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δ-Opioid receptor agonists produce antinociception and [^{35}S]GTPγS binding in μ receptor knockout mice

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Abstract

We examined the effects of [D-Pen^2,D-Pen^5]enkephalin (DPDPE), [D-Ala^2,Glu^4]deltorphin (DELT), and (+)-4-[(α*R*)-α((2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC80) on [^{35}S]GTPγS binding in brain membranes prepared from μ-opioid receptor knockout (−/−) mice. The potency and maximal response (E_{max}) of these agonists were unchanged compared to control mice. In contrast, while the potency of [D-Pen^2,pCl-Phe^4,D-Pen^5]enkephalin (pCl-DPDPE) was not significantly different, the E_{max} was reduced as compared to controls. In the tail-flick test, intracerebroventricular (i.c.v.) or intrathecal (i.th.) DELT produced antinociceptive effects in −/− mice with potency that did not differ significantly from controls. In contrast, the antinociceptive potency of i.c.v. and i.th. DPDPE was displaced to the right by 4- and 9-fold in −/− compared to control mice, respectively. Reduced DPDPE antinociceptive potency in −/− mice, taken together with reduced DPDPE- and pCl-DPDPE- stimulated G protein activity in membranes prepared from −/− mice, demonstrate that these agonists require μ-opioid receptors for full activity. However, because DELT mediated G protein activation and antinociception were both comparable between −/− and wild type mice, we conclude that the μ-opioid receptor is not a critical component of δ-opioid receptor function.

Keywords: δ-Opioid receptor; μ-Opioid receptor; μ-Opioid receptor-knockout mice; Antinociception; G protein; Opioid drug

1. Introduction

Many studies indicate that δ-opioid receptors mediate antinociception. δ-opioid receptor-selective compounds have been shown to produce antinociception that can be blocked by δ-selective antagonists (Heyman et al., 1987; Porreca et al., 1987; Shah et al., 1994) but not by μ-selective antagonists (Heyman et al., 1987). Prolonged agonist exposure produces antinociceptive tolerance to δ-opioids without significant cross-tolerance to μ-opioid receptor-selective agonists (Mattia et al., 1991). These data support a direct role for δ-opioid receptors in mediating antinociception. Furthermore, treatment of animals with antisense oligodeoxynucleotides targeted at the cloned δ-opioid receptor selectively attenuated the antinociception produced by δ-opioid, but not μ- or κ-opioid agonists, suggesting that δ receptor selective agonists such as [D-Ala^2, Glu^4]deltorphin act predominantly through δ receptors (Tseng et al., 1994; Bilsky et al., 1996; Standifer et al., 1997).

Germ line mutation of opioid receptor genes have produced transgenic mice which lack specific opioid receptors; these models have been used to evaluate the role of opioid receptors in physiological and pathological function. It was recently reported that mice with null mutation

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of the cloned μ-opioid receptor (MOR) (−/− mice) did not exhibit antinociceptive response to two putative δ-opioid receptor selective agonists, Tyr-D-Ser(*O*-*t*-butyl)-Gly-Phe-Leu-Thr-(*O*-*t*-butyl) (BUBU) (Matthes et al., 1996 and 1998) and [D-Pen^2, D-Pen^5]enkephalin (DPDPE) (Sora et al., 1997a). These observations led to the postulation that antinociceptive efficacy of δ-opioid receptor selective agonists requires the presence of μ-opioid receptors in the central nervous system. On the other hand, it may also be plausibly argued that the ligands used in this case are not highly selective for δ receptors as previously thought. The present study aims to test the hypothesis that in the −/− mice the δ-opioid receptors are functionally fully active and mediate antinociception. We have determined the activation of G proteins by δ-opioid selective agonists in both brain and spinal cord membranes prepared from control or −/− mice, and the antinociceptive actions of δ-selective agonists in both control and −/− mice. Our data indicate that the δ-opioid receptor remains functionally coupled to G proteins and produces antinociceptive actions in −/− mice. Thus, expression of μ-opioid receptors is not required for δ-opioid receptor functions.

2. Materials and methods

2.1. Generation of μ-opioid receptor knockout mice

The generation of μ-opioid receptor knockout mice in a C57/129Sv genetic background has been previously described (Sora et al., 1997b). Briefly, a 16.5-kb DNA fragment encoding part of the 5′ flanking region, the first exon and part of the first intron of the μ-opioid receptor was isolated from a mouse λ library. A deletion mutation of the fragment was produced in which the translation initiation site was omitted and this fragment was subcloned into the pPGKneo expression vector. Embryonic stem cells were then transfected with this construct and antibiotic resistant colonies screened for the mutation. Embryonic stem cells with the disrupted μ-opioid receptor were then injected into blastocysts and implanted in pseudopregnant mice. Successive offsprings were screened for loss of the μ-opioid receptor.

