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Oxytocin Blocks the Development of Heroin–Enkephalin Cross-Tolerance in Mice

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KRIVÁN, M., G. SZABÓ, Z. SARNYAI, G. L. KOVÁCS AND G. TELEGDY. *Oxytocin blocks the development of heroin-enkephalin cross-tolerance in mice.* PHARMACOL BIOCHEM BEHAV **43**(1) 187–192, 1992.—The development of cross-tolerance to an analgesic effect has been observed between a μ-receptor agonist, heroin, and a δ-receptor agonist, Met^2-Pro^5-enkephalinamide. Repeated treatments with heroin twice a day for 4 days resulted in a decreased nociceptive effect to enkephalin on day 5. The enkephalin dose-response line was shifted to the right, considered a sign of the development of cross-tolerance. Peripheral treatment with oxytocin blocked the development of heroin–enkephalin cross-tolerance. A similar effect was observed after intracerebroventricular administration of oxytocin, supporting our assumption that oxytocin blocks the development of heroin–enkephalin cross-tolerance via CNS mechanisms.

Heroin Enkephalin Met^2-Pro^5-enkephalinamide Cross-tolerance Oxytocin Analgesic effect Opiate receptor

OPIATE receptors constitute a heterogenous network in the CNS. The main opiate receptor subclasses were described by Gilbert and Martin (10,20) as μ-, κ-, and σ-receptors. A year later, the existence of a new peptide-sensitive opiate receptor subtype was assumed, named the δ-receptor (19). The above-mentioned articles and the report by Porreca et al. (24) suggested that specific opiate effects are mediated by different opiate receptor subclasses. Each of the major opiate ligand classes is selective for one of the major receptor types. For example, while morphine or heroin exerts its effects via activation of μ-opiate receptors (6), enkephalin interacts with δ-receptors (29) and dynorphine has high affinity for κ-receptors (7). However, most of these ligands do not have significant affinity solely for one opiate receptor subtype. Thus, morphine has highest affinity for μ receptors but it binds to and exerts its effects through other opiate receptor subclasses too (11).

The development of tolerance as a consequence of a prolonged exposure to opiate agents is well established. Cross-tolerance can be observed between many narcotic drugs. Previously, it was assumed that a complementary chemical structure and similar pharmacological action on the same receptor sites (22,25) are the prerequisite conditions for the development of cross-tolerance, for example, morphine vs. normorphine and morphine vs. levalorphanol. However, it has been verified that cross-tolerance may be observed between other opiate receptor subtype binding subclasses (30).

The discovery of endogenous opiate peptides (Met- and Leu-enkephalin) gave impetus to the development of synthetic enkephalin analogs. Pert et al. (23) described a synthetic enkephalin analog, D-Ala^2-Met^5-enkephalin, that appeared to be resistant to enzymatic degradation without loss of receptor affinity and yielded a long-lasting antinociceptive activity. Similarly, D-Ala^2-D-Leu^5-enkephalin exerts dose-related long-duration antinociceptive activity after ICV administration (4). Bajusz et al. (3) reported the synthesis of an even more potent analgesic peptide: Met^2-Pro^5-enkephalinamide. The analog was a 55 times more potent analgesic than morphine when injected intravenously and 49.8 times more active than morphine when applied ICV (3). Schwarzberg et al. reported that the acute tolerance of Met^2-Pro^5-enkephalin can be attenuated by oxytocin (OXT) (27).

It is well known that OXT, a nonapeptide of posterior pituitary origin, has numerous modulating effects in the CNS. OXT attenuates learning and memory processes (8,18) in animals and human volunteers (9,16) and can therefore be considered an amnestic nonapeptide. OXT diminishes the electrical self-stimulation rate (26) and drug self-administration (14). It has been noted that systemic injection of this neuropeptide inhibits the development of acute and chronic tolerance to heroin (16), β-endorphin (17), and Met^2-Pro^5-enkephalin (27). Both ICV infused and IP injected OXT dose dependently reduced food and water consumption and the time spent eating and drinking, and increased the latency to the first eating

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and drinking (2). Repeated treatment with OXT inhibited the development of tolerance not only to opiates but also to the hypothermic action of ethanol (28).

