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Tolerance to δ- But Not μ-Opioid Receptors in the Spinal Cord Attenuates Inhibition of the Tail-Flick Response Induced by β-Endorphin Administered Intracerebroventricularly in Mice

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SUH, H. H. AND L. F. TSENG. *Tolerance to δ- but not μ-opioid receptors in the spinal cord attenuates inhibition of the tail-flick response induced by β-endorphin administered intracerebroventricularly in mice.* PHARMACOL BIOCHEM BEHAV **35**(4) 807–813, 1990.—Male ICR mice were rendered tolerant by intrathecal (IT) injection once a day with either μ-agonist, D-Ala^2-NMePhe^4-Gly-ol-enkephalin (DAMGO) or δ-agonist, D-Pen^2-D-Pen^5-enkephalin (DPDPE) (toleragen) by doubling the dose each day starting from 0.125 and 1 μg for DAMGO and DPDPE, respectively, for 6 days. On day 6, the magnitude of tolerance was assessed by establishing IT dose-response lines for the effect of the chronic drug given as bolus injections (probe). The antinociception was assessed by the tail-flick and hot-plate test. Repeated IT injections of DPDPE reduced inhibition of the tail-flick and hot-plate response induced by DPDPE (ED_{50} values for DPDPE increase 10-fold) but not DAMGO. Repeated IT injections of DAMGO reduced inhibition of the tail-flick and hot-plate response induced by DAMGO (ED_{50} value for DAMGO increase 7- to 10-fold) but not DPDPE. The effects of the tolerance to μ- and δ-opioid receptor activity in the spinal cord on inhibition of the tail-flick and hot-plate response induced by intracerebroventricularly (ICV) administered β-endorphin and morphine were then studied. β-Endorphin or morphine at different doses were injected ICV 4 hr after the last IT injection of DPDPE or DAMGO. Repeated IT bolus injections of DPDPE reduced inhibition of the tail-flick response but not the hot-plate response induced by β-endorphin. On the other hand, repeated IT bolus injections of DAMGO did not affect inhibition of the tail-flick and hot-plate response induced by β-endorphin. Repeated IT bolus injections of either DPDPE or DAMGO did not affect inhibition of the tail-flick and hot-plate response induced by morphine. The results suggest that spinal δ- but not μ-opioid receptors are involved in ICV administered β-endorphin-induced inhibition of the tail-flick response. Neither spinal μ- nor δ-receptors are involved in inhibition of the tail-flick and hot-plate response induced by morphine given ICV.

β-Endorphin Morphine Chronic intrathecal injection Tolerance Cross-tolerance

WE have previously demonstrated that antinociception induced by β-endorphin administered intracerebroventricularly (ICV) is mediated by the activation of the opioid receptors in the spinal cord (20, 21, 25, 26). This is supported by the findings that the blockade of spinal opioid receptors by intrathecal (IT) injection of naloxone antagonizes inhibition of the tail-flick response induced by β-endorphin administered ICV. In biochemical studies, β-endorphin administered ICV releases Met-enkephalin from the spinal cord in anesthetized rats (27, 28, 31). We therefore postulate that analgesia produced by β-endorphin administered supraspinally is mediated by the release of Met-enkephalin from the spinal cord and subsequent activation of opioid receptors in the spinal cord.

Recently we have proposed that δ- but not μ-opioid receptors in the spinal cord are involved in ICV injected β-endorphin-induced antinociception. The inhibition of the tail-flick response induced by β-endorphin administered ICV was effectively antagonized by IT injection of selective δ-opioid receptor antagonists, ICI 174,864 and ICI 154,129, but not selective μ-opioid receptor antagonist, β-funaltrexamine (21).

Unlike β-endorphin, the inhibition of the tail-flick response induced by ICV administered morphine is not mediated by opioid

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TABLE 1

TIMES FOR MEASURING THE TAIL-FLICK AND HOT-PLATE RESPONSE AFTER INTRACEREBROVENTRICULAR (ICV) OR INTRATHECAL (IT) ADMINISTRATION OF DIFFERENT OPIOIDS

Drugs	Tail-Flick Test Time (min)	Hot-Plate Test Time (min)
ICV β-endorphin	30	15
ICV morphine	30	15
IT DAMGO	10	5
IT DPDPE	10	10

DAMGO = D-Ala^2-NMePhe^4-Gly-ol-enkephalin.
DPDPE = D-Pen^2-D-Pen^5-enkephalin.

systems in the spinal cord. The blockade of spinal opioid receptors by IT injection of naloxone or other selective opioid receptor antagonists, ICI 174,864, ICI 154,129 and β-funaltrexamine, did not affect inhibition of the tail-flick response induced by ICV administered morphine (20, 21, 25, 26).

