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Effects of serine/threonine protein phosphatase inhibitors on morphine-induced antinociception in the tail flick test in mice

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Abstract

The aim of this study was to evaluate the effects of serine/threonine protein phosphatase (PP) inhibitors on morphine-induced antinociception in the tail flick test in mice, and on [^{3}H]naloxone binding to the forebrain crude synaptosome fraction. Neither okadaic acid nor cantharidin (1–10 000 nM) displaced [^{3}H]naloxone from its specific binding sites, which indicates that they do not interact at the opioid receptor level. The i.c.v. administration of very low doses of okadaic acid (0.001–1 pg/mouse) and cantharidin (0.001–1 ng/mouse), which inhibit PP2A, produced a dose-dependent antagonism of the antinociception induced by morphine (s.c.). However, L-norokadaone (0.001 pg/mouse–1 ng/mouse, i.c.v.), an analogue of okadaic acid lacking activity against protein phosphatases, did not affect the antinociceptive effect of morphine. On the other hand, high doses of okadaic acid (10 ng/mouse, i.c.v.) and cantharidin (1 μg/mouse, i.c.v.), which also block PP1, and calyculin-A (0.1 fg/mouse–1 ng/mouse, i.c.v.), which inhibits equally both PP1 and PP2A, did not modify the morphine-induced antinociception. These results suggest that the activation of type 2A serine/threonine protein phosphatases may play a role in the antinociceptive effect of morphine, and that PP1 might counterbalace this activity.

Keywords: Antinociception; Morphine; Serine/threonine protein phosphatase; Okadaic acid; Cantharidin; Calyculin-A; L-Norokadaone; [^{3}H]Naloxone

1. Introduction

The reversible phosphorylation and dephosphorylation of proteins, catalysed by protein kinases and protein phosphatases, respectively, play an important role in the control of intracellular cell events. Serine/threonine protein phosphatases (PPs) dephosphorylate serine and threonine residues in proteins (Van Hoof et al., 1996). Numerous types of PPs are present in the brain, but the best characterized are type 1 (PP1) and type 2 (PP2) (Price and Mumby, 1999). Type 2 protein phosphatases can be further subdivided into metal ion-independent PP2A, Ca^{2+}-calmodulin-dependent PP2B, and Mg^{2+}-dependent PP2C (see Van Hoof et al., 1996, for a review). Several drugs can inhibit the functions of these phosphatases. Okadaic acid and cantharidin are cell permeable agents that inhibit PP2A in vitro at much lower concentrations than PP1 (Ishihara et al., 1989; Cohen et al., 1989, 1990; Hardie et al., 1991; Suganuma et al., 1992; Li et al., 1993; Honkanen, 1993). Calyculin-A is another cell permeable protein phosphatase inhibitor, which is equally potent against PP1 and PP2A (Ishihara et al., 1989; Suganuma et al., 1992; Honkanen et al., 1994; Girault, 1994).

It has been reported that PPs modulate several biochemical and electrophysiological processes occurring after μ-opioid receptor activation. Okadaic acid completely reverses the inhibitory effects of sufentanil upon cAMP formation in guinea pig longitudinal muscle (Wang et al., 1996). Okadaic acid also decreases the rate of recovery from desensitisation of currents induced by stimulating μ- and δ-opioid receptors (Osborne and Williams, 1995; Morikawa et al., 1998). However, there are few studies on the role of the PPs on opioid-induced effects in vivo. Bernstein and Welch (1998) have evaluated the effects of okadaic acid in tail flick test in naive and morphine-tolerant mice, and have reported that relatively high doses of this drug do not modify the effect of morphine in naive animals and increase the antinociception induced by morphine in animals which have received chronic treatment with this opioid. The effects of other inhibitors of PP1 or PP2A on morphine-induced antinociception have not been tested.