2.2. Membrane preparation

C57/129Sv (control) or −/− mice were euthanized by cervical dislocation. Whole brains and spinal cords were removed and frozen at −70°C. Frozen tissues were homogenized (10 strokes with a teflon homogenizer) in TE (10 mM Tris–HCl, 1 mM EDTA, pH = 7.4) buffer. After centrifugation the membrane pellet was homogenized (8 strokes as above) in assay buffer (25 mM Tris–HCl, 150 mM NaCl, 2.5 mM $MgCl_2$, 1 mM EDTA, 50 μM GDP, 30 μM bestatin, 10 μM captopril and 0.1 mM phenylmethylsulfonyl fluoride, pH 7.4) and incubated (30 min, 30°C) to allow endogenous ligands to dissociate from δ-opioid receptors. Membranes were then centrifuged, resuspended in assay buffer, and used at a final optical density (280 nm) of 0.8 or 0.4 in the [^{35}S]GTPγS binding assay for brain and spinal cord tissue, respectively.

2.3. [^{35}S]GTPγS binding assay

Membranes were incubated with increasing concentrations of agonist in the presence of 0.1 nM [^{35}S]GTPγS (1000–1500 Ci/mmol, NEN, Boston, MA) in assay buffer (total volume of 1 ml, duplicate samples) as a measure of agonist-mediated G protein activation. After incubation (90 min, 30°C), the reaction was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters, followed by four washes with ice-cold 25 mM Tris/120 mM NaCl, pH 7.4. Filters were pretreated with assay buffer prior to filtration to reduce nonspecific binding. Bound radioactivity was measured by liquid scintillation spectrophotometry after an overnight extraction with EcoLite® (ICN, Biomedicals, Costa Mesa, CA) scintillation cocktail.

2.4. Antinociceptive assay

2.4.1. Intrathecal injections

Intrathecal injections were made according to the method of Hylden and Wilcox (1980). The mouse was held firmly by the pelvic girdle and the vertebral column immobilized. A 30-gauge needle, attached to a 25-μl syringe, was inserted between two lower lumbar vertebrae at about a 20° angle until insertion into the vertebral canal was felt. Proper insertion was generally indicated by a reflexive straightening and lifting of the tail. The drug was injected in a volume of 5 μl.

2.4.2. Intracerebroventricular injections

Mice were lightly anesthetized with ether. A longitudinal incision was then made in the scalp and the bregma suture identified. A 25-gauge needle, attached to a 25-μl syringe, was inserted 2 mm through the skull in a position

Table 1
Characterization of [^{35}S]GTPγS binding in membranes prepared from whole brains of control (+/+) and μ-knockout (−/−) mice. Data values represent the mean ± standard error of the mean (S.E.M.) for ≥ 3 independent experiments

Drug	+/+ Membranes			−/− Membranes		
	E_{max}	EC_{50} (μM)	Hill slope	E_{max}	EC_{50} (μM)	Hill slope
DPDPE	118 ± 3	0.58 ± 0.18	0.81 ± 0.14	112 ± 2	0.56 ± 0.17	1.02 ± 0.18
pCl-DPDPE	157 ± 8	4.8 ± 1.0	0.43 ± 0.05	130 ± 3	0.72 ± 0.53	0.82 ± 0.15
SNC80	144 ± 3	0.30 ± 0.05	0.88 ± 0.03	143 ± 4	0.33 ± 0.07	0.94 ± 0.05
DELT	112 ± 2	0.76 ± 0.65	0.81 ± 0.20	114 ± 4	0.13 ± 0.05	1.3 ± 0.3

Table 2
Characterization of [^{35}S]GTPγS binding in membranes prepared from spinal cords of control (+/+) and μ-knockout (−/−) mice. Data values represent the mean ± S.E.M. for three independent experiments. Mean pharmacodynamic values could not be determined in DPDPE-stimulated −/− membranes as sigmoidal dose response curves did not converge for individual experiments as calculated using Prism ver. 2

Drug	+/+ Membranes			−/− Membranes		
	E_{max}	EC_{50} (μM)	Hill slope	E_{max}	EC_{50} (μM)	Hill slope
DPDPE	121 ± 13	8 ± 12	3.2 ± 2.6	–	–	–
SNC80	125 ± 1	0.89 ± 0.40	1.0 ± 0.1	134 ± 1	3.5 ± 1.1	0.71 ± 0.05

1 mm lateral to the midline and 2 mm caudal to bregma in order to reach the lateral ventricle. Injection depth was controlled by a plug on the needle. Drugs were injected in a volume of 5 μl.