Antinociceptive tolerance to selective opioid receptor agonist is developed after repeated injections or continuous infusion of selective opioid receptor agonists using osmotic minipumps into the subarachnoid space in rats (1, 12–14, 16, 17, 23, 24, 32, 35, 37). Tseng (24) has reported that chronic infusion of morphine into the subarachnoid space produces tolerance to morphine and partial cross-tolerance to DADLE given IT. Similar results were obtained by Russel *et al.* (16) in that chronic IT infusion of PLO17 produces tolerance to PLO17 and only partial cross-tolerance to DPDPE. In mice, it is necessary to use multiple IT injection of tolergens for induction of tolerance. The procedure prevents the infusion of the tolergens continuously to the supraspinal sites. We found in the present studies that multiple IT injections of DPDPE produced tolerance spinally to δ- but not μ-opioid receptor and repeated IT injection of DAMGO produced tolerance spinally to μ- but not δ-opioid receptor for antinociception. The present study was then designed to study the effects of tolerance to μ- or δ-opioid receptors in the spinal cord on inhibition of the tail-flick and hot-plate response induced by ICV administered β-endorphin and morphine. We found that inhibition of the tail-flick response induced by β-endorphin administered ICV was attenuated in mice injected chronically with DPDPE but not DAMGO. Tolerance to either μ- or δ-opioid receptors in the spinal cord did not affect morphine-induced inhibition of the tail-flick response. Our results give additional evidence that spinal δ- but not μ-opioid receptors are involved in β-endorphin-induced inhibition of the tail-flick response and the opioid receptors, either μ or δ, are not involved in ICV injected morphine-induced antinociception.

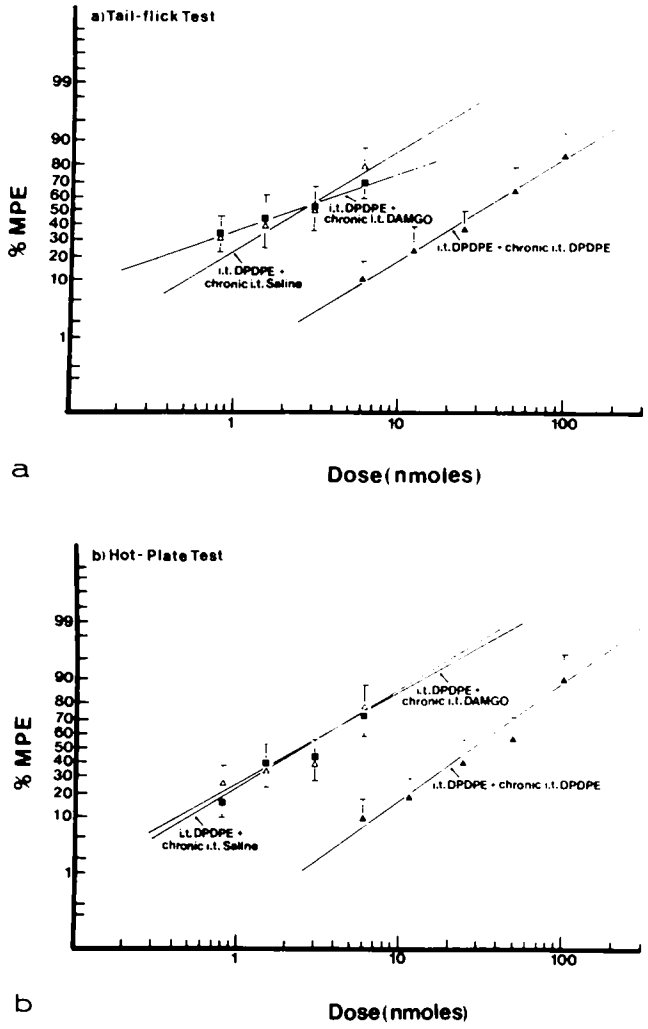

FIG. 1. Effects of repeated intrathecal (IT) bolus injections of saline and DPDPE and DAMGO on inhibition of the tail-flick (a) and hot-plate (b) response induced by DPDPE administered IT. Saline, DPDPE or DAMGO was injected IT once a day. The dose of DAMGO and DPDPE was 0.125 and 1 μg for the first injection and the dose was doubled each day for 6 days. DPDPE at different doses was injected IT 4 hr after the last IT injection of DPDPE or DAMGO. The tail-flick and hot-plate responses were tested 10 min after IT injection of DPDPE. The vertical bars are the standard error of the mean. N = 9 mice for each dose used.

METHOD

Subjects

Male ICR mice weighing 23–25 g (Harlan Sprague Dawley, Inc., Indianapolis, IN) were used. Animals were housed 5 per cage in a room maintained at 22 ± 0.5°C with an alternating 12-hour light-dark cycle. Food and water were available ad lib. Each animal was used only once.

Analgesiometric Tests

Antinociception was determined by the tail-flick (2) and hot-plate (3) tests. For measuring latency of the tail-flick response, mice were gently held by hand with their tails positioned in the apparatus (EMDIE Instrument Co., Maidens, VA, Model 6). The tail-flick response was elicited by applying a radiant heat on the dorsal surface of tail. The intensity of the radiant heat for the tail-flick response was adjusted so that the animal flicked its tail after 3 to 4.5 sec. For measuring latency of the hot-plate response, each mouse was gently placed on a 55°C copper plate (30 × 30 × 30 cm; iitc Inc., Woodland Hills, CA, Model 39 Hot-Plate) and the time for the mouse to lick the forepaw was measured. The forepaw licking response had a latency 2 to 3 seconds shorter than hindpaw licking response. Both responses were dose-dependently inhibited by β-endorphin and morphine in a similar fashion. Control latencies for the hot-plate response were approximately 9.5 sec. The hot-plate and tail-flick latencies were measured before (T_0) and after (T_1) ICV or IT injection of various opioid agonists. The inhibitions of the tail-flick and hot-plate response

TABLE 2

EFFECT OF REPEATED INTRATHECAL (IT) BOLUS INJECTIONS OF DPDPE AND DAMGO ON ED_{50} VALUES FOR DPDPE AND DAMGO GIVEN IT FOR INHIBITION OF THE TAIL-FLICK AND HOT-PLATE RESPONSE