In the present study we evaluated the effects of three inhibitors of PPs on the antinociception induced by the acute

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administration of morphine in the tail flick test. We tested the effects of two preferential inhibitors of PP2A, okadaic acid and cantharidin, which differ in their order of potency in inhibiting PP2A (Cohen et al., 1989; Li and Casida, 1992; Li et al., 1993; Honkanen, 1993), and compared them with those produced by calyculin-A, a non-selective inhibitor of PP1 and PP2A. To rule out possible false positive results, we also tested the effect of L-norokadaone, an analogue of okadaic acid that does not affect the activity of PPs (Nishiwaki et al., 1990; Honkanen et al., 1994). Furthermore, in order to assess the possible interaction at the receptor level between morphine and PP inhibitors, we performed binding experiments to determine whether these drugs affect the specific binding of [^{3}H]naloxone.

2. Methods

2.1. Animals

Female CD-1 mice (Charles River, Barcelona, Spain) weighing 25–30 g were used for all the experiments. The animals were housed 15 per cage in a temperature-controlled room (21 ± 1 °C) with air exchange every 20 min and an automatic 12-h light/dark cycle (0800–2000 h). They were fed a standard laboratory diet and tap water ad libitum until the beginning of the experiments. The animals were placed in the experimental room 2 h before the antinociceptive test, for adaptation. The experiments were performed during the light phase (0900–1500 h). Naive animals were always used. Mice were handled in accordance with the ethical principles for the evaluation of pain in conscious animals (Zimmermann, 1983) and with the European Communities Council Directive of 24 November 1986 (86/609/ECC).

2.2. Drugs and drug administration

The drug used as a prototype of μ-opioid receptor agonists was morphine hydrochloride (General Directorate of Pharmacy and Drugs, Spanish Ministry of Health), which was dissolved in ultrapure water and injected subcutaneously (s.c.). The inhibitors of serine/threonine PPs used were: okadaic acid, cantharidin and calyculin-A (all provided by Sigma Química); as a negative control we used L-norokadaone (ICN Hubber). The inhibitors of PPs and L-norokadaone were dissolved in 1% Tween 80 in ultrapure water, and were injected intracerebroventricularly (i.c.v.). Control animals received the same volume of vehicles.

The s.c. injections were done in the interscapular region, in a volume of 5 ml/kg. The i.c.v. injections were done in the right lateral cerebral ventricle in a volume of 5 μl/mouse, according to the method that we previously described in detail (Ocaña et al., 1995). Briefly, the injection site was identified according to the method reported by Haley and McCormick (1957). The drug solution was injected with a 10 μl Hamilton syringe with a sleeve around the needle to prevent the latter from penetrating more than 3 mm into the skull. After the experiments were done, the position of the injection was evaluated in each brain, and the results from animals in which the tip of the needle did not reach the lateral ventricle were discarded. The accuracy of the injection technique was evaluated and the percentage of correct injections was 99%.

2.3. Antinociception experiments

The tail flick test was used to evaluate the antinociceptive effects of the drugs. The test was performed as previously described (Ocaña et al., 1993). Briefly, the animals were restrained in a Plexiglas tube and placed on the tail flick apparatus (LI 7100, Letica). A noxious beam of light was focussed on the tail about 4 cm from the tip, and the tail flick latency was recorded automatically to the nearest 0.1 s. The intensity of the radiant heat source was adjusted to yield baseline latencies between 3 and 5 s; this intensity was never changed and any animal whose baseline latency was outside the pre-established limits was excluded from the experiments. In order to minimise injury in the animals, a cut-off time of 10 s was used.

Baseline tail flick latencies were recorded 10 min and immediately before all injections. Once baseline latencies were obtained the animals received an s.c. injection of morphine or its solvent (ultrapure water), and immediately thereafter an i.c.v. injection of a PP inhibitor (okadaic acid, cantharidin, calyculin-A), L-norokadaone or their solvent (1% Tween 80 in water). The end of the last injection was considered time 0; from this time response latency was measured again at 15, 30, 45, 60, 90 and 120 min.

