, SC).
3. In acute tolerance studies, animals were pretreated with the peptide and 1 h later a tolerance-inducing dose of morphine (60 mg/kg) was injected; 24 h later, a test dose of morphine was used to assess the antinociceptive effect.
4. In chronic tolerance and withdrawal studies, the peptide was given 1 h before pellets were implanted. The peptide treatment was repeated three times 24 h apart. On day 4, a test dose of morphine was given and the antinociceptive effect was determined. Three hours later, animals received naloxone (0.1 mg/kg, SC) and the precipitated withdrawal signs were assessed. The precipitated abstinence syndrome was measured by scoring the latency of the appearance of stereotyped jumping from a circular platform 35 cm in diameter and 70 cm high. A cutoff time of 900 s was used (6). The body temperatures and body weights of all animals were measured 1 h after injection of naloxone and changes in both parameters were calculated.

Statistical Analysis

The statistical analysis of the data was made by one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons with unequal cell size. A probability level of 0.05 was accepted as indicating significant differences.

RESULTS

ANP administered SC or ICV in graded doses (0.2-200 ng) did not influence pain perception in morphine-naive animals (Table 1).

In the first experiment, upon a single dose of morphine administration a substantial analgesic effect could be observed over the investigation period. Upon peripheral treatment with ANP, a dose of 200 ng/animal significantly decreased the analgesic effect of morphine at all time points investigated [at 30 min, $F(2, 42) = 11.27$, $p < 0.001$, 60 min, $F(2, 42) = 9.64$, $p < 0.001$, and 120 min, $F(2, 42) = 9.40$, $p < 0.001$]. By 120 min, mice pretreated with 20 ng ANP also showed a decreased nociceptive response to a challenge dose of morphine (Fig. 1).

In the second study, the morphine-induced analgesia was decreased, $F(6, 80) = 8.77$, $p < 0.001$, 30 min after ICV injection of 5, 10, 20, or 200 ng ANP. Sixty minutes after 5, 20, or 200 ng ANP administration, the morphine-induced analgesic effect was decreased, $F(6, 80) = 7.91$, $p < 0.001$. There was no difference in analgesic response by 2 h after peptide administration (Fig. 2).

TABLE 1

EFFECT OF ANP ON PAIN SENSITIVITY

		SC	
Time (min)	Control	20 ng	200 ng
15	0.75 ± 10.18 (14)	−2.29 ± 6.77 (15)	−8.40 ± 15.91 (15)
30	−2.68 ± 9.31 (14)	−16.58 ± 6.81 (15)	−7.67 ± 9.07 (15)
60	−12.04 ± 10.18 (14)	−7.33 ± 7.00 (15)	1.59 ± 4.89 (15)
120	−3.45 ± 10.07 (14)	−3.97 ± 5.92 (15)	−3.36 ± 11.72 (15)
240	−14.02 ± 13.71 (14)	−4.62 ± 3.89 (15)	−9.93 ± 7.40 (15)
		ICV	
Time (min)	Control	0.02 ng	0.2 ng
15	−5.43 ± 7.91 (22)	−0.38 ± 4.13 (12)	−16.17 ± 12.66 (9)
30	1.80 ± 6.93 (22)	−5.43 ± 5.59 (12)	−5.33 ± 7.74 (9)
60	−5.61 ± 5.79 (22)	−12.45 ± 6.11 (12)	−5.65 ± 10.66 (9)
120	−3.01 ± 7.45 (22)	−10.70 ± 6.00 (12)	3.43 ± 9.73 (9)
240	3.11 ± 10.37 (22)	−9.90 ± 5.48 (12)	0.93 ± 8.94 (9)

Values represent mean ± SEM (n) for animals treated; n = number of animals.

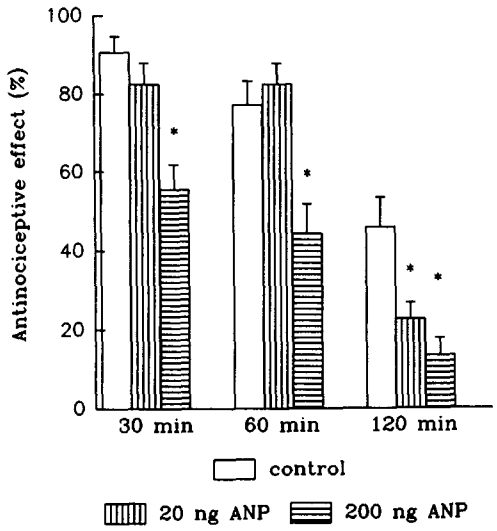

FIG. 1. Effects of ANP (SC) on acute antinociceptive action of morphine in tail-flick test. The peptide was administered just after a preliminary test of sensitivity to pain; 30 min later, an additional tail-flick test was performed; 45 min later, the test dose of morphine (4 mg/kg) was injected; pain sensitivity was measured 30, 60, and 120 min thereafter. Number of animals per group: control, 14; 20 ng ANP, 12; 200 ng ANP, 19. *Significant differences (at $p < 0.05$) between controls and the respective peptide-treated group at a given time point. Values shown are means ± SEM.

