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β-Endorphin Tolerance is Inhibited by Oxytocin

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KOVÁCS, G. L. AND G. TELEGDY. *β-Endorphin tolerance is inhibited by oxytocin.* PHARMACOL BIOCHEM BEHAV **26**(1) 57–60, 1987.—The repeated administration of β-endorphin to mice resulted in the development of tolerance to the analgesic effect of the opioid peptide. While SC injections of oxytocin failed to modify the magnitude or the duration of the analgesic effect of a single intracerebroventricular (ICV) β-endorphin injection, the development of tolerance to β-endorphin was antagonized by SC and ICV oxytocin treatment. Since both oxytocin and β-endorphin are endogenous peptides in the brain, the data raise the possibility that endogenous oxytocin might be involved in the sensitivity of the central nervous system towards repeated or prolonged opioid stimulation.

β-Endorphin Oxytocin Analgesic effect Tolerance

RECENT data indicate that neurohypophyseal neuropeptides (oxytocin and vasopressin) affect drug addiction processes [8, 12, 21]. In accordance with this idea, oxytocin inhibited the development of tolerance to morphine, heroin or enkephalin [10, 11, 16]. As in the case of exogenous opiates, tolerance develops following repeated injections of β-endorphin, an opioid peptide normally present in the brain [19,22]. While morphine or heroin exerts its analgesic effect via activation of μ-receptors in the brain [5], enkephalins interact with δ-receptors [1,24]. β-Endorphin, on the other hand, is likely to trigger ε-receptors [15,20]. It is conceivable that the stimulation of ε-receptors contributes to the supraspinal control of spinal sensory neurons [20]. In view of these data, it was considered of interest to investigate whether oxytocin is able to modify the development of the analgesic tolerance induced by central β-endorphin treatment.

METHOD

Male CFLP mice of an inbred strain, weighing 25–30 g, were maintained on a standard illumination schedule, with food and water available ad lib. Animals were anesthetized with sodium pentobarbital (Nembutal®, 40 mg/kg) and a polyethylene cannula was then inserted into the right lateral cerebral ventricle and fixed to the skull with dental acrylate. Experiments were started after a recovery period of 5 days.

β-Endorphin (β-lipotropin 61–91, human, Organon, Oss, The Netherlands) was dissolved in artificial cerebrospinal fluid and injected via the ICV cannula.

Two experimental paradigms were used: (a) The pain sensitivity was measured with the heat irradiant method of D'Amour and Smith [6] at various time intervals (0, 15, 30, 45, 60 or 120 min) after a single ICV injection of β-endorphin. (b) Mice were first rendered tolerant to β-endorphin by daily ICV injections of 5 μg of this opioid peptide (one injection daily). On the fourth day of treatment, the pain sensitivity (TF_0) was measured and β-endorphin was then injected ICV. The analgesic effect of this treatment was measured 15 min later (TF_{15}). The percentage analgesia was calculated via the formula:

$$\text{Analgesia } (\%) = \frac{TF_{15} - TF_0}{TF_{max} - TF_0} \times 100,$$

where TF_{max} indicates the cut-off time.

Graded doses of oxytocin (Richter Co., Budapest, Hungary) were injected SC or ICV 1 hr prior to the daily injections of β-endorphin.

One-way analysis of variance, followed by post hoc paired comparison (Scheffe's test), was used to analyse data in tolerant mice. Multiple ANOVA for repeated measures and split-plot ANOVA were used to evaluate the dose- and time-related effects of β-endorphin and oxytocin in experimentally naive mice. A probability level of 0.05 was accepted as indicating significant differences.

RESULTS

The effect of ICV β-endorphin treatment on the pain sensitivity is depicted in Fig. 1. Multiple ANOVA for two independent and one repeated measures revealed a significant effect of the dose of β-endorphin and of the time between treatment and testing. Since there was a significant interaction between the dose of β-endorphin with the time of treatment (most probably because of the difference in the slopes of the curves with low versus high β-endorphin treatments), the data were further analysed: two-way ANOVA was used to compare various treatment groups in the same time interval, while split-plot ANOVA was used to measure the effect of time. Two-way ANOVA revealed that oxytocin did not influence the dose-dependent antinociceptive effect of