The degree of antinociception was calculated with two procedures: (1) using latency time values according to the formula: $\%\text{antinociception}=[(LT_d - LT_v)/(LT_{max} - LT_v)] \times 100$, where LT_d and LT_v are the tail flick latency times for drug-treated and vehicle-treated animals, respectively, and LT_{max} is the maximum latency time (10 s), and (2) using the area under the curve of tail flick latency against time (AUC), which yields a global value of the antinociception induced over the 2 h experimental period, according to the formula: $\%\text{antinociception}=[(AUC_d - AUC_v)/(AUC_{max} - AUC_v)] \times 100$, where AUC_d and AUC_v are the areas under the curve for drug-treated and vehicle-treated animals, respectively, and AUC_{max} is the area under the curve of maximum possible antinociception (10 s in each determination).

2.4. Binding experiments

In these experiments we used [^{3}H]naloxone (specific activity 57.50 Ci/mmol) as the radioligand (Dupont-NEN). Unlabeled drugs were naloxone hydrochloride, okadaic acid

ammonium salt and cantharidin (all from Sigma Química). All dilutions were prepared with 50 mM Tris, pH 7.4.

Binding experiments were carried out in the crude synaptosome fraction or P_2 fraction isolated from naive mice forebrains as previously described (González et al., 2001). Briefly, mice were killed by decapitation, the brains were quickly removed and the forebrains were dissected and placed in tubes containing 10 ml ice-cold 0.32 M sucrose solution. Then each forebrain was homogenised with three strokes of a Polytron homogeniser (Model PT10-35, Kinematica) at position 3. Each stroke lasted 10 s and was separated from the next stroke by a 30-s period during which the tube was placed in ice. The homogenates were centrifuged (Avanti 30, Beckman Instruments) at $1000 \times g$ for 10 min at 4 °C; the resulting pellets were discarded and the supernatants were centrifuged again under the same conditions. The resulting supernatants were poured into a centrifuge tube, taking care not to disturb the pellets, and were centrifuged at $17000 \times g$ for 20 min at 4 °C. Then each pellet (P_2 fraction) was resuspended in buffer (Tris 50 mM, pH 7.4) and thoroughly mixed using a vortex agitator.

Protein concentrations were measured by the method of Lowry et al. (1951) with some modifications, using bovine serum albumin as the standard. The experiments were carried out with a final protein concentration of about 0.6 mg/ml, which was within the linear range of the relationship between specific binding and protein concentration (0.125–2 mg/ml).

For radioligand assays we incubated 460 μl of crude synaptosome fraction with 20 μl [^{3}H]naloxone (final concentration in the medium 2 nM) and 20 μl of unlabeled drug or its solvent at 30 °C for 30 min (Freissmuth et al., 1993). Specific binding was defined as that displaced by 10 μM unlabeled naloxone. Incubations were terminated by adding 5 ml ice-cold 50 mM Tris–HCl buffer, pH 7.4 at 4 °C. Bound and free [^{3}H]naloxone were immediately separated by rapid filtration under a vacuum through Whatman GF/B glass fibre filters with a Brandel cell harvester (Model M-12T, Brandel Instruments) and washed twice with 5 ml ice-cold buffer. The filters were transferred to scintillation counting vials containing 4 ml liquid scintillation cocktail (Optiphase Hisafe II, Wallac Scintillation Products) and left to equilibrate for at least 12 h. The radioactivity retained on the filter was measured with a liquid scintillation spectrometer (Beckman Instruments), with an efficiency of 52%. All incubations were done in triplicate and each experiment was done at least three times.

2.5. Data analysis

In the antinociception experiments, the values in the control group were compared against those obtained in the treated groups using one-way or two-ways analysis of variance (ANOVA) followed by the Newman–Keuls test. The differences between means were considered significant when the value of P was below 0.05.

The IC_{50} (concentration of unlabeled drug that inhibited 50% of specific [^{3}H]naloxone binding) and ED_{50} (dose of morphine that produced half of the maximal antinociception) values were calculated from the dose–response curves using non-linear regression analysis with the Sigma Plot 2000 version 6.00 computer program (SPSS). The AUC of tail flick latency against time was calculated with the GraphPad Prism version 3.00 computer program (GraphPad Software).