Separate groups of animals were pretreated with ANP or saline and 30 min later a priming dose of morphine (60 mg/kg) was injected. One day later, the acute tolerance to morphine was studied by measuring the analgesic response to a challenge dose of morphine (4 mg/kg). Animals treated with saline on day 1 and a day later with a challenge dose of morphine showed a significant antinociceptive effect for 60 min. Morphine-pretreated mice, on the other hand, displayed a lessened pain sensitivity to the same dose of morphine, regarded as a sign of development of tolerance to morphine (30–60 min). Compared to this control group, pretreatment with 20 or 200 ng ANP (SC) blocked the development of acute tolerance to morphine 30 min after analgesic administration, $F(5, 115) = 19.03$, $p < 0.001$. A sustained blockage [at 60 min, $F(5, 115) = 16.07$, $p < 0.001$] was observed with the 200-ng dose of ANP (Fig. 3).

In a separate experiment, animals were equipped with ICV cannulae and the previously mentioned experimental set-up was used. ANP give ICV in 20- and 200-ng/animal doses blocked the development of tolerance to morphine 30 min after a challenge dose of morphine compared to the tolerant control group. A dose of 5 or 20 ng ANP exerted a constant blockade on the development of tolerance to morphine 1 h later [at 30 min, $F(7, 122) = 22.27$, $p < 0.001$, 60 min, $F(7, 122) = 9.21$, $p < 0.001$]. All remaining doses of ANP were ineffective at all time points. Two hours after the challenge dose of morphine was given, the antinociceptive effect had disappeared in all groups (Fig. 4).

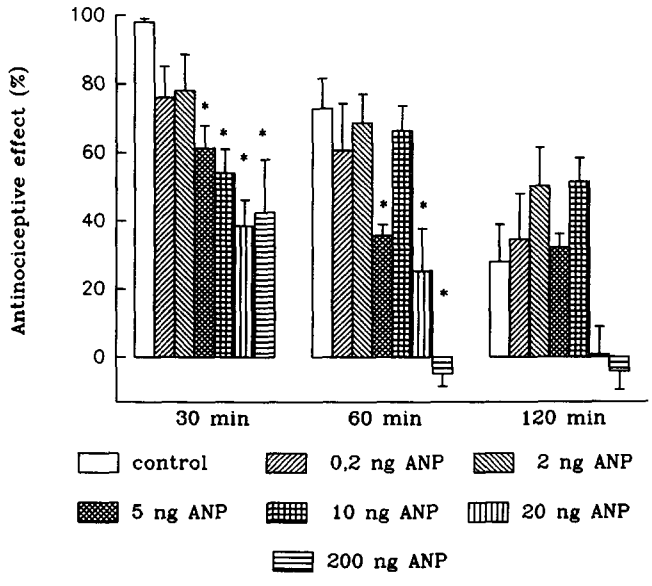

FIG. 2. Effects of ANP (ICV) on acute antinociceptive action of morphine (4 mg/kg) in tail-flick test. The procedure and abbreviations were the same as described in Fig. 1. Number of animals per group: control, 18; 0.2 ng ANP, 11; 2 ng ANP, 10; 5 ng ANP, 13; 10 ng ANP, 13; 20 ng ANP, 14; 200 ng ANP, 8.

