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Crosstolerance Between Butorphanol and Morphine in Rats

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FENG, Y. Z., M. NARITA, Y. T. TSENG, B. HOSKINS AND I. K. HO. *Crosstolerance between butorphanol and morphine in rats.* PHARMACOL BIOCHEM BEHAV **49**(3) 657-661, 1994.—To investigate the antinociceptive effects of morphine and U-50,488 after continuous administration with butorphanol, rats were intracerebroventricularly (ICV) infused with butorphanol (26 nmol/μl/h) through osmotic minipumps for 3 days. Six hours after termination of infusion, the rats were challenged with different doses of morphine or U-50,488. Antinociceptive effects, as assessed by tail-flick and acetic acid writhing tests, were measured 15 min after challenge. Development of crosstolerance to morphine was evident in butorphanol-infused animals. The study also revealed that crosstolerance to butorphanol developed in continuously ICV morphine-infused animals. Continuous ICV infusion with butorphanol produced a marked rightward shift of the antinociceptive dose–response curve resulting from U-50,488 challenge. These results showed that there is an antinociceptive crosstolerance between butorphanol and morphine, and crosstolerance to U-50,488 developed in continuously butorphanol-infused animals. The present data suggested that chronic ICV treatment with high doses of butorphanol can lead to desensitization of the antinociceptive systems mediated through the central κ as well as μ receptors in rats.

Antinociception Butorphanol Morphine U-50,488 Crosstolerance Intracerebroventricular Osmotic minipump

BUTORPHANOL tartrate is a potent mixed agonist/antagonist opioid analgesic that belongs to the group of opioids known as morphinans (8,16,20). Very little information is available regarding its crosstolerance development, especially to morphine or U-50,488 (a selective κ agonist). Although butorphanol is believed to have a lower abuse potential as an analgesic and anesthetic agent, a few cases of butorphanol abuse have been reported since its introduction in 1978 (11). Pircio et al. (20) reported that the degree of tolerance development to butorphanol and morphine was similar in writhing test in mice. Horan et al. (9) reported that tolerance developed to the antinociceptive effects (using the acetic acid writhing test) of butorphanol in butorphanol-infused rats, as evidenced by its significantly increased ED_{50} value, as compared to that of saline-treated animals. Because of the abuse potential and the capability of producing severe physical dependence when butorphanol is used in excessive dosages and for long durations (3,5,12,21), knowledge of crosstolerance development between butorphanol and other opioids is of great value. The present study demonstrates the development of crosstolerance to morphine or to U-50,488 in butorphanol-tolerant animals.

METHOD

Animals and Chemicals

Seven- to eight-week-old male Sprague–Dawley rats (Charles River, Wilmington, MA), weighing 225–250 g, were used. Animals were kept in a room with an ambient temperature of 21 ± 2°C and 12 L : 12 D cycle with free access to food and water for a week prior to experiments. Butorphanol was the generous gift of the Bristol-Myers Corporation (Syracuse, NY). Morphine was purchased from Mallinckrodt Chemical Works (St. Louis, MO). U-50,488 was obtained from Research Biochemicals Incorporated (Natick, MA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise specified.

Surgical Procedures

Rats were anesthetized with Equithensin (4.25 g chloral hydrate, 2.23 g $MgSO_4 \cdot 7H_2O$, 0.972 g sodium pentobarbital, 44.4 ml propylene glycol, 10 ml 95% ethanol, and distilled water to make a final volume of 100 ml), 3 ml/kg (IP), and

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placed in a stereotaxic frame. An indwelling stainless steel guide cannula (26 gauge, 10 mm long) was implanted into the right cerebral lateral ventricle (AP: −0.5 mm, LAT: +1.3 mm, and DV: −4.5 mm) with the bregma chosen as the stereotaxic reference point (18). Dental acrylic cement (Lang Dental MFG. Co., Wheeling, IL) was applied to the surface of the skull and a protective cap was placed around the cannula. After the acrylic had hardened, the animal was removed from the stereotaxic frame. A stylet (32 gauge stainless steel tubing) was placed into the guide cannula to allow the cannula to remain patent. The presence of c.s.f. in the guide cannula was examined to assure proper placement. After surgery, each rat was given 150,000 units of procaine penicillin G (Pfizer-pen-AS, Pfizer Corp., NY), SC, to prevent infection. One week's recovery was allowed before beginning the infusion of drugs.