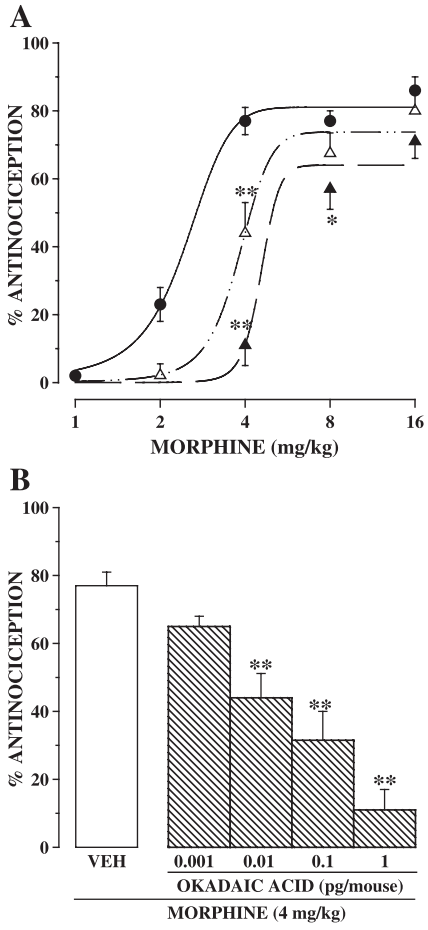

Fig. 1. Effects of the association of okadaic acid (i.c.v.) or its vehicle with morphine (s.c.) in the tail flick test in mice. (A) Effects of morphine plus vehicle (●), morphine plus okadaic acid 0.01 pg/mouse (△) and morphine plus okadaic acid 1 pg/mouse (▲). (B) Effects of the i.c.v. administration of different doses of okadaic acid (0.001–1 pg/mouse) or its vehicle on the antinociception induced by morphine (4 mg/kg, s.c.). The white column represents the effect of morphine plus vehicle, and the hatched columns represent the effects of the association of morphine with different doses of okadaic acid. The percentage of antinociception was calculated from the area under the curve of antinociception (see Methods). Each point and each column represents the mean ± S.E.M. ($n \geq 8$). Statistically significant differences in comparison to morphine plus vehicle: *$P < 0.05$; **$P < 0.01$ (two-way ANOVA [A] and one-way ANOVA [B] followed by Newman–Keuls test).

3. Results

3.1. Effect of okadaic acid and cantharidin on morphine-induced antinociception

As expected, the subcutaneous administration of morphine (1–16 mg/kg) induced a dose-dependent antinociceptive effect in the tail flick test in mice. Okadaic acid (i.c.v.) produced a dose-dependent inhibition of the effect of morphine at very low doses, displacing its dose–response curve to the right (Fig. 1A) and increasing the ED_{50} of morphine from 2.45 ± 0.23 mg/kg (morphine + vehicle) to 3.79 ± 0.33 mg/kg (morphine + okadaic acid 0.01 pg/mouse) and to 4.55 mg/kg (morphine + okadaic acid 1 pg/mouse). When several doses of okadaic acid (0.001–1 pg/mouse, i.c.v.) were associated with morphine 4 mg/kg s.c., we observed that the inhibitory effect of this agent was clearly dose-dependent and that the highest dose used (1 pg/mouse) significantly decreased the effect of morphine from 77 ± 4% to 11 ± 6% (Fig. 1B).