2.4.3. Tail flick test

Antinociceptive activity was determined in C57/129Sv (control) or −/− mice by using the nociceptive warm water tail flick reflex as previously described (Bilsky et al., 1996). The tail flick test was performed by placing the tail of the mouse in a heated water bath maintained at 55°C. The latency until tail withdrawal from the bath, or a rapid flick, is determined once before (control) and at regular intervals after injection. A 15-s cut-off was employed to avoid tissue damage. Mice with tail flick baseline latencies of > 5 s were excluded from these studies.

2.5. Drugs

[D-Ala^2,NMePhe^4, Gly-ol^5]enkephalin (DAMGO), DPDPE and [D-Ala^2, Glu^4]deltorphin (DELT) were pur-

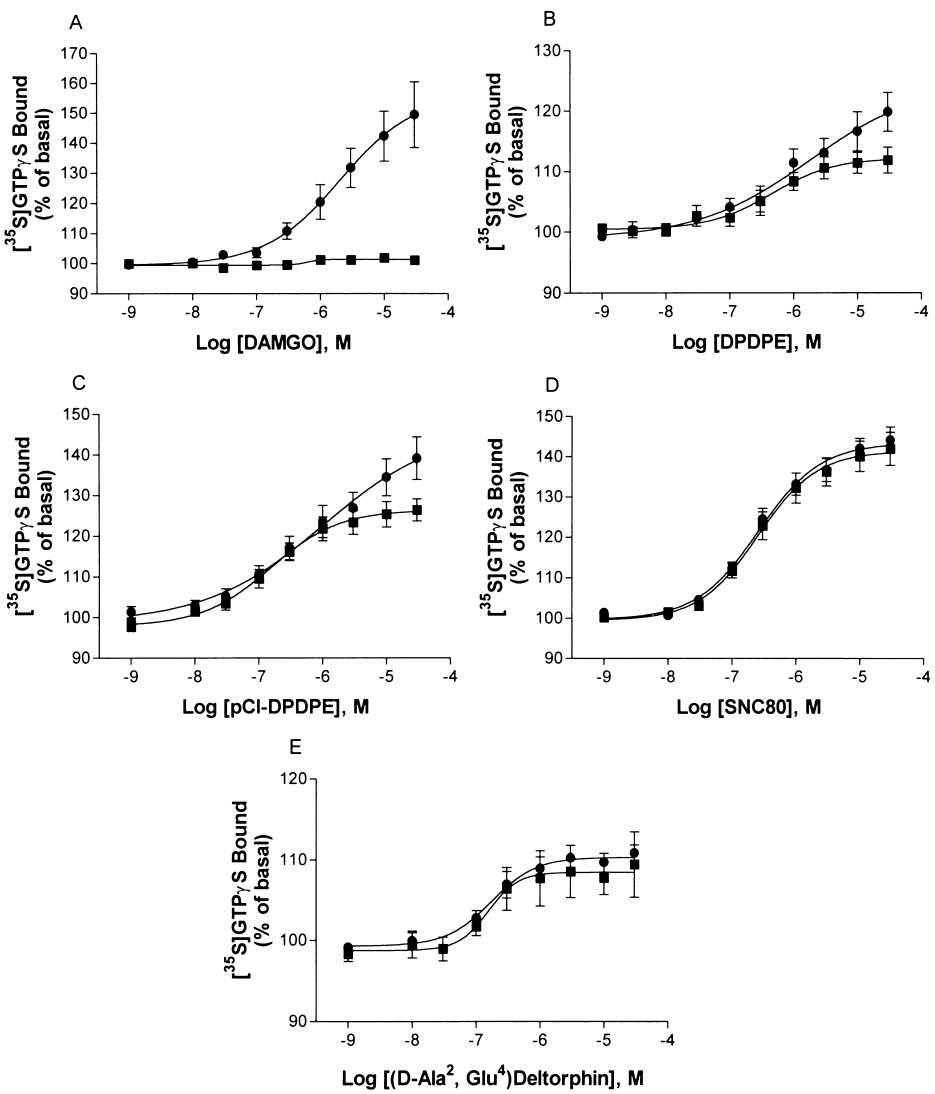

Fig. 1. The effect of opioid agonists on [^{35}S]GTPγS binding to membranes prepared from control (●) and −/− (■) mouse brains. Membranes were incubated with [^{35}S]GTPγS (0.1 nM) in the presence of (A) DAMGO, (B) DPDPE and (C) pCl-DPDPE, (D) SNC80 and (E) DELT. Each data point represents the mean ± S.E.M. at each drug concentration from multiple experiments. $N = 4$ (DAMGO), 4 (DPDPE), 5 (pCl-DPDPE), 6 (SNC80) and 3 (DELT).

