Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 3, pp. 591–594, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00145-X

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

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Received 27 April 1993

KRIVÁN, M., G. SZABÓ, Z. SARNYAI, G. L. KOVÁCS AND G. TELEGDY. *Oxytocin blocks the development of heroin-fentanyl cross-tolerance in mice.* PHARMACOL BIOCHEM BEHAV **52**(3) 591–594, 1995.—The development of cross-tolerance to an analgesic effect was observed between two μ-receptor agonists, heroin and fentanyl. Repeated treatments with heroin twice a day for 4 days resulted in a decreased nociceptive effect to fentanyl on day 5. The fentanyl dose-response line shifted to the right, and was considered to be a sign of the development of cross-tolerance. Peripheral treatment with oxytocin did not block the development of heroin–fentanyl cross-tolerance. However, intracerebroventricular administration of oxytocin blocked the development of tolerance, causing a leftward shift in the dose-response curve and supporting the assumption that oxytocin blocks the development of heroin–fentanyl cross-tolerance via CNS mechanisms.

Heroin Fentanyl Cross-tolerance Oxytocin Analgesic effect Opiates Narcotics Mice

THE DEVELOPMENT of tolerance as a consequence of prolonged exposure to opiate agents is well established. Cross-tolerance can be observed among many narcotic drugs. Previously, it was assumed that a complementary chemical structure and similar pharmacologic action on the same receptor sites (17,18) are the prerequisite conditions for the development of cross-tolerance (e.g., 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 (23).

Previous studies from this laboratory revealed the development of cross-tolerance of a μ-receptor agonist, heroin, to a δ-receptor agonist, Met^2-Pro^5-enkephalin (13). As a continuation of our studies on the development of opiate cross-tolerance, the effect of heroin–fentanyl cross-tolerance was investigated; both narcotics showed μ-receptor agonist activity. Fentanyl is a potent opioid frequently used in analgesia and classified as a pure μ-agonist (14–16,22). It is also known that fentanyl traces can be found in heroin from drug markets. A recent outbreak of drug overdose death associated with fentanyl-laced heroin in drug addicts shows that this drug can have a devastating effect among individuals who already abuse other narcotics such as opiates (5).

It is well known that oxytocin (OT), a nonapeptide of posterior pituitary origin, has numerous modulatory effects in the CNS. OT attenuates learning and memory processes (3,12) in animals and human volunteers (4,10), and can therefore be considered an amnestic nonapeptide. Both intracerebroventricular (ICV)-infused and intraperitoneal-(IP) injected OT dose-dependently reduced food and water consumption and the time spent eating and drinking, and increased the latency to the first eating and drinking (1). Repeated treatment with OT inhibited the development of tolerance to the hypothermic action of ethanol (21). Oxytocin diminishes the electrical self-stimulation rate (19) and drug self-administration (7). It has been noted that systemic injection of this neuropeptide inhibits the development of acute and chronic tolerance to heroin (9,10), β-endorphin (11), and Met^2-Pro^5-enkephalin (20), and the cross-tolerance of heroin to enkephalin (13).

Because heroin is one of the most frequently abused narcotic analgesic and traces of fentanyl can be found in heroin from the drug market, in the present study we investigated whether cross-tolerance can develop between heroin and fentanyl. We also set out to study the effects of OT on heroin-fentanyl cross-tolerance.

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METHOD

Adult male CFLP mice (n = 344) of an inbred strain (LATI, Gödöllö, Hungary), weighing 25 ± 5 g, were used. They were maintained on a standard illumination schedule of 12 L : 12 D (lights on at 0700 h). 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 the 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 light beam focused 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 fentanyl or heroin and the control tail-flick latencies were between 1.5 and 2.2 s; and TF_{30} is the corresponding value 30 min later.