	ED_{50} (nmoles/mouse)* Chronic IT		
	Saline	DPDPE	DAMGO
a. Tail-flick test			
IT DPDPE	2.09 (1.04–4.20)‡	21.23† (10.23–42.34)	2.17 (0.08–5.82)
IT DAMGO	0.0083 (0.0049–0.0139)	0.0087 (0.0047–0.0160)	0.0833† (0.0445–0.1560)
b. Hot-plate test			
IT DPDPE	2.76 (1.36–5.59)	29.12† (15.23–53.48)	2.80 (1.52–5.20)
IT DAMGO	0.0099 (0.0051–0.0190)	0.0108 (0.0062–0.0189)	0.0691† (0.0389–0.1227)

*Mice were injected IT daily with saline, DAMGO or DPDPE. The dose was started from 0.125 and 1 μg for DAMGO and DPDPE, respectively, and the dose was doubled each day for 6 days. ED_{50} values were calculated by the method described by Litchfield and Wilcoxon (11).
†Significantly different from saline control, $p<0.05$.
‡Numbers in parentheses indicate the 95% confidence intervals.

were expressed as "Percent Maximum Possible Effect (% MPE)" which was calculated as $[(T_1-T_0)/(T_2-T_0)] \times 100$, where the cutoff time (T_2) was set at 10 and 30 sec for the tail-flick and hot-plate tests, respectively. To calculate ED_{50} values, at least 3 drug doses were used and 7–10 mice were used for each dose. ED_{50} values and their 95% confidence intervals were determined by the method of Litchfield and Wilcoxon (11) with the aid of a computer program described by Tallarida and Murray (22).

Experimental Protocol

Intrathecal injection was made according to the procedure of Hylden and Wilcox (6) using a 25-μl Hamilton syringe with 30-gauge needle and ICV administration was performed following the method described by Haley and McCormick (4). Injection volume was 4 and 5 μl for IT and ICV injection, respectively. Mice were injected IT with either saline (4 μl), DPDPE or DAMGO (toleragen) once a day by doubling the dose each day starting from 0.125 and 1 μg for DAMGO and DPDPE, respectively, for 6 days. Various doses (probe) of DPDPE and DAMGO were injected IT and β-endorphin or morphine was injected ICV 4 hr after the last chronic IT injection. The tail-flick and hot-plate responses were tested at various times (see Table 1) after IT or ICV injection of opioid agonists. Based on the preliminary time course study, the times chosen were those at which each drug produces its maximal tail-flick and hot-plate inhibition. Injection sites were verified by injecting the same volume of 1% methylene blue and examined the distribution of the injected dye in the ventricular space and the spinal cord. The dye injected ICV was found to be distributed in ventricular space and ventral surface of the brain. No dye was found in the thoracic and lumbar areas of the spinal cord. The dye injected IT was distributed both rostrally and caudally of the injected site (about 2 cm) and no dye was found in the brain and upper cervical portion of the spinal cord. Since opioid peptides were injected once a day, it was not likely that the injected opioid peptide would infuse to the brain.

Drugs

Drugs used are morphine sulfate (Mallinckrodt Chemical Works, St. Louis, MO), β-endorphin (Peninsula Laboratories Inc., Belmont, CA), DPDPE (D-Pen^2-D-Pen^5-enkephalin) and DAMGO (D-Ala^2-NMePhe^4-Gly-ol-enkephalin) (Bachem Inc., Torrance, CA). All drugs used for injection were dissolved in a sterile saline.

RESULTS

Effects of Repeated IT Injections of DPDPE and DAMGO on Inhibition of the Tail-Flick and Hot-Plate Response Induced by DPDPE and DAMGO Administered IT

Four hours after the last IT injection of DPDPE and DADLE, both control latencies of the tail-flick and hot-plate response in mice chronically injected IT with DPDPE and DAMGO were not significantly different from those of mice chronically injected IT with saline. In mice injected with saline, DPDPE and DAMGO, respectively, the latencies are 3.85 ± 0.10, 3.81 ± 0.09 and 3.75 ± 0.08 sec in the tail-flick test and 9.6 ± 0.30, 9.8 ± 0.4 and 9.6 ± 0.32 sec in the hot-plate test ($N = 36$, four groups of 9 each; mean ± S.E.M.). No animal showed changes in spontaneous behavior or paralysis after repeated IT injection of saline, DPDPE or DAMGO. The repeated IT bolus injection of DPDPE (toleragen) reduced inhibition of both tail-flick and hot-plate response induced by IT administered DPDPE but not DAMGO (probe). The dose-response lines for DPDPE-induced inhibition of the tail-flick and hot-plate response were shifted parallel to the right and the ED_{50} values for DPDPE were significantly increased 10.2- and 10.6-fold in the tail-flick and hot-plate assay, respectively, in mice chronically injected IT with DPDPE (Fig. 1a and b, Table 2). On

