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Effects of Secretin on Acute and Chronic Effects of Morphine

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BABARCZY, E., G. SZABÓ AND G. TELEGDY. *Effects of secretin on acute and chronic effects of morphine.* PHARMACOL BIOCHEM BEHAV 51(2/3) 469–472, 1995.—The effects of intracerebroventricularly (ICV) administered secretin on the analgesic, tolerance-inducing, and dependence-inducing actions of morphine were investigated, in adult, male CFLP mice. Secretin administered doses ICV did not itself affect pain sensitivity in a heat-radiant tail flick test. However, it depressed the acute nociceptive effect of a single subcutaneous (SC) dose of morphine (4 mg/kg) after ICV (1 or 10 ng/animal) secretin administration. A dose of 10 ng secretin facilitated the development of acute morphine tolerance. On the other hand, none of the doses applied had any influence on chronic morphine tolerance, where animals were implanted SC with a morphine- containing pellet and the pain sensitivity was measured 3 days later. Morphine withdrawal signs were also evaluated by injecting naloxone. In a 100-ng dose, secretin increased the latency of the withdrawal jumping response; the peptide did not modify the other abstinence signs. These data suggest that central secretin administration can modify the analgesic effect of morphine.

Morphine Tolerance Dependence Secretin Pain Tail flick

SECRETIN, a 27 amino acid peptide, has been identified by different techniques in the central nervous system of vertebrates (2,12,13). The effects of secretin on the periphery are well characterized, whereas those on the central nervous system were largely unknown until secretin receptors (5) and secretin-sensitive brain adenylate cyclase activity (15) were described. Recently, the cDNAs and genes for secretin were detected (8), and its distribution within the brain has been described (6).

Relatively few data are available on the effects of secretin on behavioral processes. Secretin injected ICV significantly increased defecation and decreased open field activity and novel-object approaches. The peptide has no effect on stereotypic behavior (gnawing, grooming, or rearing) in rats (3). No data have been reported on the effects of secretin on pain sensitivity, but a structural analogue, vasoactive intestinal peptide (VIP), has been shown to produce analgesia (7) by action on both opioid- and nonopioid-modulated pain pathways.

The present paper reports an investigation of the effects of secretin on pain sensitization, its acute interaction with morphine, its interaction with the development of acute and chronic tolerance, and the development of morphine withdrawal upon ICV peptide administration.

METHOD

Animals

Male CFLP mice (30 ± 5 g) of an outbred strain (LATI, Gödöllő, Hungary) were used. They were kept under a standard light–dark cycle (lights on between 0600 and 1800 h) with food and water available ad lib. At least a week of habituation was allowed before the beginning of experiments. The animals were kept and treated according to the rules of the Ethical Committee for the Protection of Animals in Research (Albert Szent-Györgyi Medical University).

Surgery

For ICV cannulation, 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. The experiments started 4 days after ICV cannulation. Upon conclusion of the 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.

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TABLE 1
EFFECTS OF SECRETIN ON PAIN SENSITIVITY

Time (min)	Control	Secretin 0.01 μg	Secretin 0.1 μg	Secretin 1 μg
15	2.37 ± 1.38 (11)	4.13 ± 1.06 (8)	3.21 ± 1.52 (8)	−1.15 ± 2.51 (10)
30	2.25 ± 1.69 (11)	4.83 ± 0.74 (8)	3.34 ± 1.39 (8)	−3.03 ± 2.43 (10)
45	5.14 ± 1.27 (11)	4.92 ± 1.21 (8)	2.02 ± 1.33 (8)	−2.55 ± 2.13 (10)
60	1.36 ± 1.30 (11)	2.18 ± 1.19 (8)	0.83 ± 1.77 (8)	−5.48 ± 2.74 (10)

Values are means ± SEM for treated animals; number of animals shown in parentheses.

For chronic tolerance and withdrawal studies, animals were lightly anesthetized with ether, and a morphine pellet was implanted SC into the sacral area through a small section in the neck area.

Treatments

For ICV treatment, the peptide was dissolved in artificial cerebrospinal fluid and injected in a volume of 2 μl. In all experiments involving measurement of the antinociceptive effect, morphine-HCl (Alkaloida, Tiszavasvári, Hungary; 4 mg/kg, SC) was used. In acute tolerance studies, 100 mg/kg of morphine-HCl (SC) was used as a tolerance-inducing dose. In chronic tolerance and withdrawal studies, pellets containing 35 mg morphine were implanted. The precipitated withdrawal syndrome was induced with naloxone-HCl, 1 mg/kg SC (Sigma, St. Louis, MO).