In the present study, we investigated whether cross-tolerance can develop between heroin and Met^2-Pro^5-enkephalin. We also set out to study the effects of OXT on heroin–enkephalin cross-tolerance.

METHOD

Adult, male CFLP mice (N = 404) of an inbred strain (LATI, Gödöllő, Hungary), weighing 25 ± 5 g, were used. They were maintained on a standard illumination schedule of 12 L:12 D (lights on at 7:00 a.m.). Mice were housed 10 per cage with standard food and water available ad lib. Animals were anesthetized with Nembutal (sodium pentobarbital, 40 mg/kg, IP) and a stainless steel 18-ga guide cannula was implanted into the right lateral cerebral ventricle and fixed to the skull with dental acrylate (the coordinates for the cannula placement were: 1 mm posterior to the bregma, 1.2 mm lateral to the sagittal suture and 2.5 mm deep from the top of skull). The experiments were started 5 days after surgery.

Measurement of Nociceptive Sensitivity

The nociceptive sensitivity of animals was determined by using a tail-flick method. The nociceptive stimulus was a standard intensity lightbeam focuses from a distance of 3 cm onto the root of the tail. Mice could alleviate the pain by pulling away their tails.

The antinociceptive effect was calculated via the following formula:

$$\text{antinociceptive effect} = \frac{TF_{30} - TF_0}{TF_{max} - TF_0} \times 100,$$

where TF_{max} is the maximum cutoff time (20 s), TF_0 is the tail-flick latency before injection of enkephalin or heroin (the control tail-flick latencies were between 1.5–2.2 s), and TF_{30} is the corresponding value 30 min later.

Development of Chronic Heroin Tolerance

Chronic heroin tolerance was induced with increasing doses of heroin (diacetylmorphine hydrochloride; Diosynth, Appeldoorn, Holland) injected SC twice a day for 4 days (2, 4, 4, and 6 mg/kg, SC, heroin). On the fifth day, the antinociceptive effect of a test dose of heroin (0.1, 0.25, or 0.5 mg/kg, SC) was measured in heroin-pretreated and heroin-naive control groups. The latter group was treated with 0.9% saline twice a day for 4 days.

Development of Heroin–Enkephalin Cross-Tolerance

Heroin tolerance was induced in the same manner as described above. On the fifth day, however, the antinociceptive effect of Met^2-Pro^5-enkephalin (donated by Dr. Botond Penke) was tested. The enkephalin analog, dissolved in artificial cerebrospinal fluid (CSF), was administered into the lateral cerebral ventricle in a dose of 0.1, 0.25, 0.4, or 0.5 μg/animal; 30 min after enkephalin injection, the tail-flick latency was measured again.

Modification of Heroin-Enkephalin Cross-Tolerance by OXT

Oxytocin (G. Richter Pharmaceutical Co., Budapest, Hungary) treatment was administered in three schedules:

1. One group of animals was treated with OXT (0.05 μg/animal, SC) 30 min before each heroin treatment for 4 days. The last treatment was given 30 min before measurement of the cross-tolerance with an ICV dose of enkephalin.
2. In the second schedule, the effect of a single dose of OXT (0.5 μg/animal, SC) on the cross-tolerance was investigated. OXT was administered peripherally 30 min before the test dose of enkephalin was injected.
3. In the third schedule, the effect of chronic ICV OXT administration on the development of cross-tolerance was studied. Twice a day for 4 days, 30 min prior to heroin treatment, OXT (0.005 μg/2 μl/animal, ICV) was injected.