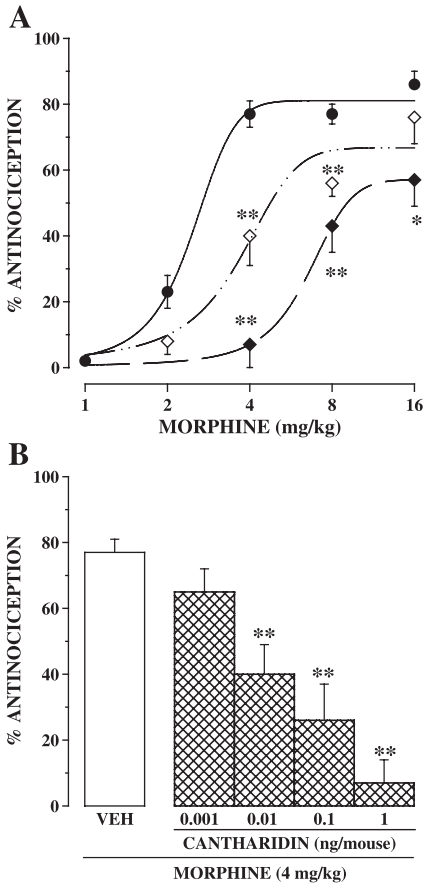

Fig. 2. Effects of the association of cantharidin (i.c.v.) or its vehicle with morphine (s.c.) in the tail flick test in mice. (A) Effects of morphine plus vehicle (●), morphine plus cantharidin 0.01 ng/mouse (◇) and morphine plus cantharidin 1 ng/mouse (◆). (B) Effects of the i.c.v. administration of different doses of cantharidin (0.001–1 ng/mouse) or its vehicle on the antinociception induced by morphine (4 mg/kg, s.c.). The white column represents the effect of morphine plus vehicle, and the cross-hatched columns represent the effect of the association of morphine with different doses of cantharidin. The percentage of antinociception was calculated from the area under the curve of antinociception (see Methods). Each point and each column represents the mean ± S.E.M. ($n \geq 8$). Statistically significant differences in comparison to morphine plus vehicle: $^{*}P < 0.05$; $^{**}P < 0.01$ (two-way ANOVA [A] and one-way ANOVA [B] followed by Newman–Keuls test).

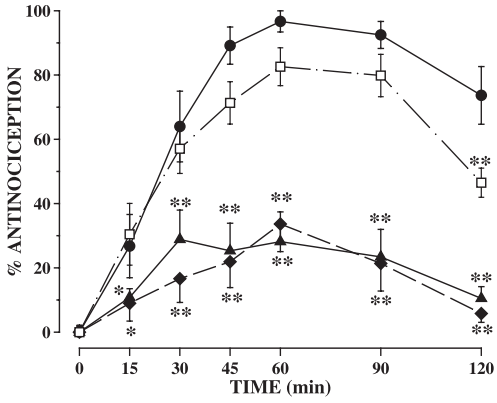

Fig. 3. Time course of the antinociceptive effects obtained with the tail flick test in mice treated with the association of morphine 4 mg/kg, s.c. plus: vehicle i.c.v. (●), L-norokadaone 1 ng/mouse, i.c.v. (□), okadaic acid 1 pg/mouse, i.c.v. (▲) and cantharidin 1 ng/mouse, i.c.v. (◆). The percentage of antinociception represents the percentage of the maximum tail flick latency at each time evaluated (see Methods). Each point represents the mean ± S.E.M. ($n \geq 8$). Statistically significant differences in comparison with morphine plus vehicle: $^{*}P < 0.05$; $^{**}P < 0.01$ (two-way ANOVA followed by Newman–Keuls test).

Cantharidin also produced a dose-dependent inhibition of the effect of morphine, although the doses required to produce these effects were higher that those of okadaic acid. This drug (administrated i.c.v.) displaced the dose–response curve of morphine (s.c.) to the right and increased the ED_{50} from 2.45 ± 0.23 mg/kg (morphine + vehicle) to 3.69 ± 0.82 and 6.55 mg/kg (morphine + cantharidin 10 pg/mouse and 1 ng/mouse, respectively) (Fig. 2A). The effect of a wide range of doses of cantharidin (0.001–1 ng/mouse, i.c.v.) on the antinociception induced by morphine (4 mg/kg, s.c.) was also tested. Fig. 2B showed that this inhibitor

Table 1
Effect of inhibitors of PPs (i.c.v.) or vehicle (i.c.v.) on antinociception induced by morphine 4 mg/kg (s.c.)