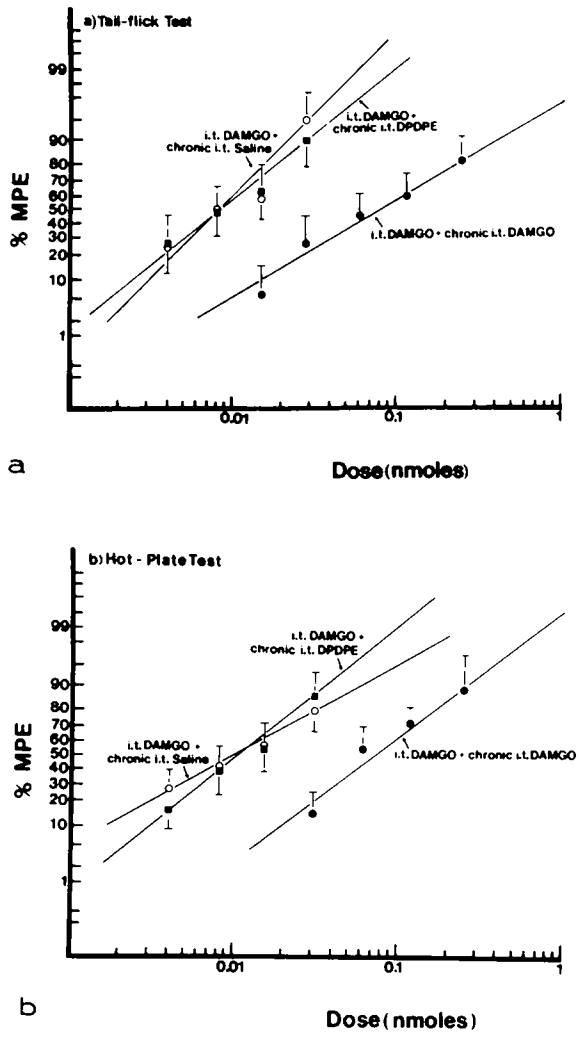

FIG. 2. Effects of repeated intrathecal (IT) bolus injections of saline and DPDPE and DAMGO on inhibition of the tail-flick (a) and hot-plate (b) response induced by DAMGO administered IT. Saline, DPDPE or DAMGO was injected IT once a day. The dose of DAMGO and DPDPE was 0.125 and 1 μg for the first injection and the dose was doubled each day for 6 days. DAMGO at different doses was injected IT 4 hr after the last IT injection of DPDPE or DAMGO. The tail-flick response was tested 10 min and the hot-plate response was tested 5 min after IT injection of DAMGO. The vertical bars are the standard error of the mean. N = 9 mice for each dose used.

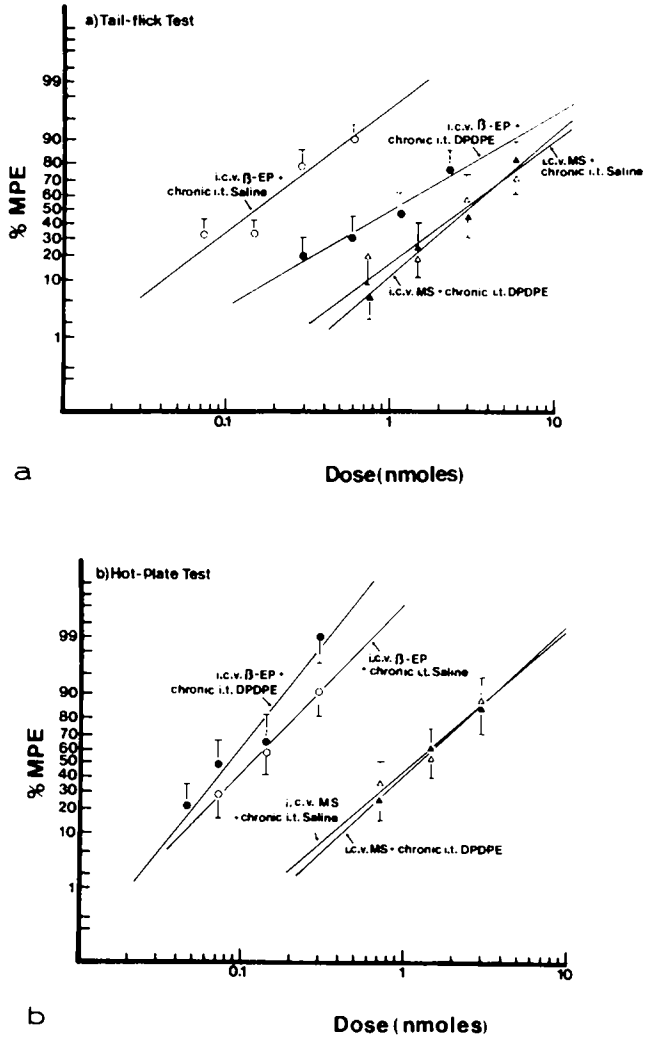

FIG. 3. Effects of repeated intrathecal (IT) bolus injections of DPDPE on inhibition of the tail-flick (a) and hot-plate (b) response induced by β-endorphin and morphine administered intracerebroventricular (ICV). Saline or DPDPE was injected IT once a day. The dose of DPDPE was 1 μg for the first injection and the dose was doubled each day for 6 days. β-Endorphin or morphine at different doses was injected ICV 4 hr after the last IT injection of DPDPE. The hot-plate and tail-flick responses were tested 15 and 30 min, respectively, after ICV injection of β-endorphin or morphine. The vertical bars are the standard error of the mean. N = 9 mice for each dose used.

the other hand, dose-response lines for DAMGO-induced inhibitions and the ED_{50} values for DAMGO were not changed in mice chronically injected with DPDPE (Fig. 2a and b, Table 2).