Mice subjected to previous ICV injections were decapitated after experiments, and the position of the cannula was controlled visually by ICV injection of methylene blue into the decapitated heads. Animals with misplaced cannulae were not included in the final evaluation.

Statistical Evaluation of Data

For statistical analysis of the results, linear regression lines were computed on the basis of Bolton's method (5). The linearity and parallelism of the dose-response lines were calculated and the relative potency was determined by comparing the dose-response lines. The relative potency was expressed as a ratio between two drugs that give the same response (5).

RESULTS

Measurement of Control Tail-Flick Latencies

Control tail-flick latencies (TF_0) were measured before the test dose of heroin or enkephalin was given. None of the pretreatments altered the initial tail-flick latencies among the groups (data not shown; p = 0.66–0.98 for Figs. 1–4).

Development of Tolerance to Heroin

Repeated administration of heroin shifted the heroin dose-response curve to the right as compared to the saline-pretreated control group. In the tolerant group, 2.82 times more heroin was necessary to produce the same analgesic effect as that in heroin-naive control animals. The shift to the right of the heroin dose-response curve demonstrates the development of tolerance to heroin in heroin-pretreated animals (Fig. 1).

Development of Heroin–Enkephalin Cross-Tolerance

In the next step, the effect of enkephalin was investigated in heroin-tolerant mice. Following the chronic heroin treatment, the analgesic effect of enkephalin was tested. While enkephalin gave a near-maximum analgesic effect (99%) in nontolerant control mice at a dose of 0.4 μg/animal, the same dose of enkephalin in heroin-tolerant mice had a lower effect (58%). After chronic heroin treatment, the enkephalin dose-response curve shifted to the right, which demonstrates the development of cross-tolerance between heroin and enkephalin. In the tolerant group, 2.45 times more enkephalin was needed to produce the same analgesic response (Fig. 2).

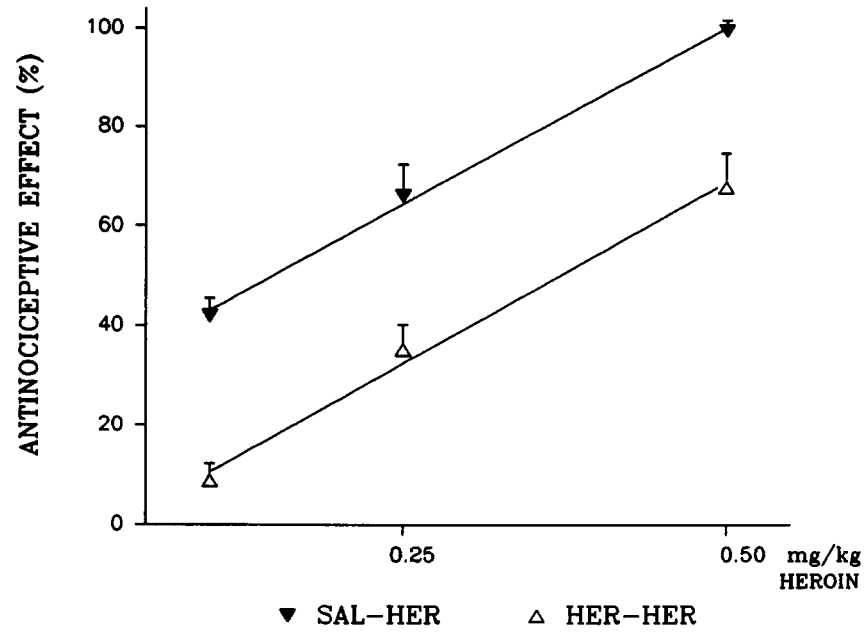

FIG. 1. Dose-response curves for test doses of heroin (0.1, 0.25, or 0.5 mg/kg, SC) in mice treated chronically with saline or heroin. SAL, 0.9% NaCl SC twice a day for 4 days; HER, heroin 2, 4, 4, and 6 mg/kg SC twice a day for 4 days; HER, test doses of heroin on the fifth day. Number of animals per group: SAL-HER, 27; HER-HER, 27. Values shown are means ± SEM.