Procedures

All experiments were started with an initial tail flick latency measurement, pain sensitivity being measured immediately before and 15, 30, 45, and 60 min after the test morphine challenge. The heat-radiant tail flick method of D'Amour and Smith (4) was used with identical equipment. The antinociceptive effect was expressed according to the equation:

$$ANTINOCICEPTIVE\ EFFECT = \frac{TF_n - TF_0}{TF_{max} - TF_0} \times 100$$

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 measurement *n* (15, 30, 45, or 60) min after morphine injection, and TF_{max} indicates the cutoff time (20 s). The control tail flick latencies (TF_0) were between 2.5 and 3.0 s in all groups.

The following experiments were conducted:

1. The effects of secretin on pain sensitivity were studied.
2. In studies with secretin on the acute antinociceptive effect of a single dose of morphine, the peptide was given ICV 15 min prior to the test dose of morphine (4 mg/kg, SC), and the pain sensitivity was assessed 30 and 60 min later.
3. In acute tolerance studies, animals were pretreated with the peptide and 15 min later a tolerance-inducing dose of morphine (100 mg/kg, SC) was injected; 6 h later, a test dose of morphine (4 mg/kg) was used to assess the antinociceptive effect.
4. In chronic tolerance and withdrawal studies, the peptide was given 15 min before the morphine-containing pellets were implanted. The peptide treatment was repeated three times, 24 h apart. On day 4, a test dose of morphine (4 mg/kg) was given and the antinociceptive effect was determined. Three hours later, the animals received naloxone (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 (10). The body temperatures and body weights of all animals were measured 30 and 60 min after injection of naloxone, and the changes in both parameters were calculated.

Statistical Analysis

Statistical analysis of the data was made by ANOVA. For significant ANOVA values, groups were compared by Tukey's test for multiple comparisons with unequal cell size. A probability level of 0.05 was accepted as indicating significant differences.

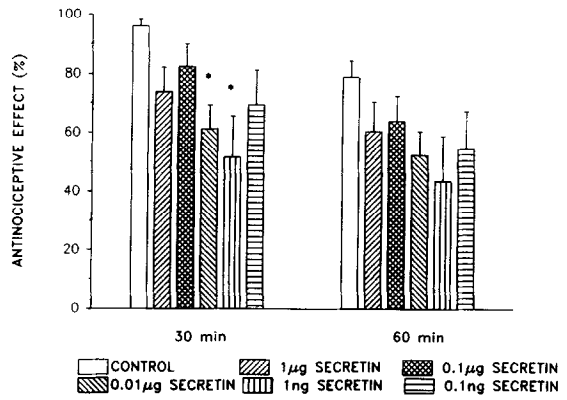

FIG. 1. Effects of secretin ICV on acute antinociceptive action of morphine (4 mg/kg) in tail flick test. The peptide was given ICV 15 min prior to the test dose of morphine, and the pain sensitivity was assessed 30 and 60 min later. Control, $n = 27$; 1 μg secretin, $n = 16$; 0.1 μg secretin, $n = 16$; 0.01 μg secretin, $n = 24$; 1 ng secretin, $n = 8$; 0.1 ng secretin, $n = 8$. Two doses of secretin (1 and 10 ng) inhibited morphine analgesia 30 min after narcotic challenge ($p < 0.01$). The peptide effect dissipated by 1 h. Values shown are means ± SEM.