Chronic IT injection of DAMGO reduced inhibition of both tail-flick and hot-plate response induced by IT administered DAMGO but not DPDPE. The dose-response lines for DAMGO-induced inhibition of the tail-flick and hot-plate response were shifted parallel to the right and the ED_{50} values for DAMGO were significantly increased 10- and 7-fold in the tail-flick and hot-plate assay, respectively, in mice chronically injected IT with DAMGO (Fig. 2a and b, Table 2). On the other hand, the dose-response lines for DPDPE-induced inhibitions and the ED_{50} values for DPDPE were not changed in mice chronically injected IT with DAMGO (Fig. 1a and b, Table 2).

Effects of Repeated IT Injections of DPDPE on Inhibition of the Tail-Flick and Hot-Plate Response Induced by β-Endorphin and Morphine Administered ICV

Mice were injected IT with 4 μl of saline, DPDPE, or DAMGO once a day for 6 days. Various doses of β-endorphin and morphine were injected ICV 4 hr after the last chronic IT injection of saline, DPDPE or DAMGO. As depicted in Fig. 3a and b, β-endorphin at doses from 0.07 to 0.58 nmoles caused a dose-dependent increase of inhibition of the tail-flick and hot-plate response in mice chronically injected IT with saline for 6 days. Repeated IT injection of DPDPE decreased the potency of β-endorphin (ICV) in the tail-flick test, as shown in Table 3. The

TABLE 3

EFFECT OF REPEATED INTRATHECAL (IT) BOLUS INJECTION OF DPDPE AND DAMGO ON ED_{50} VALUES FOR β-ENDORPHIN AND MORPHINE GIVEN INTRACEREBROVENTRICULARLY (ICV) FOR INHIBITION OF THE TAIL-FLICK AND HOT-PLATE RESPONSE

	ED_{50} (nmoles/mouse)*		
		Chronic IT	
	Saline	DPDPE	DAMGO
a. Tail-flick test			
ICV β-EP	0.15 (0.08–0.27)‡	1.05† (0.48–2.31)	0.10 (0.06–0.18)
ICV MS	2.87 (1.47–5.65)	3.01 (1.68–6.98)	1.28 (0.05–3.17)
b. Hot-plate test			
ICV β-EP	0.12 (0.06–0.20)	0.08 (0.02–0.13)	0.18 (0.09–0.37)
ICV MS	1.14 (0.84–1.91)	1.20 (0.87–2.10)	1.82 (0.96–3.50)

*Mice were injected IT daily with saline, DAMGO or DPDPE. The doses was started from 0.125 and 1 μg for DAMGO and DPDPE, respectively, and the dose was doubled each day for 6 days. ED_{50} values were calculated by the method described by Litchfield and Wilcoxon (11).

†Significantly different from saline control, $p<0.05$.

‡Numbers in parentheses indicate the 95% confidence intervals.

dose-response line for β-endorphin was shifted parallel to the right in the tail-flick assay and the ED_{50} value for β-endorphin-induced inhibition of the tail-flick response was increased 6.8-fold. In contrast, chronic IT injection of DPDPE did not affect inhibition of the tail-flick response induced by morphine administered ICV (Fig. 3a and Table 3). Repeated IT injection of DPDPE did not affect inhibition of the hot-plate response induced by either ICV administered β-endorphin or morphine (Fig. 3b and Table 3).

Effects of Repeated IT Injections of DAMGO on Inhibition of the Tail-Flick and Hot-Plate Response Induced by β-Endorphin and Morphine Administered ICV

Repeated IT injections with DAMGO did not affect inhibition of the tail-flick and hot-plate response induced by either ICV administered β-endorphin or morphine (Fig. 4a, b and Table 3)

DISCUSSION

The development of tolerance to opioid receptors by chronic administration of opioid agonists into the spinal subarachnoid space has been reported previously (1, 12–14, 16, 17, 23, 24, 32, 37). We found in the present studies that repeated bolus IT injections of DPDPE and DAMGO produced tolerance to δ- and μ-opioid receptors, respectively, in the spinal cord in both the tail-flick and hot-plate tests. Mice made tolerant to a δ-opioid agonist by chronic bolus IT injection of DPDPE did not show cross-tolerance to DAMGO injected IT, and mice made tolerant to μ-opioid agonist by chronic bolus IT injection of DAMGO did not exhibit cross-tolerance to DPDPE injected IT in inhibiting the tail-flick test. It is likely that DPDPE selectively acts on δ-opioid receptors, while DAMGO selectively acts on μ-opioid receptors for the induction of tolerance. These results are consistent with studies of Tung and Yaksh (32) in that there was no cross-tolerance between δ- and μ-opioid agonists in the development of tolerance in the spinal cord. The lack of cross-tolerance between DAMGO and DPDPE in the present experiment is slightly different from the studies of Russel *et al.* (16). Russel *et al.* (16) found that continuous infusion of PLO17, a selective mu agonist, produced tolerance to PLO17 and partial cross-tolerance to DPDPE given IT.