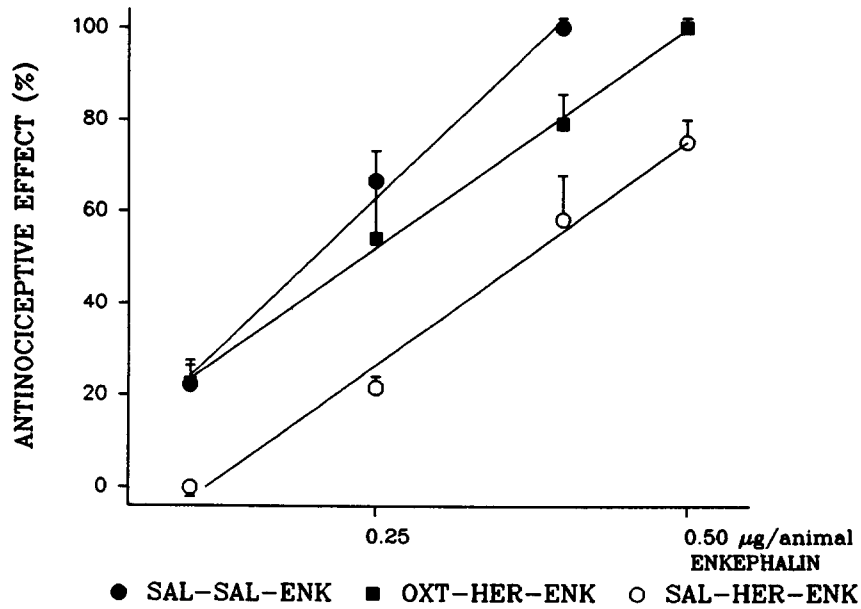

FIG. 2. Development of heroin-enkephalin cross-tolerance. Effect of chronically SC administered OXT on heroin-enkephalin cross-tolerance. SAL, HER, see Fig. 1; ENK, test doses of enkephalin (0.1, 0.25, 0.4, or 0.5 μg/animal) on the fifth day; OXT, 0.05 μg/animal oxytocin SC twice a day for 4 days, 30 min before saline or heroine. Number of animals per group: SAL-SAL-ENK, 41; OXT-HER-ENK, 41; SAL-HER-ENK, 50.