Drugs	% Antinociception[a]
Morphine + vehicle	72.24 ± 9.72
Morphine + okadaic acid 1 pg/mouse	12.55 ± 6.83[b]
Morphine + okadaic acid 10 ng/mouse	52.96 ± 13.03[NS]
Morphine + cantharidin 1 ng/mouse	6.13 ± 6.97[b]
Morphine + cantharidin 1 μg/mouse	50.6 ± 9.01[NS]

NS: The differences were not statistically significant in comparison to the vehicle-treated group ($P > 0.05$; two-way ANOVA followed by Newman–Keuls test).

[a] Percent antinociception was calculated from the area under the curve of antinociception (see Methods) and expressed as the mean ± S.E.M. ($n \geq 8$).

[b] Differences statistically significant in comparison to the vehicle-treated group ($P < 0.01$; two-way ANOVA followed by Newman–Keuls test).

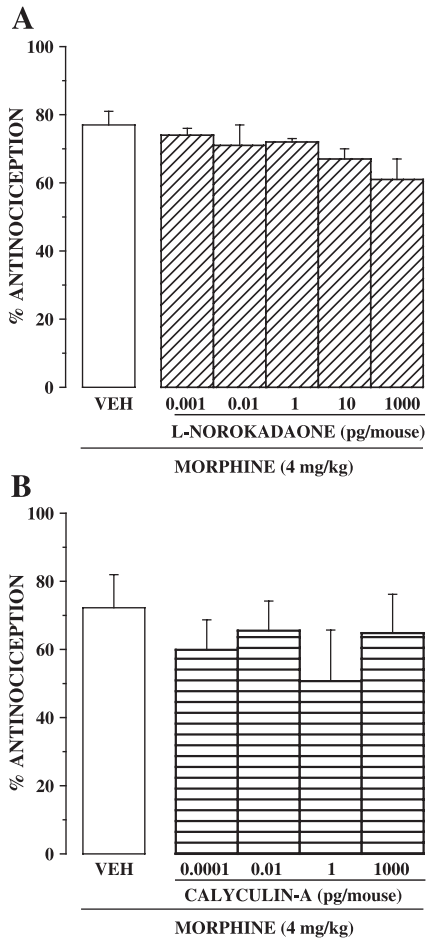

Fig. 4. (A) Antinociceptive effects induced by the association of morphine 4 mg/kg, s.c. with vehicle i.c.v. (white bar) and morphine 4 mg/kg, s.c. plus different doses of L-norokadaone i.c.v. (hatched bars). (B) Antinociceptive effects induced by the association of morphine 4 mg/kg, s.c. with vehicle i.c.v. (white bar) and morphine 4 mg/kg, s.c. plus different doses of calyculin-A (horizontal hatched bars). The effects were evaluated with the tail flick test in mice and calculated from the area under the curve of antinociception (see Methods). Each column represents the mean ± S.E.M. ($n = 8–12$). There were no statistically significant differences in comparison to morphine plus vehicle (one-way ANOVA test).

of PP produced a dose-dependent antagonism; the maximum effect was observed with 1 ng/mouse of cantharidin, which reduced the effect of morphine 4 mg/kg from 77 ± 4% to 7 ± 6%.

When the time courses of the effects were plotted, it was evident that okadaic acid 1 pg/mouse and cantharidin 1 ng/mouse significantly reduced the antinociception produced by morphine at 4 mg/kg from 15 to 120 min after injection (Fig. 3).

When okadaic acid and cantharidin were associated to morphine (4 mg/kg, s.c.) at much higher doses that previously shown to exert maximum inhibition on the effects of morphine (10 ng/mouse and 1 μg/mouse for okadaic acid and cantharidin, respectively), their effects on antinociception induced by morphine were non-significant (Table 1).

The effects on the pain threshold produced by the i.c.v. administration of PPs alone were also studied. Neither of the PP inhibitors at the doses used (okadaic acid 1 pg/mouse and 10 ng/mouse; cantharidin 1 ng/mouse and 1 μg/mouse) modified the tail flick latency values of mice when given alone, and had no hyperalgesic or analgesic effects (data not shown).