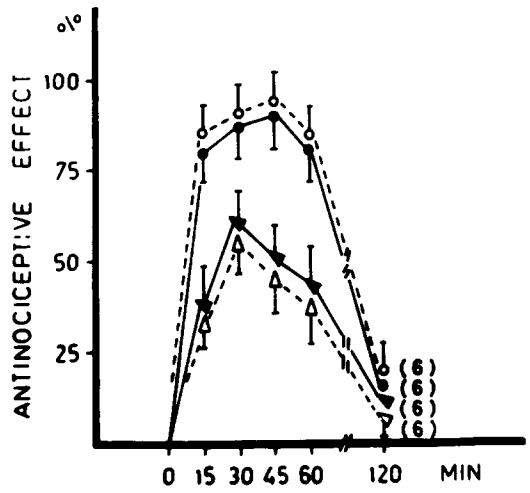

FIG. 1. Failure of oxytocin to alter the analgesic effect of a single injection of β-endorphin. Oxytocin or saline was injected SC 1 hr prior to the ICV injection of β-endorphin. The dose of oxytocin was 0.02 mg/kg. Each group consists of 6 experimental animals and the pain sensitivity in each mouse was determined 6 times. △; SC saline + 1 μg β-endorphin ICV, ▲; SC oxytocin + 1 μg β-endorphin ICV, ○; SC saline + 5 μg β-endorphin ICV, ●; SC oxytocin + 5 μg β-endorphin ICV. Vertical bars illustrate the SEM. Data on statistical analysis are listed below.

MULTIPLE ANOVA FOR TWO INDEPENDENT AND ONE REPEATED MEASURE

	Sum Squares	Degree of Freedom	Mean Square	F-Test Ratio	Sign.
Factor A	537.07	1	537.07	51.42	0.001
Factor B	1.74	1	1.74	0.17	NS
A × B	4.60	1	4.60	0.44	NS
Error	208.89	20	10.44	—	—
Factor C	2388.31	5	477.66	436.05	0.001
A × C	294.20	5	58.84	53.71	0.001
B × C	4.44	5	0.88	0.81	NS
A × B × C	3.53	5	0.71	0.64	NS
Error	109.54	100	1.09	—	—

A: dose of β-endorphin, B: oxytocin, C: time.

β-endorphin at any time interval studied. Split-plot ANOVA was performed separately for groups of low and high doses of β-endorphin. The antinociceptive effect of β-endorphin was time-dependent both following low amounts, $F(5,50)=106.8$, $p<0.001$, and high amounts, $F(5,50)=389.5$, $p<0.001$, of the neuropeptide. Pain sensitivity was back to the control range 120 min following β-endorphin treatment.

Figure 2 illustrates the effect of SC oxytocin treatment on the analgesic tolerance induced by β-endorphin. The data indicated that the analgesic effect of 5 μg β-endorphin (measured 15 min after β-endorphin treatment) was significantly different in the various treatment groups, $F(4,43)=7.53$, $p<0.05$. Whereas 5 μg β-endorphin caused an analgesia of 80% in the intact control mice, the same treatment resulted in a significantly attenuated (35%) analgesic

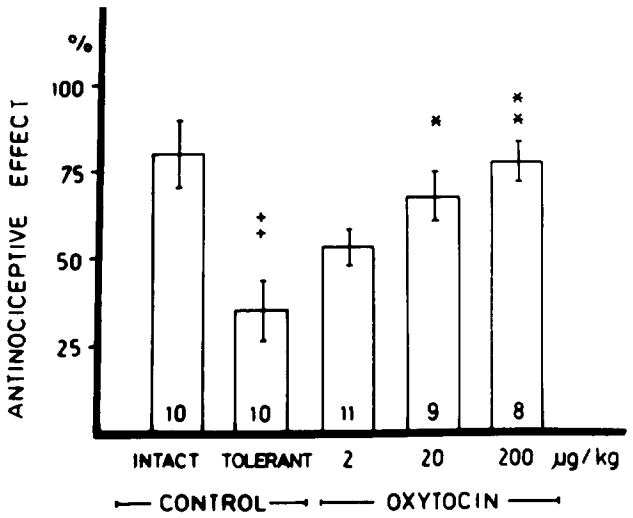

FIG. 2. The influence of systemic oxytocin treatment on the analgesic tolerance induced by β-endorphin. Intact: Mice received single daily ICV injections of artificial cerebrospinal fluid for 3 consecutive days. The data illustrate the analgesic effect of 5 μg β-endorphin, injected ICV on the fourth day of treatment. The analgesic effect was measured 15 min after the injection of the opioid peptide. Tolerant: Mice received single daily ICV injections of 5 μg β-endorphin for 4 consecutive days. On day 4, the analgesic effect of β-endorphin was measured 15 min after treatment. Oxytocin: Mice rendered tolerant to β-endorphin received SC injections of graded doses of oxytocin 1 hr prior to the daily treatment with β-endorphin. ++ Significantly different from tolerant control (for details, see text); * and ** significantly different from tolerant control (for details, see text).