Development of Heroin-Fentanyl Cross-Tolerance

Chronic heroin tolerance was induced as described earlier (11), with increasing doses of heroin (diacetylmorphine hydrochloride; Diosynth, Appeldoorn, Holland) injected subcutaneously (SC) twice a day for 4 days (0800 and 1600 h) with increasing daily doses of 1, 2, 2, and 4 mg/kg. Control animals received 0.9% saline. On the 5th day, the baseline tail-flick value and antinociceptive effect of fentanyl were tested. Fentanyl, dissolved in 0.9% saline, was administered in a dose of 40–100 μg/kg animal, SC; 30 min after the fentanyl injection, the tail-flick latency was measured again.

Modification of Heroin-Fentanyl Cross-Tolerance by OT

Oxytocin (Gedeon Richter Pharmaceutical Co., Budapest, Hungary) was administered according to two schedules. One group of animals was treated with OT (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 SC dose of fentanyl on day 5. Control animals received 0.9% saline. In the second schedule, the effect of chronic ICV OT administration on the development of cross-tolerance was studied. Twice a day for 4 days, 30 min before heroin treatment, OT (0.005 μg/2 μl per animal, ICV) was injected. Control animals received an equivalent amount of artificial cerebrospinal fluid (CSF). Heroin-fentanyl cross-tolerance was measured on day 5. The following four groups were used in our experiments: a) saline/CSF + saline + fentanyl (SAL/CSF-SAL-FE); b) saline/CSF + heroin + fentanyl (SAL/CSF-HE-FE); c) oxytocin + saline + fentanyl (OT-SAL-FE); d) oxytocin + heroin + fentanyl (OT-HE-FE).

Mice subjected to previous ICV injections were decapitated after the 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 (2). The linearity and parallelism of the dose-response lines were calculated, and the relative potency and ED_{50} were determined by comparing the dose-response lines. The relative potency is expressed as a ratio between two drugs (e.g., control vs. experimental) that gave the same response (2).

RESULTS

Measurement of Control Tail-Flick Latencies

Control tail-flick latencies (TF_0) were measured before the test dose of heroin or fentanyl was given. None of the pretreatments altered the initial tail-flick latencies in the various groups (data not shown).

Development of Heroin-Fentanyl Cross-Tolerance

The effects of fentanyl were investigated in heroin-tolerant mice. Following the chronic heroin treatment, the analgesic effect of fentanyl was tested. Whereas fentanyl gave a near-maximum analgesic effect (99%) in the nontolerant control mice (SAL-SAL-FE) at a dose of 80 μg/animal, the same dose of fentanyl in heroin-tolerant mice (SAL-HE-FE) had a lower effect (50%). After chronic heroin treatment, the fentanyl dose-response curve shifted to the right, which demonstrated the development of cross-tolerance between heroin and fentanyl. In the tolerant group, 1.57 times more fentanyl was needed to produce the same analgesic response (Fig. 1). ED_{50} was 76.43 (63.05–92.65) for SAL-HE-FE group compared to 50.12 (43.80–57.34) in SAL-SAL-FE. A similar shift to the right was observed in animals given ICV treatment between the nontolerant [CSF-SAL-FE, ED_{50} 50.01 (44.05–56.77)] and tolerant [CSF-HE-FE, ED_{50} 71.21 (66.33–76.46)] mice (Fig. 2).

Effects of SC OT on the Development of Heroin-Fentanyl Cross-Tolerance

In this experiment, the modulatory effects of SC OT on heroin-fentanyl cross-tolerance were examined. Chronic peripheral OT administration did not alter the acute antinociceptive response to fentanyl [OT-SAL-FE, ED_{50} 51.79 (41.79–61.19), relative potency: 1.04], and did not block the development [OT-HE-FE, ED_{50} 72.17 (62.24–83.67), relative potency: 1.39] of heroin-fentanyl cross-tolerance. The fentanyl dose-response curves showing cross-tolerance with heroin were shifted modestly to the left after multiple SC OT injections. The ED_{50} and relative potency values of fentanyl in the OT-pretreated group were not significantly different from those for the respective control animals (SAL-HE-FE) (Fig. 1).