chased from RBI (Natick, MA). (+)-4-[(α*R*)-α((2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*, *N*-diethylbenzamide (SNC80) was synthesized in the laboratory of Dr. Kenner C. Rice (NIH, Bethesda, MD). [D-Pen^2, pCl-Phe^4, D-Pen^5]enkephalin (pCl-DPDPE) was synthesized in the laboratory of Dr. Victor J. Hruby (University of Arizona, Tucson, AZ). Morphine sulfate was purchased from Sigma (St. Louis, MO).

2.6. Data analysis

Mean E_{max}, EC_{50}, and Hill slope values reported in Tables 1 and 2 were determined using the pharmacodynamic values calculated from individual experiments. Data from each experiments were fit to a variable slope sigmoidal dose response (four parameter fit) curves using Prism ver.2 (GraphPad, San Diego, CA). Statistically significant differences between E_{max}, EC_{50} and Hill slope values were determined by comparing the means using a t test. $N \geq 3$ for all experiments. Figs. 1 and 2 represent variable slope sigmoidal dose response curves where each data point represents the mean value of pooled experiments.

In the tail flick test, the data are converted to % antinociception by the formula: 100 × (test latencybaseline − latency)/(15 − baseline latency). Dose–response curves are generated from data collected at the time of peak effect. The A_{50}, the dose producing 50% maximum pharmacological effect, and its confidence intervals are determined by linear regression analysis of the log dose–response curve. Significant differences in potency or percent antinociception between two treatments were determined by *t*-test (Tallarida and Murray, 1987).

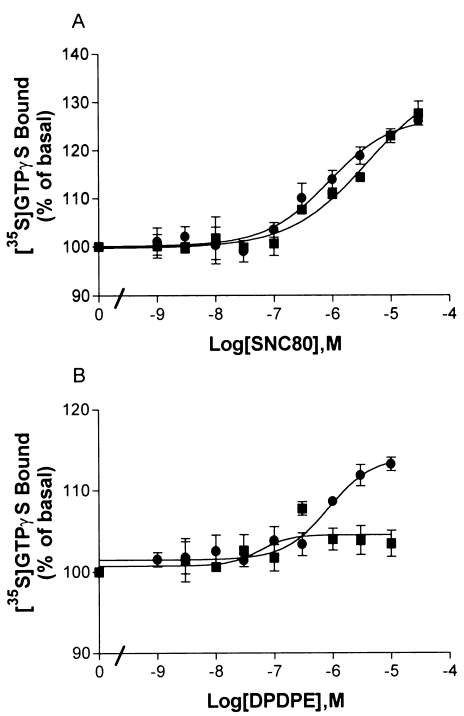

Fig. 2. The effect of opioid agonists on [^{35}S]GTPγS binding to spinal cord membranes prepared from control (●) and −/− (■) mouse. Membranes were incubated with [^{35}S]GTPγS (0.1 nM) in the presence of (A) SNC80 and (B) DPDPE. Each data point represents the mean ± S.E.M. at each drug concentration from multiple. $N = 3$.

3. Results

We examined G-protein activation by the μ-opioid receptor-selective agonist DAMGO, and the opioid agonists DPDPE, pCl-DPDPE, SNC80 and DELT in whole brain membranes prepared from control and −/− mice. These latter drugs have previously been shown to be highly δ-opioid receptor-selective (Kramer et al., 1993; Knapp et al., 1996). Activation was determined as drug-induced binding of [^{35}S]GTPγS to membranes (Fig. 1). In membranes prepared from control mouse brains, mean maximal responses of 118, 157, 144 and 112% basal [^{35}S]GTPγS binding were calculated using Prism ver. 2 for DPDPE, pCl-DPDPE, SNC80 and DELT, respectively. These drugs had mean potency values of 0.58, 4.8, 0.30 and 0.76 μM, respectively (Table 1).