The model of the selective development of the tolerance to μ- and δ-opioid receptor after repeated IT bolus injection of selective opioid receptor agonist was then used to determine the type of opioid receptors in the spinal cord which mediate the β-endorphin-induced inhibition of the tail-flick response. We found that inhibition of the tail-flick response induced by ICV administered β-endorphin was attenuated in mice made tolerant to δ-opioid receptors in the spinal cord by repeated bolus IT injection of DPDPE for 6 days. On the other hand, the β-endorphin-induced inhibition of the tail-flick response was not attenuated in mice made tolerant to μ-opioid receptors in the spinal cord by repeated IT injection of DAMGO. The results indicate that δ- but not μ-opioid receptors in the spinal cord are involved in β-endorphin-induced inhibition of the tail-flick response. The results of the present studies are in agreement with our previous finding that chronic IT injection of D-Ala^2-D-Leu^5-enkephalin (DADLE) attenuated inhibition of the tail-flick response induced by β-endorphin administered ICV (29). The finding that δ- but not μ-opioid receptors in the spinal cord are involved in β-endorphin-induced inhibition of the tail-flick response is supported by other studies. Blockage of spinal δ-opioid receptors by IT injection of ICI 174,864 and ICI 154,129, selective δ-opioid receptor antagonists (5,15), effectively antagonized inhibition of the tail-flick response induced by ICV administered β-endorphin (21). Moreover, a single ICV injection of β-endorphin reduced IT injected DPDPE- but not DAMGO-induced inhibition of the tail-flick response, indicating that ICV injection of β-endorphin produces tolerance to δ- but not μ-opioid receptor activity in the spinal cord (21).

We previously reported that β-funaltrexamine (33,34) injected IT antagonized inhibition of the tail-flick response induced by

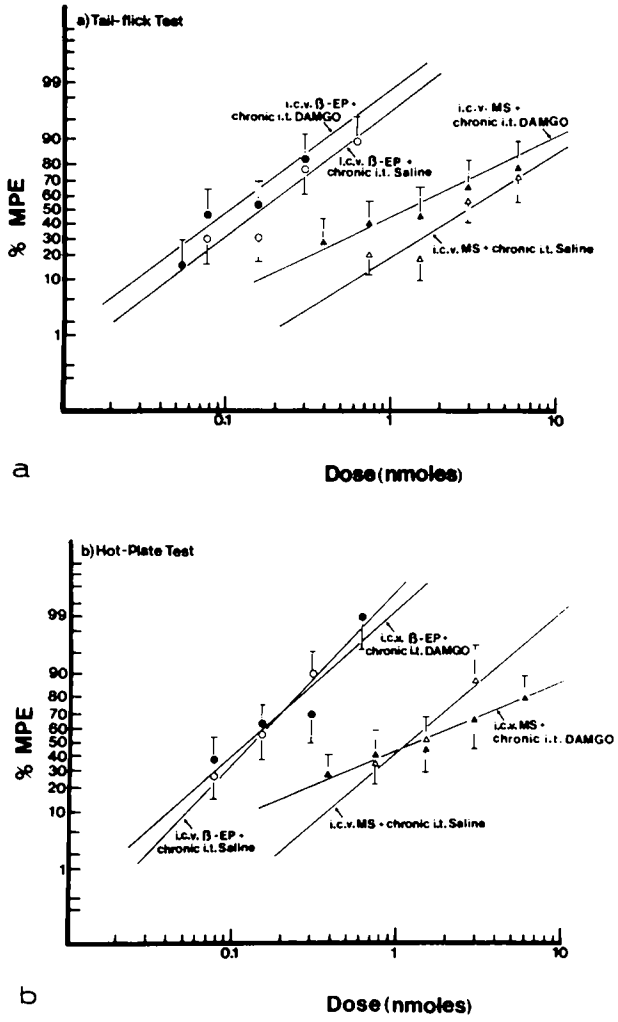

FIG. 4. Effects of repeated intrathecal (IT) bolus injections of DAMGO on inhibition of the tail-flick (a) and hot-plate (b) response induced by β-endorphin and morphine administered intracerebroventricularly (ICV). Saline or DAMGO was injected IT once a day. The dose of DAMGO was 0.125 μg for the first injection and the dose was doubled each day for 6 days. β-Endorphin or morphine at different doses was injected ICV 4 hr after the last IT injection of DAMGO. The hot-plate and tail-flick responses were tested 15 and 30 min, respectively, after ICV injection of β-endorphin or morphine. The vertical bars are the standard error of the mean. N = 9 mice for each dose used.

β-endorphin administered ICV (18). However, the dose of β-funaltrexamine used was 2.5 μg which was later found to be not selective for the μ-opioid receptor; it blocked both δ- and μ-opioid receptors in the spinal cord (Tseng, unpublished observation). In another study (21), β-funaltrexamine at a dose of 0.025 μg, which blocked IT injected DAMGO- but not DPDPE-induced inhibition of the tail-flick response, did not affect inhibition of the tail-flick response induced by ICV administered β-endorphin. The results indicate that μ-opioid receptors in the spinal cord are not involved in ICV administered β-endorphin-induced antinociception.

It has been postulated that β-endorphin-induced antinociception is mediated by the release of Met-enkephalin from the spinal cord. Tseng *et al.* (27, 28, 31) have demonstrated that β-endorphin administered ICV releases Met-enkephalin from the spinal cord in anesthetized rat. Antibodies to Met-enkephalin injected IT effectively antagonize inhibition of the tail-flick response induced by β-endorphin (30). Inhibition of the degradation of Met-enkephalin by IT injection of thiorphan and bestatin, potentiates inhibition of the tail-flick response induced by β-endorphin administered ICV (Tseng, manuscript submitted).