Effects of ICV Administered OT on Heroin-Fentanyl Cross-Tolerance

The effects of OT applied into the lateral cerebral ventricle on cross-tolerance were studied. In animals subjected to previous chronic peripheral heroin treatment (CSF-HE-FE), the

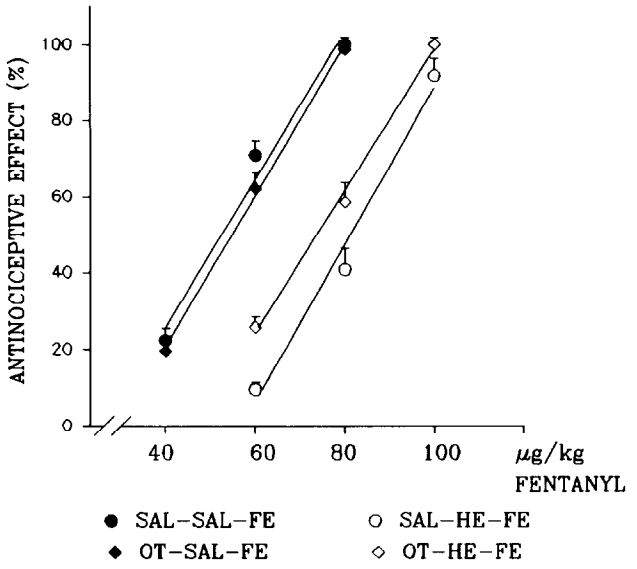

FIG. 1. Development of heroin-fentanyl cross-tolerance. Effects of SC chronically administered OT on heroin-fentanyl cross-tolerance. SAL, 0.9% NaCl, SC, twice a day for 4 days; HE, heroin, 2, 4, 4 and 6 mg/kg, SC, twice a day for 4 days; FE, test doses of fentanyl (0.1, 0.25, 0.4, or 0.5 μg/animal) on day 5; OT, 0.05 μg/animal OT, SC, twice a day for 4 days, 30 min before saline or heroin. Numbers of animals per group: SAL-SAL-FE: 32; OT-HE-FE: 33; SAL-HE-FE: 33; and OT-SAL-FE: 33. Values shown are means ± SEM.

fentanyl dose-response curve was shifted significantly to the right as compared with the curve for the saline-treated group (CSF-SAL-FE) (relative potency = 1.42). After chronic ICV OT administration, the fentanyl dose-response curve was shifted to the left [OT-HE-FE, ED_{50} 51.58 (43.06–61.79)] and the relative potency of fentanyl was decreased to 1.06, showing that ICV OT treatment impaired the development of heroin-fentanyl cross-tolerance. OT itself [OT-SAL-FE, ED_{50} 51.05 (44.65–58.36), relative potency: 1.03] did not change the antinociceptive effect of fentanyl (Fig. 2).

DISCUSSION

In a previous study, repeated injections of heroin shifted the heroin dose response curve to the right as compared to the saline-pretreated control group. For tolerant animals, 2.82 times more heroin was necessary to produce similar analgesic effect as that in heroin-naive animals (13).

The development of tolerance upon repeated administration of opiate-like agents is well established. The exact mechanism of cross-tolerance, however, is not known. Cross-tolerance can be observed between opiate agonists of different opiate receptor types. The earlier assumption that only those substances show cross-tolerance which are pharmacologically similar and which exert their effects on the same receptor type has changed during the past few years. Our previous study revealed cross-tolerance between a δ-receptor agonist, Met^2-Pro^5-enkephalinamide, and a μ-receptor agonist, heroin (13). It is likely that a common molecular mechanism for the μ- and δ-receptors can account for the development of cross-tolerance.

In the present experiment, the development of heroin-fentanyl cross-tolerance was demonstrated. In comparison with our previous study, in which the relative potency of the δ-receptor agonist Met^2-Pro^5-enkephalinamide to heroin was 2.45, in the present experiment the relative potency of fentanyl was lower. This observation is consistent with the assumption that agents such as fentanyl with high intrinsic activity will produce less tolerance, and therefore, a smaller shift in the analgesia dose-effect curve than those with low efficacy (6).