3.2. Effect of L-norokadaone on morphine-induced antinociception

L-norokadaone did not exert any effect in our model when it was administrated alone (data not shown) or in association with morphine. As illustrated in Figs. 3 and 4A, it was unable to modify the antinociception induced by s.c. injections of morphine, although we tested a wide range of doses from 1 fg/mouse to 1 ng/mouse.

3.3. Effect of calyculin-A on morphine-induced antinociception

Calyculin-A did not modify the latency times in the tail flick test when it was associated with the morphine vehicle (data not shown) or when several doses of calyculin-A (0.0001–1000 pg/mouse, i.c.v.) were associated with morphine 4 mg/kg, s.c. (Fig. 4B).

3.4. Binding experiments

Naloxone induced a concentration-dependent displacement of tritiated naloxone from its specific binding sites in mice forebrain synaptosomes, with an IC_{50} of 3.47 ± 1.01 nM. In contrast, neither okadaic acid nor cantharidin (1–10 000 nM) significantly modified [^{3}H]naloxone binding at any concentration tested (Fig. 5), and we therefore deduce that neither had any affinity for the opioid receptor.

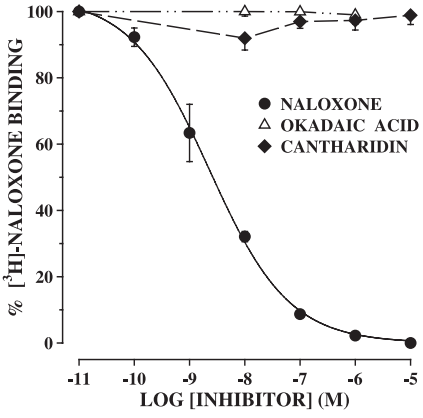

Fig. 5. Displacement produced by naloxone (●), but not by okadaic acid (△) or cantharidin (◆), of 2 nM [^{3}H]naloxone specifically bound to crude synaptosome fractions obtained from mice forebrains. Incubations were performed at 30 °C for 30 min. The final protein concentration was about 0.6 mg/ml. Each point represents the mean ± S.E.M. ($n \geq 3$).

4. Discussion

In the present study, the i.c.v. administration of very low doses of okadaic acid and cantharidin inhibited morphine-induced antinociception in mice in a dose-dependent way. However, neither okadaic acid nor cantharidin modified the specific binding of [^{3}H]naloxone to forebrain membranes, which argues against a direct action of these inhibitors on opioid receptors. On the other hand, several findings suggest that the inhibition of serine/threonine PPs can be the main mechanism involved in these effects. Firstly, both drugs have the same efficacy, but different potencies (okadaic acid>cantharidin) in inhibiting PPs (Li and Casida, 1992; Honkanen, 1993), and in our experiments they also antagonized morphine antinociception with the same efficacy, okadaic acid being more potent than cantharidin. Moreover, the okadaic acid analogue L-norokadaone, which does not inhibit PPs (Nishiwaki et al., 1990; Honkanen et al., 1994), was unable to modify the antinociceptive effect of morphine. These data argue in favour of the idea that specific inhibition of serine/threonine PPs underlies the interaction between morphine and okadaic acid/cantharidin. In addition, if we assume that the volume of cerebrospinal fluid in mice is about 100 μl, as did other authors (Bernstein and Welch, 1998), the final concentration of inhibitors of PPs in the brain after the i.c.v. injection of doses that induced the maximum inhibitory effect in our experiments (i.e. 1 pg/mouse and 1 ng/mouse of okadaic acid and cantharidin, respectively) would be 0.124 nM for okadaic acid and 51 nM for cantharidin. These concentrations are within the range of IC_{50} values for the inhibition of PP2A with okadaic acid (0.1–0.3 nM) and cantharidin (40–160 nM) (Cohen et al., 1989, 1990; Li et al., 1993; Honkanen, 1993; Girault, 1994). Thus, although it is not appropriate to draw strong inferences from in vitro drug potencies to more complex in vivo systems, it can be considered that the concentrations of okadaic acid and cantharidin which antagonize morphine-induced antinociception in our experiments may inhibit type 2A PP.