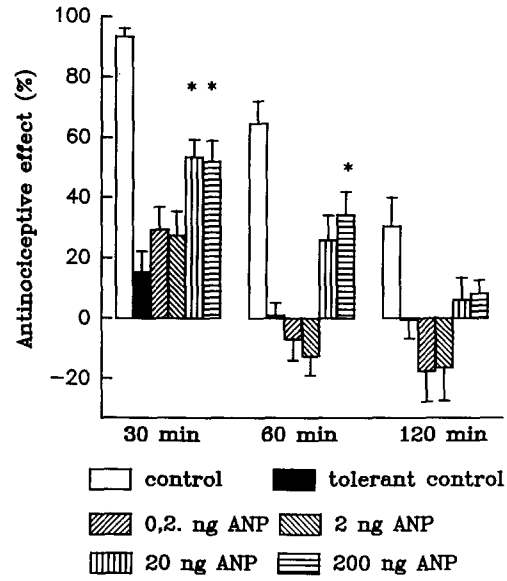

FIG. 3. Effects of ANP (SC) on development of acute tolerance to morphine. On day 1, animals were treated with peptide or vehicle and 1 h later a tolerance-inducing dose of morphine (60 mg/kg) was injected; 24 h later, a test dose of morphine (4 mg/kg) was administered after a preliminary test of sensitivity to pain; the nociceptive effect was checked 30, 60, and 120 min thereafter. If there were significant differences (at $p < 0.05$) between control and tolerant control groups at a given time point, asterisks (*) indicate significant differences (at $p < 0.05$) between tolerant control and peptide-treated groups. Number of animals per group: control, 22; tolerant control, 22; 0.2 ng ANP, 15; 2 ng ANP, 15; 20 ng ANP, 23; 200 ng ANP, 24.

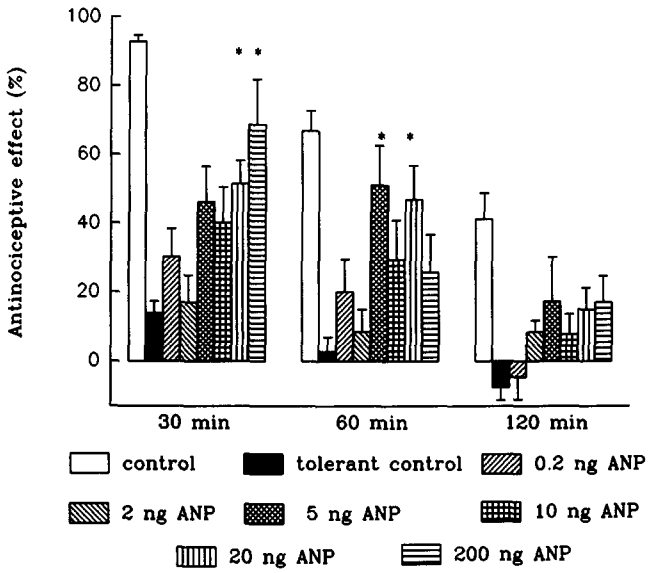

FIG. 4. Effects of ANP (ICV) on development of acute tolerance to morphine. The procedure and abbreviations were the same as described in Fig. 3. Number of animals per group: control, 31; tolerant control, 26; 0.2 ng ANP, 9; 2 ng ANP, 9; 5 ng ANP, 13; 10 ng ANP, 14; 20 ng ANP, 19; 200 ng ANP, 9.

Chronic morphine tolerance was blocked by 20 or 200 ng ANP (ICV) during a 120-min investigation period [at 30 min, $F(3, 91) = 13.10$, $p < 0.001$, 60 min, $F(3, 91) = 9.44$, $p < 0.001$, and 120 min, $F(3, 91) = 6.20$, $p < 0.001$] (Fig. 5).

In the last experiment, the influence of ICV administered ANP was investigated on the development of naloxone-precipitated withdrawal syndromes. In both doses tested (200 and 20 ng), the peptide did not modify the occurrence of any of the abstinence signs checked (Table 2).

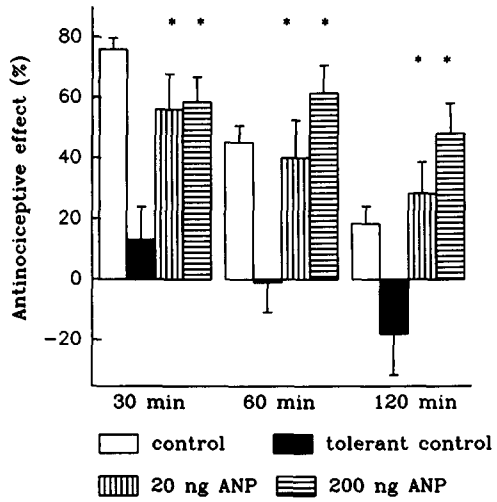

FIG. 5. Effects of ANP (ICV) on development of chronic tolerance to morphine. On day 1, peptide was given 1 h before implantation of a morphine-containing pellet (containing 35 mg morphine HCl) and ANP treatment was repeated on 3 consecutive days; on day 4, a test dose (4 mg/kg) of morphine was given just after a preliminary test of sensitivity to pain; the nociceptive effect was checked 30, 60, and 120 min thereafter. The abbreviations were the same as described in Fig. 3. Number of animals per group: control, 38; tolerant control, 30; 20 ng ANP, 13; 200 ng ANP, 14.