No effect by chronic IT administration of DPDPE or DAMGO on the tail-flick antinociception after ICV administered morphine indicates that the antinociception induced by morphine administered ICV is not mediated by either δ- or μ-opioid receptors in the spinal cord. The release of endogenous opioid peptides and the opioid receptors appear not to be involved in morphine-induced inhibition of the tail-flick response. This postulation is supported by other evidence. 1) The blockade of the spinal opioid receptors by naloxone did not affect inhibition of the tail-flick response induced by morphine administered ICV (20, 25, 26). 2) Morphine administered ICV did not cause a release of Met-enkephalin from the spinal cord (27). 3) Thiorphan or bestatin, inhibitors of Met-enkephalin degradation enzymes, injected IT did not affect inhibition of the tail-flick response induced by morphine administered ICV (Tseng, manuscript submitted). 4) The blockade of spinal δ- or μ-opioid receptors by IT injection of selective δ- or μ-opioid receptor antagonists, ICI 174,864, ICI 154,129 and β-funaltrexamine, did not affect inhibition of the tail-flick response induced by morphine given ICV (21). 5) Single ICV injection of morphine did not produce acute tolerance to spinal δ- or μ-opioid agonists (21).

Repeated IT injections of DPDPE did not affect inhibition of the hot-plate response induced by ICV administered β-endorphin. Since the hot-plate response is primarily a supraspinally organized response (7), β-endorphin might inhibit the hot-plate response at the supraspinal sites and decreased δ receptor activity in the spinal cord by repeated IT injection of DPDPE should not affect the ICV administered β-endorphin-induced inhibition of the hot-plate response.

Analgesic effects induced by morphine and β-endorphin have been postulated to be mediated by different neuronal mechanisms. The blockade of spinal α_2-adrenergic or serotonergic receptors by IT injection of yohimbine, phentolamine or methysergide effectively antagonized inhibition of the tail-flick response induced by morphine administered ICV (8–10, 20, 35, 36), but not β-endorphin (20). Intrathecal pretreatment with desipramine or fluoxetine, norepinephrine and serotonin uptake inhibitors, potentiated inhibition of the tail-flick response induced by morphine, but not by β-endorphin administered ICV (19). Pretreatment of mice with DSP4, 6-hydroxydopamine or 5,6-dihydroxytryptamine effectively antagonized inhibition of the tail-flick response induced by morphine, but not β-endorphin administered ICV (unpublished observation). These data support the view that antinociception induced by morphine administered ICV is mediated by spinopetal noradrenergic and serotonergic system, whereas spinal monoaminergic systems are not involved in ICV administered β-endorphin-induced antinociception.

The present study supports our previous hypothesis that β-endorphin and morphine administered ICV elicit their analgesia by activating different descending pain inhibitory systems, and δ- but not μ-opioid receptors in the spinal cord are involved in antinociception induced by ICV administered β-endorphin.