The development of cross-tolerance was assessed by dose-response studies using several doses of fentanyl; given the high number of animals used, a full dose-effect experiment was not performed with OT. The OT dose, however, was chosen on the basis of previous experiments in which OT inhibited the development of narcotic tolerance (11–13). Peripheral treatment with OT was ineffective in the present experiment, although a similar dose of OT inhibited the development of tolerance to heroin (9), heroin-enkephalinamide cross-tolerance (13), and narcotic and alcohol tolerance (11,12,21). It is interesting that OT showed a bell-shaped dose-response effect in behavioral experiments (10), and a lack of effect upon peripheral administration of OT may relate to this phenomenon. The present results demonstrate that OT inhibits the development of heroin-fentanyl cross-tolerance after central administration. Repeated peptide treatment or treatment concurrent with opiate administration was necessary to demonstrate a block in the development of cross-tolerance. Previous cross-tolerance studies demonstrated that a single dose of OT given just before the test of cross-tolerance was insufficient (13).

The effect of OT on the development of narcotic tolerance is not yet fully understood. A connection between OT and the dopaminergic system has been described, indicating the regulatory role of dopamine (DA) in central OT secretion. Chronic administration of OT reduces the use and release of DA, and also the density of DA-binding sites in the mouse forebrain (8), which region contains DA-ergic terminals of the mesolimbic DA-ergic projection as well as OT-ergic binding

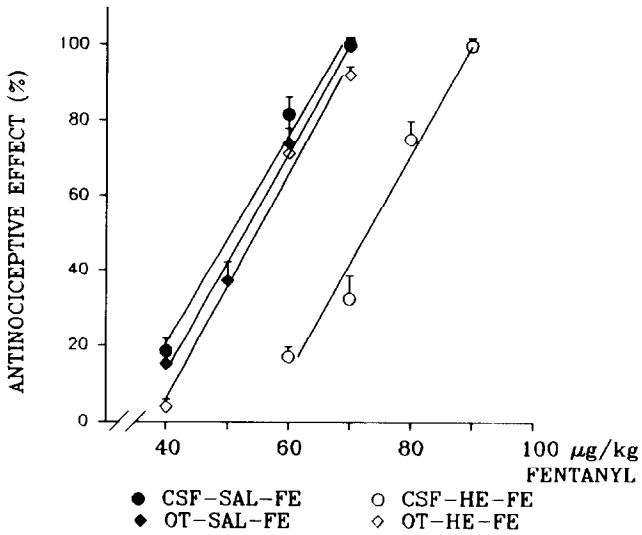

FIG. 2. Effects of ICV chronically administered OT on heroin-fentanyl cross-tolerance. OT, 0.005 μg/animal OT, ICV, twice a day for 4 days, 30 min before heroin or saline; CSF, twice a day for 4 days, ICV. For other abbreviations, see Fig. 1. Numbers of animals per group: CSF-SAL-FE: 48; OT-HE-FE: 39; CSF-HE-FE: 84; and OT-SAL-FE: 42.

sites. It is likely that OT and the opiates are able to interact in the DA-ergic system. The inhibitory effect of OT treatment on morphine tolerance and dependence can be abolished by a DA receptor antagonist, pimozide (8).

The present results showing that only OT given centrally was effective in inhibiting heroin–fentanyl cross-tolerance (peripheral administration was ineffective) strengthens our supposition that OT may act through CNS structures, although a full range of protection doses of the peptide was not used.

ACKNOWLEDGEMENTS

This work was supported by the Hungarian Ministry of Social Welfare (T-70/1990) and the National Scientific Research Foundation (OTKA) (T/3, 1354).