Under ether anesthesia, animals were implanted SC with osmotic minipumps (Alzet 2001, Alza Corp., Palo Alto, CA) between the scapulae. A 4-cm piece of tygon tubing (0.38 mm inner diameter, Cole-Palmer, Chicago, IL) was applied to connect the minipump to a piece of L-shaped stainless steel injector tubing (32 gauge, 30 mm long), with one end having the same length as the guide cannula. Each drug solution was filtered through a 0.2 μm Acrodisk syringe filter (Gelman Scientific, Ann Arbor, MI) before it was introduced into the minipump. All of the delivery apparatus was assembled under sterile conditions. Minipumps were primed overnight at room temperature in normal saline so that optimal flow rate (1 μl/h) was obtained.

Administration Schedule and Induction of Tolerance

Rat tail-flick test. Rats were infused ICV continuously with saline (1 μl/h), butorphanol, or morphine (26 nmol/μl/h) for 3 days. The duration and dosing regimens of infusion were determined to be optimal in previous experiments (13). Drugs were dissolved in sterile physiological saline. All drug doses, calculated as the free base, were expressed as nmol/μl/h for the infusion or nmol/rat for the assessment of dose-response curves for tolerance development after 3 days of infusion. Six hours after termination of infusion, saline-infused rats were challenged with butorphanol (8.7, 13, 26, or 52 nmol/5 μl, ICV), morphine (0.53, 1.06, 1.6, or 2.6 nmol/5 μl, ICV) or U-50,488 (200, 500, or 1,000 nmol/5 μl, ICV); butorphanol-infused rats were challenged with morphine (6.5, 13, 26, 32, or 65 nmol/5 μl, ICV), or U-50,488 (1,000, 2,500, or 5,000 nmol/5 μl, ICV) and morphine-infused rats were challenged with butorphanol (26, 65, 130, or 260 nmol/5 μl, ICV). These doses were found, in preliminary experiments, to be equally effective analgesic doses of these drugs as measured by the tail-flick assay. Tail-flick latencies were measured 15 min after injections.

Acetic acid writhing test. Rats were infused ICV continuously with saline, butorphanol, or morphine for 3 days as previously described. Six hours after termination of infusion, saline-infused rats were challenged with butorphanol (2.17, 6.5, 26, or 65 nmol/5 μl, ICV) or morphine (0.18, 0.54, or 2.17 nmol/5 μl, ICV); butorphanol-infused rats were challenged with morphine (6.5, 13, or 26 nmol/5 μl, ICV) or U-50,488 (1,000, 2,500, or 5,000 nmol/5 μl, ICV) and morphine-infused rats were challenged with butorphanol (65, 130, or 260 nmol/5 μl, ICV). Fifteen minutes later the rats were given 0.6% acetic acid, 10 ml/kg, IP. Ten minutes following the administration of acetic acid, the animals were individually sequestered and observed for a 10-min period for the presence of a writhe (defined as a characteristic stretching of the hind limbs and/or constriction of the abdominal musculature).

Analgesic Test

Antinociceptive effects were assessed by the tail-flick (1,4) and acetic acid writhing tests (7). In the tail-flick test, pretreatment tail-flick latency for each rat was determined three times at 5-min intervals, and the mean was designated as the baseline latency (BL). Lamp voltage was adjusted to obtain mean BL values of approximately 3.6–4.3 s (mean BL and SD were 3.9 ± 0.14 s) and held constant throughout all experiments. A cutoff point of 10 s was chosen to minimize thermal damage to the tail. Any animal having a baseline latency to tail flick of greater than 4.5 s or less than 3.0 s was excluded from the study to eliminate any false positive antinociceptive score. The ED_{50}s and 95% confidence intervals were determined according to the method of Litchfield and Wilcoxon (15). Each ani-