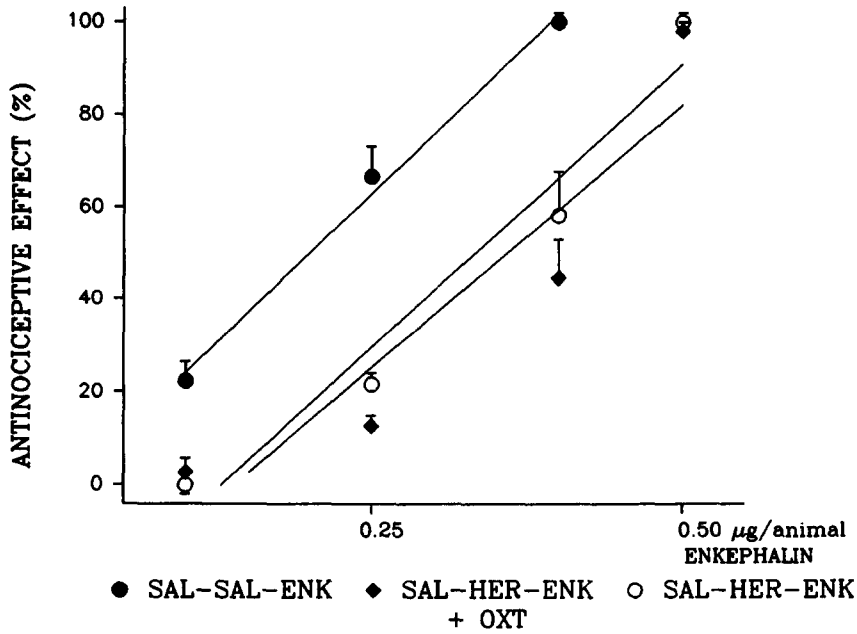

FIG. 3. Effect of a single high dose of SC administered OXT on heroin-enkephalin cross-tolerance. SAL, HER, ENK, see Figs. 1 and 2; OXT, 0.5 μg/animal oxytocin SC 30 min before the test doses of enkephalin. Number of animals per group: SAL-SAL-ENK, 29; SAL-HER-ENK + OXT, 42; SAL-HER-ENK, 43.

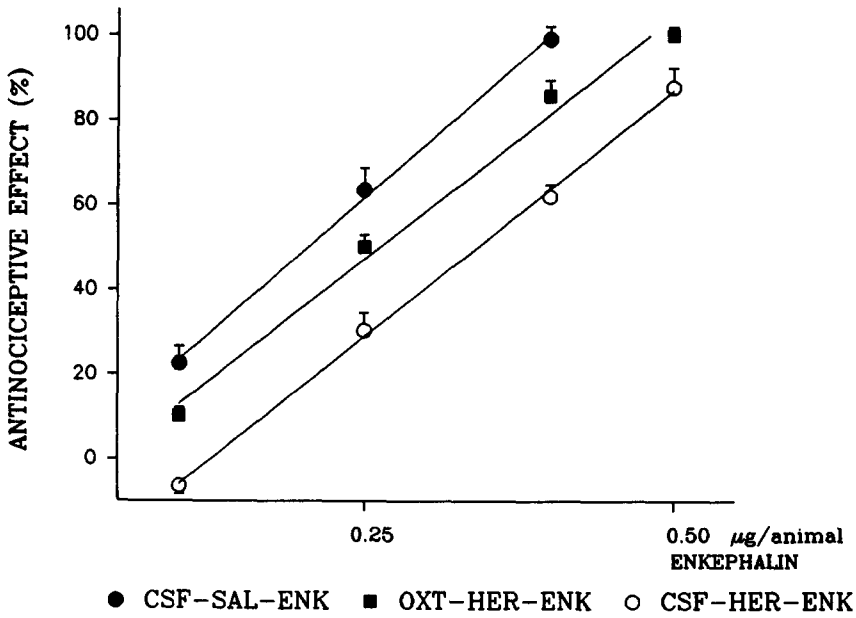

FIG. 4. Effect of chronically ICV administered OXT on heroin-enkephalin cross-tolerance. SAL, HER, ENK, see Figs. 1 and 2; OXT, 0.005 μg/animal oxytocin ICV twice a day for 4 days, 30 min before heroin or saline; CSF, twice a day for 4 days ICV. Number of animals per group: CSF-SAL-ENK, 31; OXT-HER-ENK, 38; CSF-HER-ENK, 35.

Effect of SC OXT on the Development of Heroin-Enkephalin Cross-Tolerance

In the next experiment, the modulatory effect of OXT on heroin-enkephalin cross-tolerance was examined. Chronic peripheral OXT administration altered the degree of heroin-enkephalin cross-tolerance. The enkephalin dose-response curves showing cross-tolerance with heroin shifted to the left after multiple SC OXT injection. The relative potency value of enkephalin in the OXT-pretreated group was significantly less (1.35) than that in exclusively heroin-treated animals (2.45) (Fig. 2).

To decide which phase of cross-tolerance is influenced by OXT, a single high-dose peripheral OXT injection was given prior to ICV enkephalin administration (Fig. 3). This treatment did not cause a significant shift in the enkephalin dose-response curve between heroin-treated controls and animals treated with a single dose of OXT. The relative potency of the acute OXT treatment as compared to the SAL-HER-ENK control group was 1.06.