REFERENCES

1. Arletti, R.; Benelli, A.; Bertolini, A. Influence of oxytocin on feeding and drinking behavior. Abstract of the 11th Annual Meeting of the European Neuroscience Association 50:19; 1988.
2. Bolton, S. Pharmaceutical statistics. Practical and clinical applications. New York: Marcel Decker; 1984.
3. Drago, F.; Bohus, B.; DeWied, D. Interaction between vasopressin and oxytocin in the modulation of passive avoidance retention of the rat. Neurosci. Lett. 7:260; 1981.
4. Ferrier, B. M.; Kenneth, D. J.; Devlin, M. C. Influence of oxytocin on human memory process. Life Sci. 27:2311–2317; 1980.
5. Hibbs, J.; Perper, J.; Vinek, C. L. An outbreak of designer drug-related deaths in Pennsylvania. JAMA 265:1011–1013; 1991.
6. Jang, Y.; Yoburn, B. C. Evaluation of receptor mechanisms mediating fentanyl analgesia and toxicity. Eur. J. Pharmacol. 197: 135–141; 1991.
7. Kovács, G. L.; Borthaiser, Z.; Telegdy, G. Oxytocin reduced intravenous heroin self-administration in heroin tolerant rats. Life Sci. 37:17–26; 1986.
8. Kovács, G. L.; Faludi, M.; Falkay, G.; Telegdy, G. Peripheral oxytocin treatment modulates central dopamine transmission in the mouse limbic structures. Neurochem. Int. 9:481–485; 1986.
9. Kovács, G. L.; Faludi, M.; Telegdy, G. Oxytocin diminishes heroin tolerance in mice. Psychopharmacology 86:377–379; 1985.
10. Kovács, G. L.; Telegdy, G. Role of oxytocin in memory and amnesia. Pharmacol. Ther. 18:375–395; 1982.
11. Kovács, G. L.; Telegdy, G. β-Endorphin tolerance is inhibited by oxytocin. Pharmacol. Biochem. Behav. 26:57–60; 1987.
12. Kovács, G. L.; Vécsei, L.; Telegdy, G. Opposite action of oxytocin to vasopressin in passive avoidance behavior in rat. Physiol. Behav. 20:801–802; 1978.
13. Kriván, M.; Szabó G.; Sarnyai Z.; Kovács, G. L.; Telegdy. G. Oxytocin blocks the development of heroin-enkephalin cross-tolerance in mice. Pharmacol. Biochem. Behav. 43:187–192; 1992.
14. Lord, J. A. H.; Waterfield, A. A.; Hughes, J.; Kosterlitz, H. W. Endogenous opiate peptides, multiple agonists and receptors. Nature 267:495; 1977.
15. Magnan, J.; Paterson, S. J.; Tavani, A.; Kosterlitz, H. W. The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. Naunyn-Schmied. Arch. Pharmacol. 319:197–205; 1982.
16. Maguire, P.; Tsai, N.; Cometta-Morini, C.; Upton, C.; Loew, G. Pharmacological profiles of fentanyl analogs at μ, δ and κ opiate receptors. Eur. J. Pharmacol. 213:219–225; 1992.
17. Paktor, J.; Waught, J. L. Differential analgesic cross-tolerance morphine between lipophilic and hydrophilic narcotic agonist. Life Sci. 34:13–21; 1984.
18. Rosow, C. E.; Miller, J. M.; Poulsen-Burke, J.; Cochin, J. Opiates and thermoregulation in mice. J. Pharmacol. Exp. Ther. 223: 702–708; 1983.
19. Schwarzberg, H.; Hartman, G.; Kovács, G. L.; Telegdy, G. The effect of intraventricular administration of oxytocin and vasopressin on self-stimulation in rats. Acta Physiol. Hung. 47:127–131; 1976.
20. Schwarzberg, H.; Kovács, G. L.; Penke, B.; Telegdy, G. Effect of oxytocin on acute enkephalin tolerance in mice. Neuropeptides 7:247–250; 1986.
21. Szabó, G.; Kovács, G. L.; Székeli, S.; Telegdy, G. The effects of neurohypophyseal hormones on tolerance to the hypothermic effect of ethanol. Alcohol 2:567–574; 1985.
22. Yeadon, M.; Kitchen, I. Comparative binding of μ and σ selective ligands in whole brain and pons/medulla homogenates from rat: Affinity profiles of fentanyl derivatives. Neuropharmacology 27: 345–348; 1988.
23. Young, G. A.; Khazan, N. Differential tolerance and cross tolerance to repeated daily injection of mu and kappa opiate agonist in the rat. Neuropharmacology 23:505–509; 1984.