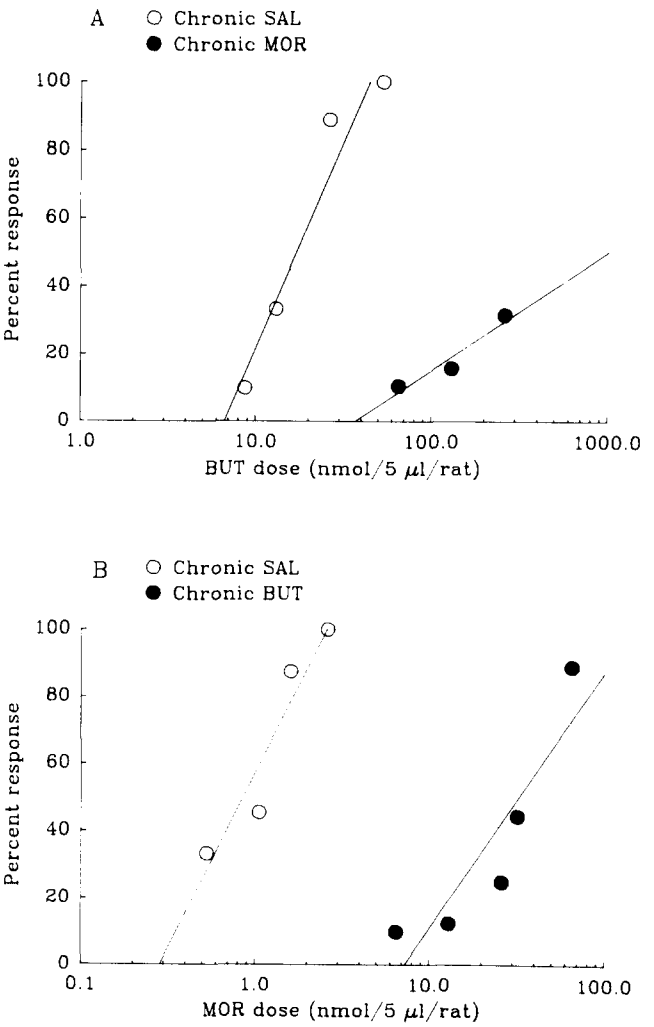

FIG. 1. Crosstolerance dose-response curves assessed by the tail-flick test. (A) Rats were infused ICV continuously with either saline (1 μl/h) or morphine (26 nmol/μl/h) for 3 days and challenged ICV with butorphanol. (B) Rats were infused ICV continuously with either saline (1 μl/h) or butorphanol (26 nmol/μl/h) for 3 days and challenged ICV with morphine. Each group cortained 8–10 rats.

mal was used once, and 8–10 animals were used per dose. For the acetic acid writhing test, rats that did not writhe were considered antinociceptive or responders. The percent response (number of responders/number in dosing group) was evaluated at each dose, and the ED_{50}s and 95% confidence intervals were determined by the method of Litchfield and Wilcoxon (15).

Statistics

Analysis of the dose-response curves and calculation of the ED_{50}, tests for parallelism, and relative potency were obtained using the Pharmacological Calculations System of Tallarida and Murray (23). Degrees of tolerance and crosstolerance development were defined as the relative potency ratios between the control and butorphanol, morphine, or U-50,488 groups. Differences were considered significant at $p < 0.05$.

RESULTS

In the tail-flick test, crosstolerance to butorphanol developed in morphine-tolerant rats, with an ED_{50} value of 666.33 nmol/5 μl/rat. In contrast, in rats treated with saline, the ED_{50} was 15.42 nmol/5 μl/rat. The butorphanol challenge dose-response curves did not differ significantly from parallelism between the morphine-tolerant rats and the saline treated rats ($p > 0.05$), and the relative potency ratio between the control and morphine groups was 40.91 (Fig. 1A, Table 1). Crosstolerance to morphine developed in butorphanol-tolerant rats, with an ED_{50} value of 31.51 nmol/5 μl/rat. In contrast, in rats treated with saline, the ED_{50} was 0.82 nmol/5 μl/rat. The morphine challenge dose-response curves did not differ significantly from parallelism between the butorphanol-tolerant rats and the saline-treated rats ($p > 0.05$), and the relative potency ratio between the control and butorphanol groups was 38.34 (Fig. 1B, Table 1). Crosstolerance to U-50,488 developed in butorphanol-tolerant rats, with an ED_{50} value of > 10,000 nmol/5 μl/rat. In contrast, in rats treated with saline, the ED_{50} was 386.81 nmol/5 μl/rat. The U-50,488 challenge dose-response curves did not differ significantly from parallelism between the butorphanol-tolerant rats and the saline-treated rats ($p > 0.05$) and the relative potency ratio between the control and U-50,488 groups was 21.87 (Fig. 3A, Table 1).