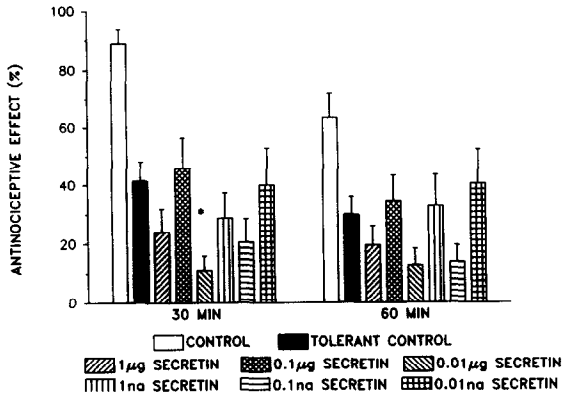

FIG. 2. Effects of secretin ICV on development of acute tolerance to morphine. Animals were pretreated with the peptide, 15 min later a tolerance-inducing dose of morphine (100 mg/kg, SC) was injected, and 6 h later a test dose of morphine (4 mg/kg) was used to assess the antinociceptive effect. Control, $n = 15$; tolerant control, $n = 28$; 1 μg secretin, $n = 13$; 0.1 μg secretin, $n = 13$; 0.01 μg secretin, $n = 16$; 1 ng secretin, $n = 12$; 0.1 ng secretin, $n = 12$; 0.01 ng secretin, $n = 8$. There was a significant difference between the control and tolerant control animals 30 and 60 min after an acute morphine challenge. Secretin in a small dose of 10 ng facilitated the development of acute tolerance to morphine ($p < 0.01$). The enhancement caused by secretin dissipated by 1 h after a test dose of morphine.

RESULTS

Effects of Secretin on Tail Flick Latency

Graded doses of secretin (0.01, 0.1, or 1 μg) administered ICV at 15, 30, 45, or 60 min had no analgesic effect themselves (Table 1).

Effects of Secretin on a Challenge Dose of Morphine

A single dose of morphine caused a near-maximal analgesic effect. Two doses of secretin (1 or 10 ng) inhibited morphine analgesia 30 min after narcotic challenge, $F(5, 93) = 4.53$, $p < 0.01$. The peptide effect dissipated by 1 h, $F(5, 93) = 2.04$, $p < 0.08$ (Fig. 1).

TABLE 2

EFFECTS OF SECRETIN ON CHRONIC MORPHINE TOLERANCE

	Time	
	30 min	60 min
Control	76.50 ± 14.68 (5)	41.46 ± 14.76 (5)
Tolerant Control	8.57 ± 4.17 (7)*	12.96 ± 4.60 (7)
0.01 μg Secretin	1.62 ± 2.89 (5)	−2.91 ± 2.38 (5)
0.1 μg Secretin	18.45 ± 9.02 (7)	13.64 ± 6.85 (7)
1 μg Secretin	7.91 ± 2.42 (5)	3.55 ± 3.93 (5)

Values are means ± SEM for treated animals; number of animals shown in parentheses.

*$p < 0.05$ compared with control group (by Tukey test).

Effects of Secretin on Acute Morphine Tolerance

Acute tolerance was observed 6 h after a tolerance-inducing dose of morphine was given. Acute tolerance could be observed throughout the whole investigation period. Secretin in a small dose of 10 ng facilitated the development of acute tolerance to morphine, $F(7, 109) = 9.38$, $p < 0.01$. As in the previous study, no clear dose-effect relationship was observed (Fig. 2). The enhancement caused by secretin dissipated by 1 h after a test dose of morphine, but there was a significant difference between the control and tolerant control groups, $F(7, 109) = 4.46$, $p < 0.01$.

Effects of Secretin on Chronic Tolerance and Withdrawal

Chronic tolerance to morphine was observed in animals implanted with morphine pellets. Graded doses of secretin had no effect on the development of tolerance (Table 2).

In naloxone-precipitated withdrawal studies, a significant induced weight loss was observed in tolerant control mice and animals treated with 1 mg/kg naloxone for the time of withdrawal studies, $F(4, 51) = 4.30$, $p < 0.01$. A transitory decrease in body temperature was detected in animals treated with secretin (10–100 ng), $F(4, 51) = 3.50$, $p < 0.01$. Neither

TABLE 3

EFFECTS OF SECRETIN ON NALOXONE-PRECIPITATED WITHDRAWAL

			Secretin		
	Control	Tolereant Control	0.01 μg	0.1 μg	1 μg
Basal body weight(g)	27.02 ± 0.79	26.11 ± 0.84	27.43 ± 0.77	26.33 ± 1.31	26.32 ± 1.23
30-min body weight difference (g)	−0.67 ± 0.09	−1.32 ± 0.14*	−1.47 ± 0.12*	−1.13 ± 0.17	−1.04 ± 0.15
60-min body weight difference (g)	−0.75 ± 0.09	−1.40 ± 0.14*	−1.58 ± 0.13*	−1.20 ± 0.15	−1.15 ± 0.14
Basal temperature (°C)	37.94 ± 0.13	37.94 ± 0.18	38.19 ± 0.13	37.60 ± 0.16	37.39 ± 0.17
30-min temperature difference (°C)	0.07 ± 0.12	−0.51 ± 0.20	−0.95 ± 0.22*	−0.77 ± 0.20*	−0.79 ± 0.26
60-min temperature difference (°C)	0.13 ± 0.10	0.14 ± 0.21	−0.38 ± 0.19	−0.25 ± 0.18	−0.23 ± 0.23
Jumping latency (s)	N/A	427.6 ± 77.8	463.8 ± 103.2	841.8 ± 58.1†	394.2 ± 84.2