DISCUSSION

ANP did not affect the pain perception when it was given alone either SC or ICV. This might indicate the lack of an acute interaction of ANP at the opiate receptor site. However, when ANP was injected before a challenge dose of morphine it caused a dose-related attenuation in pain sensitivity that lasted for 2 h. The ICV injection of the peptide caused basically similar changes, although several differences were observed. A dose of ANP at least 10 times lower proved an effective blocker of pain sensitivity upon central administration. On the other hand, the blocking effect of ANP was not consistent over the investigation period, particularly if the peptide was given in lower doses. The reason for the difference is not understood but may be related to a bell-shaped dose-response curve, frequently seen after peptide treatments. The overall effect of ANP was also shorter because its action had dissipated by 120 min.

The development of tolerance to morphine was investigated in two experimental approaches. Morphine-pretreated animals displayed a lessened pain sensitivity for the same dose of morphine (4 g/kg). The morphine dose was selected to give a significant difference between the control (antinociceptive effect: 80–85%) and the tolerant control groups (antinociceptive effect: 15–18%) 30 min after injection. Acute and chronic tolerance to morphine were influenced similarly following ANP administration. Acute tolerance was affected by the same dose range of ANP after peripheral and central administration. In tolerance studies, ANP is somewhat more potent after ICV administration, although its effect was not consistent over time. The mechanisms of the lessened analgesia and tolerance after a challenge dose of morphine are poorly understood. One can speculate that the peptide treatment might alter the opiate binding to the cells.

ANP may have a physiologically relevant role in the central regulation of thirst and water balance mechanisms (5,11,12,17). The ICV administration of ANP antiserum induced a spontaneous water intake in rats and increased urinary output and urinary potassium excretion in euhydrated rats, this effect persisting for 24 h after ICV antiserum treatment (8). ANP is present in the CNS and has an inhibitory action on angiotensin-induced water intake and vasopressin release (4,10,13,15,16). Thus, ANP and vasopressin may act in opposite manners. The development of tolerance to the analgesic action of morphine is facilitated by vasopressin in mice (7). In rats, a similar behavioral effect has been observed (18). In our studies, ANP blocked the development of tolerance to morphine, showing that vasopressin and ANP exert opposite action on acquisition of tolerance to morphine.

It is known that ANP is removed from the peripheral circulation rapidly (19). Data are not available on the metabolism of peripherally administered ANP. However, in vitro studies (19) suggest it is very unlikely that a direct peptide effect can persist for up to 1 day. In our studies, on the other hand, a clear ANP effect could be observed in acute tolerance studies. Similar observation was described on the effect of ANP on passive avoidance behavior (2).

Previous experiments showed that a pellet containing a large amount of morphine (6) is sufficient to render animals dependent upon morphine. The withdrawal signs were evi-

TABLE 2

EFFECT OF ANP ON NALOXONE-PRECIPITATED WITHDRAWAL

	Control	Tolerant Control	20 ng ANP	200 ng ANP
Basal weight (g)	29.58 ± 0.58	28.43 ± 0.87	30.75 ± 1.19	28.91 ± 0.80
15 min body weight difference (g)	−0.33 ± 0.08	−0.85 ± 0.14*	−1.04 ± 0.20*	−0.90 ± 0.12*
30 min body weight difference (g)	−0.37 ± 0.08	−1.09 ± 0.15*	−1.32 ± 0.24*	−1.27 ± 0.17*
60 min body weight difference (g)	−0.44 ± 0.08	−1.23 ± 0.15*	−1.56 ± 0.24*	−1.39 ± 0.17*
Basal temperature (°C)	38.58 ± 0.12	37.99 ± 0.45	38.50 ± 0.26	38.39 ± 0.16
Temperature difference (°C)	−0.27 ± 0.14	−0.82 ± 0.43	−0.92 ± 0.18	−0.74 ± 0.16
Jumping latency (min)	N/A	10.52 ± 1.02	12.66 ± 1.23	10.45 ± 1.31

Values represent mean ± SEM for animals tested. Number of animals per group: control, 29; tolerant control, 22; 20 ng ANP, 13; 200 ng ANP, 15. N/A, no jumping was detected in the control group.

*$p < 0.05$ compared to control group (ANOVA followed by Tukey test).

denced by a loss in body weight, decrease in temperature, and spontaneous jumping from a table after naloxone administration. The development of morphine withdrawal signs was not influenced by ANP. Neither naloxone-induced jumps nor changes in body weight and temperature were affected by central administration of the peptide. The development of tolerance to and dependence upon narcotic analgesics are not necessarily parallel (9) and it is likely that ANP acts differently on this process.

From these experiments, it can be concluded that ANP affects the development of tolerance and leaves the dependence process unaffected.

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