In the acetic acid writhing test, crosstolerance to butorphanol developed in morphine-tolerant rats, with an ED_{50} value of 165.36 nmol/5 μl/rat. In contrast, in rats treated with saline, the ED_{50} was 15.72 nmol/5 μl/rat. The butorphanol challenge dose-response curves did not differ significantly from parallelism between the morphine-tolerant rats and the saline treated rats ($p > 0.05$), and the relative potency ratio between the control and morphine groups was 10.45 (Fig. 2A, Table 1). Crosstolerance to morphine developed in butorphanol-tolerant rats, with an ED_{50} value of 10.76 nmol/5 μl/rat. In contrast, in rats treated with saline, the ED_{50} was 0.70 nmol/5 μl/rat. The morphine challenge dose-response curves did not differ significantly from parallelism between the butophanol-tolerant rats and the saline-treated rats ($p > 0.05$), and the relative potency ratio between the control and butorphanol groups was 16.85 (Fig. 2B, Table 1). Crosstolerace to U-50,488 developed in butorphanol-tolerant rats, with an ED_{50} value of 3,533.39 nmol/5 μl/rat. In contrast, in rats treated with saline, the ED_{50} was 308.71 nmol/5 μl/rat. The U-50,488 challenge dose-response curves did not differ significantly from parallelism between the butorphanol-tolerant rats and the saline treated rats ($p > 0.05$) and the relative potency ratio between the control and U-50,488 groups was 10.44 (Fig. 3B, Table 1).

DISCUSSION

Butorphanol is a potent mixed agonist-antagonist opioid analgesic agent belonging to the group of morphine deriva-

TABLE 1

CROSSTOLERANCE DEVELOPMENT AMONG BUTORPHANOL, MORPHINE, AND U-50,488

Drug Infused	Dose* nmo/μl/h	Drug† Challenge	ED_{50} (95% Confidence Intervals) nmol/5 μl/Rat	Degree of Tolerance Development‡
SAL§	–	BUT	15.42 (10.30–23.11)	–
MOR§	26	BUT	666.33 (299.03–1484.76)¶	40.91
SAL§	–	MOR	0.82 (0.56–1.20)	–
BUT§	26	MOR	31.51 (21.45–46.29)¶	38.34
SAL#	–	BUT	15.72 (8.09–30.54)	–
MOR#	26	BUT	165.36 (94.44–289.52)¶	10.45
SAL#	–	MOR	0.70 (0.28–1.80)	–
BUT#	26	MOR	10.76 (6.85–16.93)¶	16.85
SAL§	–	U-50,488	386.81 (185.30–807.47)	–
BUT§	26	U-50,488	>10,000¶	21.87
SAL#	–	U-50,488	308.71 (149.68–636.71)	–
BUT#	26	U-50,488	3,533.39 (1,660.89–7,516.96)¶	10.44

*MOR and BUT were infused ICV continuously for 72 h.

†Drug challenge was administered 6 h after the termination of infusion.

‡Degree of tolerance development = relative potency ratio between the opioid-treated groups and saline-treated groups.

§Antinociceptive effect as assessed by the tail-flick test.

#Antinociceptive effect as assessed by the acetic acid writhing test.

¶Significant shift of the dose-response curve as compared to saline-infused control group ($p < 0.01$).