Effect of ICV Administered OXT on Heroin-Enkephalin Cross-Tolerance

Finally, the effect of OXT applied into the lateral cerebral ventricle on cross-tolerance was studied. In animals subjected to previous chronic peripheral heroin treatment, the enkephalin dose-response curve was shifted significantly to the right as compared to the curve for the heroin-treated group (relative potency = 2.27). After chronic ICV OXT administration, the dose-response curve was shifted to the left and the relative potency of enkephalin decreased to 1.27, showing that ICV OXT treatment impaired the development of heroin-enkephalin cross-tolerance. This effect was similar to the peripheral OXT effect on the cross-tolerance, but in the present experiment the dose of OXT was one tenth of the peripheral dose (Fig. 4). An acute OXT injection itself caused a slight shift to the right in the enkephalin dose-response curve with a relative potency of 1.1 (data not shown).

DISCUSSION

The development of tolerance upon repeated administration of opiate-like agents is well established. Cross-tolerance can be observed between opiate agonists of different opiate receptor types. The earlier assumption, that only those substances show cross-tolerance that are pharmacologically similar and exert their effects on the same receptor type, has changed during the past few years. For example, a μ-receptor agonist, morphine, interacts with ethylketocyclozacine, which exhibits higher affinity to κ-receptors, and with the SWKF 10047, a specific ligand for the σ-opiate receptor subtype. Cross-tolerance had been described between substances that exert their effects on an entirely different receptor type, for example, a μ-receptor agonist, morphine, and a GABA receptor agonist, THIP (1).

The exact mechanism of cross-tolerance, however, is not known. Our study revealed an interaction between a δ-receptor agonist, Met^2-Pro^5-enkephalinamide, and a μ-receptor agonist, heroin. Cross-tolerance to enkephalin can develop in heroin-tolerant mice if heroin has agonist activity not only for μ-receptors but also for δ-receptors. On the other hand, one can assume that chronic treatment with heroin might alter the binding characteristics of δ-opiate receptors. It is also possible that the two opiate agonists induce analgesia by acting through separate, but functionally related, pathways at a subcellular level. The mechanism of development of cross-tolerance at a subcellular level is poorly understood. It is known that μ- and δ-opiate receptors are negatively coupled by a GTP-binding protein to adenylate cyclase and decrease cyclic AMP formation (12,13). On the other hand, μ- and δ-receptors increase K^+ conductance in the cell membrane and are not linked to changes in phosphatidyl inositol turnover in the brain and perhaps in other tissues (21). A common molecular mechanism for the μ- and δ-receptors, therefore, can account for the development of cross-tolerance.

OXT reportedly inhibits narcotic tolerance (16,18,19). The present results demonstrate that OXT inhibits the development of heroin-enkephalin cross-tolerance after both peripheral and central administration. Sustained OXT administration was necessary to demonstrate a block in the development of cross-tolerance. A single dose of OXT given just prior to the test of cross-tolerance was insufficient.

A connection between the OXT and dopaminergic system has been described, indicating that dopamine (DA) stored in and released from the neural lobe may inhibit OXT secretion. Chronic administration of OXT reduces the utilization and release of DA and also the density of DA binding sites in the mouse forebrain (15). This region contains DAergic terminals of the mesolimbic DAergic projection, as well as OXTergic binding sites. It is likely that OXT and the opiates are able to interact on the DAergic system. The inhibitory effect of OXT treatment on morphine tolerance and dependence can be abolished by a DA receptor antagonist, pimozide.

A single dose of OXT did not affect the antinociceptive effect of enkephalin in mice. This observation is an agreement with previous biochemical studies showing that an acute injection of OXT has no influence on the stimulation-induced release of DA (15).

The effect of OXT given centrally was more effective than after peripheral administration, supporting our supposition that OXT may act through CNS structures, although a full range of protection doses of OXT were not used. Elucidation of the exact mechanism awaits further experiments.

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