The only previous study of the interaction between PP inhibitors and acutely administered morphine was reported by Bernstein and Welch (1998). These authors did not observe any significant modulation of the morphine-induced antinociception by okadaic acid administered i.c.v. in morphine-naive animals, and found a slight but non-significant increase in the effects of morphine by intrathecal okadaic acid. These results apparently contradict ours; however, these authors injected higher doses of this drug (0.04–2 μg/mouse) than we did. In our experiments, okadaic acid and cantharidin antagonized the antinociceptive effects of morphine at very low doses; however, when we injected doses of PP inhibitors several times greater than those that produced maximum antagonism of the effects of morphine (i.e. 10 ng of okadaic acid and 1 μg of cantharidin), we found that the modification of the morphine-induced antinociception was not significant. A possible explanation for these dual effects may be that high doses of okadaic acid and cantharidin produce other actions in addition to the inhibition of PP2A.

Okadaic acid and cantharidin inhibit PP1 at high concentrations, with an IC_{50} of 15–500 and 400–1700 nM, respectively (Cohen et al., 1989; Ishihara et al., 1989; Li et al., 1993; Honkanen, 1993). The doses of okadaic acid and cantharidin that do not modify morphine antinociception would be expected to produce concentrations in the brain in the μM range in our study, and even higher in that by Bernstein and Welch (1998). These concentrations would be expected to block not only PP2A but also PP1. Therefore, blockade of PP1 may counterbalance the effect of PP2A blockade in morphine-induced antinociception. To test this hypothesis we evaluated the effect of calyculin-A, which inhibits both PP1 and PP2A with similar potency (IC_{50} around 1 nM for both PPs) (Ishihara et al., 1989; Suganuma et al., 1992; Honkanen et al., 1994; Girault, 1994). We observed that this drug had no effect on morphine-induced antinociception, even though a wide range of doses was used. These data apparently support the above-mentioned hypothesis.

The mechanisms that may be involved in the interaction between inhibitors of PPs and morphine cannot be determined with our experimental approach. However, several possibilities may be considered. The μ-opioid agonist sufentanil decreases cAMP formation in guinea pig myenteric plexus, this effect being reversed to enhancement by okadaic acid (Wang et al., 1996). The antinociceptive effects of morphine were inhibited when mice were treated with forskolin (Suh et al., 1996) or with 3-isobutyl-1-methylxhantine (Suh et al., 1995), drugs that induce an increase in intracellular cAMP levels through different mechanisms. Thus, treatment with okadaic acid may antagonize morphine-induced inhibition of cAMP, and this may in turn affect morphine-induced antinociception. Furthermore, okadaic acid, at concentrations that inhibit PP2A, reduced outward K^+ currents (Bialojan and Takai, 1988) and completely reversed the stimulatory effects of somatostatin and angiotensin II on several types of K^+ channels (White et al., 1991; Kang et al., 1994). Bearing in mind that K^+ channel opening plays a role in morphine-induced antinociception (Ocaña et al., 1990, 1995; Narita et al., 1992; Welch and Dunlow, 1993; Raffa and Martínez, 1995), it is possible that an inhibitory effect of okadaic acid on K^+ channels may inhibit the antinociceptive effects of morphine. Another possible target for PP2A inhibitors in the brain are the L-type Ca^{2+} channels. Both protein kinase A (PKA) and PP2A are integral components of the α subunit of these channels, determining the phosphorylation level and thereby channel activity (Davare et al., 2000). The L-type Ca^{2+} channels are involved in the antinociception induced by morphine (Del Pozo et al., 1990; Dierssen et al., 1990; Dogrul et al., 2001), and a change in their activity by PP2A inhibitors may influence the effects of this opioid. Obviously, further experiments need be performed to elucidate

the mechanism of interaction between inhibitors of PPs and morphine.

In conclusion, this study shows that okadaic acid and cantharidin, but not L-norokadaone, antagonize morphine-induced antinociception and supports a role for type 2A serine/threonine protein phosphatases in the antinociceptive effects of this opioid.

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