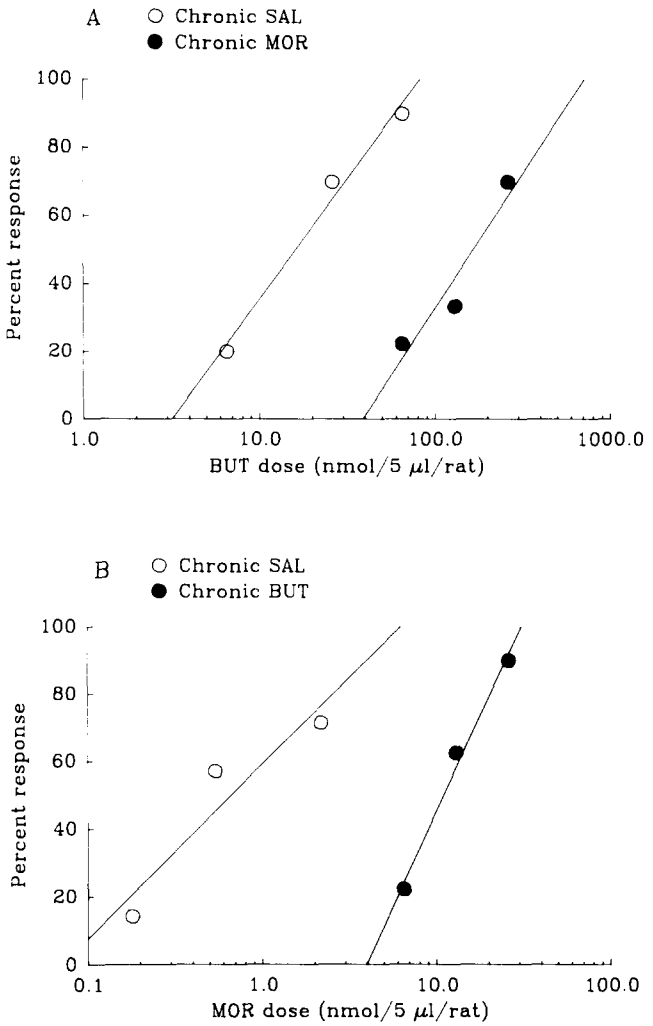

FIG. 2. Crosstolerance dose-response curves assessed by the acetic acid test. (A) Rats were infused ICV continuously with either saline (1 μl/h) or morphine (26 nmol/μl/h) for 3 days and challenged ICV with butorphanol. (B) Rats were infused ICV continuously with either saline (1 μl/h) or butorphanol (26 nmol/μl/h) for 3 days and challenged ICV with morphine. Each group contained 8–10 rats.

tives known as morphinans (16). The pharmacology of this compound, in terms of its actions on the opioid receptor system, is complex and may be due to its apparent multiplicity of actions on the opioid receptor system. In in vitro radioligand displacement studies, butorphanol was shown to be a potent competitor of ^{3}H-DAGO (μ), ^{3}H-DPDPE (δ), and ^{3}H-EKC (κ) binding, exhibiting 3, 10, and 30 times more activity, respectively, than the prototypical μ agonist, morphine (10). Additionally, butorphanol exhibits some pharmacological differences as well as similarities when compared to morphine in vivo (10). These reports indicate that butorphanol may have the intrinsic activity not only at μ but also at δ and κ receptors.

In studies from our laboratories, antinociceptive tolerance to butorphanol was evident using the tail-flick and acetic acid writhing methods in rats that had been infused ICV with butorphanol for 48 h or longer, and higher doses of butorphanol (13, 26, and 52 nmol/μl/hr) were required to induce substantial degrees of tolerance, as assessed by antinociceptive responses after challenge injections (6). Additionally, the degree of tolerance to butorphanol was less than that of morphine in the tail-flick test, while the degree of tolerance to butorphanol or to morphine was similar in the acetic acid writhing test.

In the present study, crosstolerance developed between morphine and butorphanol in both the tail-flick and acetic-acid writhing tests. The degree of rightward shift after chronic administration of butorphanol or morphine was similar in both antinociceptive tests. Recently, a profound degree of crosstolerance conferred to butorphanol in morphine-tolerant rats on schedule-controlled behavior has been reported (19). Our data support their finding. More interestingly, we found that chronic pretreatment with butorphanol produced a marked rightward shift in the antinociceptive dose-response curve for the selective κ agonist, U-50,488, indicating that crosstolerance to κ agonists develops in continuously ICV butorphanol-infused animals. It is well-known that chronic treatment with a μ agonist has no effect on the intrinsic efficacy of a κ agonist (28). The present study provides further evidence