Values are means ± SEM for tested animals. Numbers of animals per group: control, $n = 10$; tolerant control, $n = 13$; 0.01 μg secretin, $n = 10$; 0.1 μg secretin, $n = 13$; 1 μg secretin, $n = 10$.

N/A: no jumping was detected in the control group

There were no significant differences among basal body weights or basal temperatures.

*$p < 0.05$ compared with control group (by Tukey test).

†$p < 0.05$ compared with tolerant control group (by Tukey test).

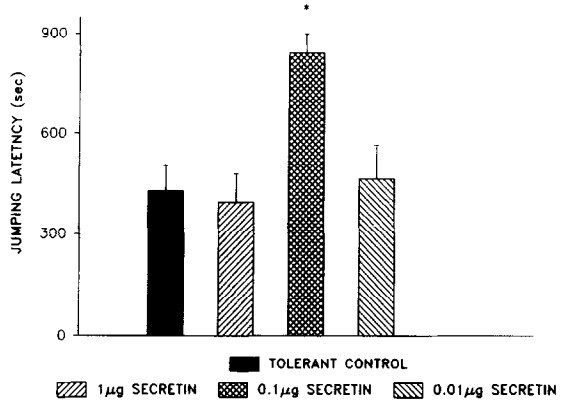

FIG. 3. Effects of different doses of secretin on withdrawal jumping response. The peptide was given 15 min before the morphine-containing pellets (containing 35 mg morphine-HCl) were implanted. The peptide treatment was repeated three times 24 h apart. On day 4, the animals received naloxone (1 mg/kg, SC) and the precipitated abstinence syndrome was measured by scoring the latency of the appearance of stereotyped jumping from a circular platform. A cutoff time of 900 s was used. Tolerant control, $n = 13$; 1 μg secretin, $n = 10$; 0.1 μg secretin, $n = 13$; 0.01 μg secretin, $n = 10$. Naloxone-precipitated withdrawal jumping occurred significantly later in animals treated with 0.1 μg secretin ($p < 0.01$).

basal body weight nor temperature differed between the groups at the beginning of the experiments (Table 3).

Naloxone-precipitated withdrawal jumpings, however, occurred significantly later in animals treated with 0.1 μg secretin, $F(4, 51) = 11.01, p < 0.01$ (Fig. 3).

DISCUSSION

The present results revealed an interaction between centrally administered secretin and morphine. Dose-related studies were carried out to determine the effects of secretin on pain sensitivity, on acute morphine analgesia and tolerance, and on chronic tolerance and withdrawal. Secretin did not influence analgesia, but depressed the analgesic effect of a challenge dose of morphine; however, the effect was not dose releated. Also, secretin facilitated the development of acute tolerance to morphine. Chronic tolerance, on the other hand, was not affected by secretin.

Naloxone-precipitated withdrawal symptoms were not evenly affected by secretin. A dose of 100 ng secretin significantly increased the hypothermic response and prolonged the onset of withdrawal jumping, but left the body weight unaffected during withdrawal. In contrast, a dose of 10 ng peptide caused a marked loss in body weight, but left other withdrawal symptoms unchanged.

Though graded doses of secretin were used in these studies, not linear dose-effect curves, but rather an inverted bell-shaped dose-response relationship was observed. In acute studies, a 10-ng dose of secretin produced the most prominent block in nociceptive effect. Higher and lower doses of the peptide were equally ineffective. The inverted bell-shaped dose-effect phenomenon is known from the literature (10,14), but as yet the explanation is unknown. A similar U-shaped dose-effect curve was observed for the withdrawal symptoms, with a shift to higher doses of secretin necessary to influence withdrawal signs.

Previous data are available from our laboratory on the effects of other neuropeptides on pain sensitivity. It has been found that atrial natriuretic peptide and oxytocin diminish the development of acute and chronic morphine tolerance and dependence, suggesting a potential endogenous role for these neuropeptides in narcotic addiction (1,11).

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