ACKNOWLEDGEMENT

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REFERENCES

1. Advokat, C.; Burton, P.; Tyler, C. B. Investigation of tolerance to chronic intrathecal morphine infusion in the rat. Physiol. Behav. 39:161–168; 1987.
2. D'Amour, F. F.; Smith, G. L. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74–79; 1941.
3. Eddy, N. B.; Leimbach, D. Synthesis analgesics. II. Dithienylbutenyl and dithienylbutylamides. J. Pharmacol. Exp. Ther. 107:385–393; 1953.
4. Haley, T. J.; McCormick, W. G. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. 12:12–15; 1957.
5. Heyman, J. S.; Mulvaney, S. A.; Mosberg, H. I.; Porrecca, F. Opioid δ-opioid receptor involvement in supraspinal and spinal antinociception in mice. Brain Res. 420:100–108; 1987.
6. Hylden, J. L. K.; Wilcox, G. L. Intrathecal morphine in mice: A new technique. Eur. J. Pharmacol. 67:313–316; 1980.
7. Jensen, T. S.; Yaksh, T. L. II. Examination of spinal monoamine receptors through which brainstem opiate-sensitive systems act in the rat brain. Brain Res. 363:114–127; 1986.
8. Kuraishi, Y.; Fukui, K.; Shiomi, H.; Akaike, A.; Takagi, H. Microinjection of opioids into the nucleus reticularis gigantocellularis of the rat. Analgesia and increase in the normetanephrine level in the spinal cord. Biochem. Pharmacol. 27:2756–2758; 1978.
9. Kuraishi, Y.; Harada, Y.; Takagi, H. Noradrenaline regulation of pain-transmission in the spinal cord mediated by α-adrenoceptors. Brain Res. 174:333–336; 1979.
10. Kuraishi, Y.; Harada, Y.; Aratani, S.; Satoh, M.; Takagi, H. Separate involvement of the spinal noradrenergic and serotonergic systems in morphine analgesia: the difference in mechanical and thermal analgesic tests. Brain Res. 273:245–252; 1983.
11. Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. 96:99–113; 1949.
12. Loomis, C. W.; Jhamandas, K.; Milne, B.; Cervenko, F. Monoamine and opioid interactions in the spinal analgesia and tolerance. Pharmacol. Biochem. Behav. 26:445–451; 1987
13. Loomis, C. W.; Milne, B.; Cervenko, F. Determination of cross-tolerance in rat spinal cord using intrathecal infusion via sequential mini-osmotic pumps. Pharmacol. Biochem. Behav. 26:131–139; 1987.
14. Milne, B.; Cervenko, F.; Jhamandas, K.; Loomis, C.; Sutak, M. Analgesia and tolerance to intrathecal morphine and norepinephrine infusion via implanted mini-osmotic pumps in the rat. Pain 22: 165–172; 1985.
15. Porreca, F.; Heyman, J. S.; Mosberg, H. I.; Omnaas, J. R.; Vaught, J. L. Role of μ- and δ-spinal opioids receptors J. Pharmacol. Exp. Ther. 240:150–158; 1987.
16. Russell, R. D.; Leslie, J. B.; Su, Y.-F.; Watkins, W. D.; Chang, K.-J. Continuous intrathecal opioid analgesia: tolerance and cross-tolerance of μ and δ spinal opioid receptors. J. Pharmacol. Exp. Ther. 240:150–158; 1987
17. Solomon, R. E.; Gebhart, G. F. Intrathecal morphine and clonidine: Antinociceptive tolerance and cross-tolerance and effects on blood pressure. J. Pharmacol. Exp. Ther. 245:444–454; 1988.
18. Suh, H. H.; Tseng, L. F. Intrathecal β-funaltrexamine antagonizes intracerebroventricular β-endorphin- but not morphine-induced analgesia in mice. J. Pharmacol. Exp. Ther. 245:587–593; 1988.
19. Suh, H. H.; Tseng, L. L.-F. Intrathecal adminstration of thiorphan, bestatin, desipramine and fluoxetine differentially potentiate the antinociceptive effects induced by β-endorphin and morphine, administered intracerebroventricularly. Neuropharmacology, in press; 1989.
20. Suh, H. H.; Fujimoto, J. M.; Tseng, L. F. Differential mechanisms mediating β-endorphin- and morphine-induced analgesia in mice. Eur. J. Pharmacol. 168:61–70; 1989.
21. Suh, H. H.; Tseng, L. F. Spinal δ- but not μ-opioid receptors are involved in tail-flick inhibition induced by β-endorphin given intracerebroventricularly in mice. FASEB J. 3:A279; 1989.
22. Tallarida, R. I.; Murray, R. B. Manual of pharmacologic calculation with computer programs. New York: Springer-Verlag; 1981.
23. Tseng, L. F. Tolerance and cross-tolerance to morphine after chronic spinal D-Ala^2-D-Leu^5-enkephalin infusion. Life Sci. 31:987–992; 1982.
24. Tseng, L.-F. Partial cross tolerance to D-Ala^2-D-Leu^5-enkephalin after chronic spinal morphine infusion. Life Sci. 32:2545–2550; 1983.
25. Tseng, L. F.; Fujimoto, J. M. Evidence that spinal endorphin mediates intraventricular β-endorphin-induced tail-flick inhibition and catalepsy. Brain Res. 302:231–237; 1984.
26. Tseng, L. F.; Fujimoto, J. M. Differential actions of intrathecal naloxone on blocking the tail-flick inhibition induced by intraventricular β-endorphin and morphine. J. Pharmacol. Exp. Ther. 232:74–79; 1985.
27. Tseng, L. F.; Higgins, M. J.; Hong, J-S.; Hudson, P. M.; Fujimoto, J. M. Release of immunoreactive Met-enkephalin from the spinal cord by intraventricular β-endorphin but not morphine in anesthetized rats. Brain Res. 343:60–69; 1985.
28. Tseng, L. F.; King, R. C.; Fujimoto, J. M. Release of immunoreactive Met-enkephalin by intraventricular β-endorphin in anesthetized rats. Regul. Pept. 14:181–192; 1986.
29. Tseng, L. F.; Suh, H. H. Effects of chronic intrathecal D-ala^2-D-Leu^5-enkephalin and morphine on intracerebroventricular β-endorphin- and morphine-induced analgesia in mice. Fed. Proc. 46:38; 1987.
30. Tseng, L. L.-F.; Suh, H. H. Intrathecal Met-enkephalin antibody blocks analgesia induced by intracerebroventricular β-endorphin but not morphine in mice. Eur. J. Pharmacol. 173:171–176; 1989.
31. Tseng, L. F.; Towell, J. F.; Fujimoto, J. M. Spinal release of immunoreactive Met-enkephalin by intraventricular β-endorphin and its analogs in anesthetized rats. J. Pharmacol. Exp. Ther. 237:65–74; 1986.
32. Tung, A. S.; Yaksh, T. L. In vivo evidence for multiple opiate receptors mediating analgesia in the rat spinal cord. Brain Res. 247:75–83; 1982.
33. Ward, S. L.; Portoghese, P. S.; Takemori, A. E. Pharmacological characterization in vivo of the novel opiate, β-funaltrexamine. J. Pharmacol. Exp. Ther. 220:494–498; 1983.
34. Ward, S. L.; Takemori, A. E. Relative involvement of μ-, κ-, and δ-receptor mechanisms in opiate-mediated antinociception in mice. J. Pharmacol. Exp. Ther. 224:525–530; 1983
35. Wigdor, S.; Wilcox, G. L. Central and systemic morphine-induced antinociception in mice: Contribution of descending serotonergic and noradrenergic pathways. J. Pharmacol. Exp. Ther. 242:90–95; 1987.
36. Yaksh, T. L. Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effect of morphine in the periaqueductal gray. Brain Res. 160:180–185; 1979.
37. Yaksh, T. L.; Kohl, R. L.; Rudy, T. L. Induction of tolerance and withdrawal in rats receiving morphine in the spinal subarachnoid space. Eur. J. Pharmacol. 42:275–284; 1977.