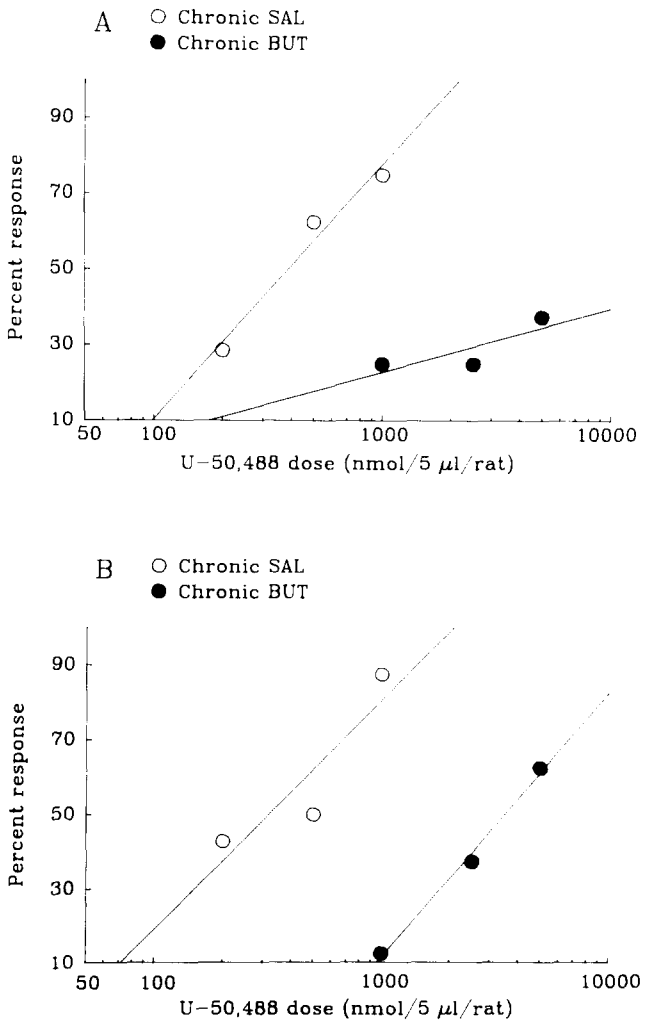

FIG. 3. Crosstolerance dose-response curves. Rats were infused ICV continuously with either saline (1 μl/h) or butorphanol (26 nmol/μl/h) for 3 days and challenged ICV with U-50,488. Antinociceptive effects were determined using the tail-flick test (A), and the acetic acid writhing test (B). Each group contained 8–10 rats.

that κ as well as μ receptors become desensitized after chronic administration of butorphanol.

A decrease in opioid receptor binding capacity (downregulation) in vivo after chronic agonist administration has been reported for μ (24,27) and δ (22,25,26) receptors. Moreover, Bhargava et al. (2) reported that the κ receptor was desensitized and downregulated in vivo as adaptation to chronic activation by κ agonists with a high intrinsic activity. In our previous binding studies, chronic infusion with high doses of butorphanol increased K_D values (in the cortex and striatum), decreased B_{max} (in the cortex) of ^{3}H-U-69,593 (κ) binding, and shifted the K_i of the selective κ antagonist, nor-binaltorphimine, against ^{3}H-U-69,593 binding in the cortex by more than 10-fold (14). These findings indicated that a reduced number of μ and κ receptors resulting from chronic infusion with butorphanol would mean a reduced efficacy of morphine and U-50,488, respectively. However, it doesn't necessarily follow that the intrinsic desensitization caused by butorphanol results from downregulation of the receptors. Nishino et al. (17) reported that opioid tolerance could be dissociated from receptor downregulation. In our rats, antinociceptive tolerance to μ agonists was rapidly developed, and, subsequently, μ receptor downregulation was produced. Thus, receptor downregulation may not be essential for the development of tolerance to opioids.

Our studies suggest that chronic ICV treatment with high doses of butorphanol can lead to desensitization of the antinociceptive systems mediated through the central μ and κ opioid receptors in rats.

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