response in mice rendered tolerant to β-endorphin. The difference between the two groups was statistically different, $F(1,18)=13.7195$, $p<0.05$. Graded doses of oxytocin (2–200 μg/kg SC, injected 1 hr prior to the daily injection of β-endorphin) inhibited the development of analgesic tolerance. Thus a test dose of 5 μg β-endorphin caused an analgesic effect which was significantly larger than the corresponding effect of the neuropeptide in the tolerant control mice, $F(3,34)=9.0023$, $p<0.05$. Paired comparison revealed that the analgesic effect of β-endorphin was significantly higher in the tolerant mice pretreated with 20 μg/kg ($F=4.82$, $p<0.05$) or 200 μg/kg ($F=7.68$, $p<0.05$) oxytocin than in the tolerant controls.

The effect of ICV oxytocin treatment is shown in Fig. 3. Statistical analysis revealed significant differences between the various treatment groups, $F(2,22)=10.5167$, $p<0.05$. ICV injections of oxytocin (50 pg 1 hr prior to the daily β-endorphin treatment) significantly attenuated the β-endorphin tolerance. This is evident from the finding that the analgesic effect of a test dose of β-endorphin was significantly ($F=5.4875$, $p<0.05$) higher than in the tolerant control animals.

DISCUSSION

The present data indicate that the administration of oxytocin, a neuropeptide of hypothalamo-pituitary origin

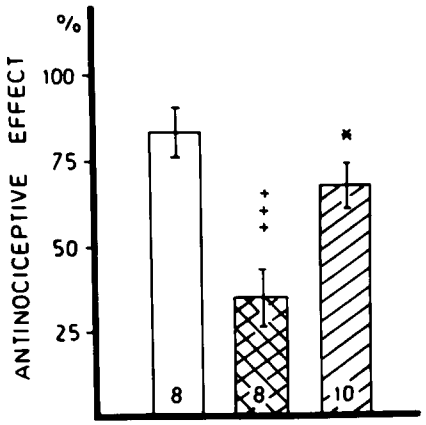

FIG. 3. The effect of intracerebroventricular oxytocin treatment on the analgesic tolerance induced by β-endorphin. Open column: intact control; hatched column: tolerant control; striped column: oxytocin (50 pg ICV) injection 1 hr prior to the daily injection of β-endorphin. +++ Significantly different from intact control; * significantly different from tolerant control (for other details, see text and Fig. 2).

which is also present in extrahypothalamic brain structures (for a summary, see [4,14]), attenuated tolerance to the analgesic effect of β-endorphin. In agreement with this suggestion, the analgesic effect of a test dose of β-endorphin was close to that measured in non-tolerant mice when oxytocin was given shortly prior to the daily injection of β-endorphin. This effect of SC oxytocin was dose-dependent. While a dose of 2 μg/kg was ineffective, 20 and 200 μg/kg of the neuropeptide significantly attenuated tolerance. This finding is in agreement with previous data indicating a similar inhibitory effect of oxytocin on the analgesic tolerance to morphine [11], heroin [9,11] or enkephalin [16]. It is of interest that whereas oxytocin attenuated β-endorphin-induced tolerance, the analgesic effect of a single β-endorphin injection was not affected by this neurohypophyseal neuropeptide. This result suggests that the interaction of oxytocin with the opioid peptide is not likely to take place at the level of endogenous opiate receptors.

Oxytocin passes the blood-brain barrier with considerable difficulty only, but minute amounts of the neuropeptide may reach target sites in the central nervous system [2, 7, 13]. It has to be stressed, however, that both oxytocin and β-endorphin are normally present in the brain and the spinal cord [3, 14, 20]. It was of interest, therefore, to observe that central (ICV) oxytocin also attenuated tolerance to β-endorphin.

Further studies are required to resolve the problem of whether oxytocin inhibits the development or attenuates the expression of tolerance to β-endorphin. Another explanation of our findings could be related to the fact that tolerance is a state-dependent phenomenon [17,18] This means that there is a strong association between the drug administration procedure and the systemic effects of the drug. Theoretically, it could be that the difference between oxytocin-treated and control animals is due to such state-dependent factors. However, since oxytocin was injected daily during the developmental phase of tolerance and the pain sensitivity in the tolerant mice was measured under identical treatment conditions, such a state-dependent effect is not likely in the present experiments.

At any event, however, the data suggest that oxytocin might be involved in regulation of the sensitivity of the central nervous system to repeated (or prolonged) exposure to β-endorphin. Since β-endorphin modulates the release of oxytocin (for a summary, see [23]), it is conceivable that neurohypophyseal neuropeptides and opioid peptides have an interrelated role in adaptive neuronal mechanisms.

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