We also examined G protein activation mediated by these drugs in membranes prepared from −/− mouse brains. These mice previously demonstrated undetectable μ-opioid receptor levels and unaltered δ-opioid receptor levels as compared to control (C57/129Sv) animals (Sora et al., 1997b). In contrast to control membranes, the μ-selective agonist DAMGO did not stimulate G proteins in −/− brain membranes thus confirming the absence of functional μ-opioid receptors (Fig. 1A). DPDPE, pCl-DPDPE, SNC80 and DELT all stimulated [^{35}S]GTPγS binding in −/− brain membranes (Fig. 1B–E). There were no significant changes in the Hill Slopes of DPDPE, SNC80 and DELT as compared to control brain membranes. However, the Hill Slope of pCl-DPDPE was shifted toward a value of one in the −/− membranes (Table 1) suggesting the loss of a low potency drug interaction with the μ-opioid receptor. The EC_{50} of pCl-DPDPE-stimulated [^{35}S]GTPγS binding was also significantly shifted towards lower drug concentrations in −/− mouse brain membranes. EC_{50} values for the other drugs were not significantly different when comparing results from control and −/− membranes. The mean maximal responses elicited by DPDPE, pCl-DPDPE, SNC80 and DELT were 112, 130, 143 and 109% [^{35}S]GTPγS binding of basal, respectively, in brain membranes from −/− mice. The calculated E_{max} value of pCl-DPDPE-stimulated [^{35}S]GTPγS binding was significantly reduced in membranes prepared from −/− mice as compared with controls. No significant reduction in the E_{max} of DPDPE, SNC80 or DELT was observed when brain membranes from control and −/− mice were compared.

In previous studies we observed that morphine pretreatment caused cross-tolerance to the antinociceptive activity of i.th. DPDPE; i.c.v. DPDPE-mediated analgesia was not affected (Porreca et al., 1987). As these findings suggest differences in the mechanism of DPDPE-mediated antinociception at spinal and supraspinal sites, we examined SNC80- and DPDPE-stimulated [^{35}S]GTPγS binding to spinal cord membranes prepared from control and −/− mice (Fig. 2, Table 2). Both DPDPE and SNC80 stimulated [^{35}S]GTPγS binding in membranes prepared from control spinal cord with E_{max} values of 121 and 125% basal binding, respectively. The E_{max} of SNC80 in −/− membranes was not significantly different than controls (Table 2). Conversely, DPDPE stimulated such low levels of [^{35}S]GTPγS binding in −/− spinal cord membranes that nonlinear regression analysis of individual experiments failed to converge a sigmoidal dose–response curve. Hence a statistical comparison of the pharmacodynamic parameters describing DPDPE-stimulated G protein activation in control and −/− spinal cord membranes was not possible.

SNC80 stimulated [^{35}S]GTPγS binding in control spinal cord membranes with lower E_{max} values as compared to control brain membranes (Tables 1 and 2). These findings are consistent with the lower expression levels of δ-opioid receptors in the spinal cord as compared to the brain (Shah et al., 1994). In contrast, the E_{max} values of DPDPE-stimulated [^{35}S]GTPγS binding in membranes from control brain and control spinal cord membranes were not statistically different by t test at the $P < 0.05$ level. In control studies, DAMGO (10 μM)-stimulated [^{35}S]GTPγS binding was 186% of basal in control spinal cord membranes; binding was eliminated in −/− membranes, confirming the loss of μ-opioid receptors in the spinal cord. These findings are consistent with a previous report showing a loss of μ-opioid receptor immunoreactivity in the spinal cord of this −/− mouse strain (Sora et al., 1997b).

Intracerebroventricular administration of morphine (30 nmol) produced 100% antinociception in control, but only 11% antinociception in −/− mice, consistent with the loss of functional μ receptors in these animals (Fig. 3A). Higher i.c.v. doses of morphine could not be tested due to the consistent production of seizures in the −/−, but not the control animals. Similarly, i.th. morphine (30 nmol) produced 89% antinociception in control mice but no significant antinociception in −/− mice (Fig. 3B). Intracerebroventricular administration of DELT produced dose-related antinociception in both groups that did not differ significantly in potency. The A_{50} values were 15 (8.3–26.7) and 4 (0.9–17.9) nmol in control and −/− animals, respectively; these values were not significantly different (Fig. 3C). Similarly, i.th. administration of DELT produced a robust antinociceptive response in both the control and −/− mice. I.th. DELT-mediated antinocicep-

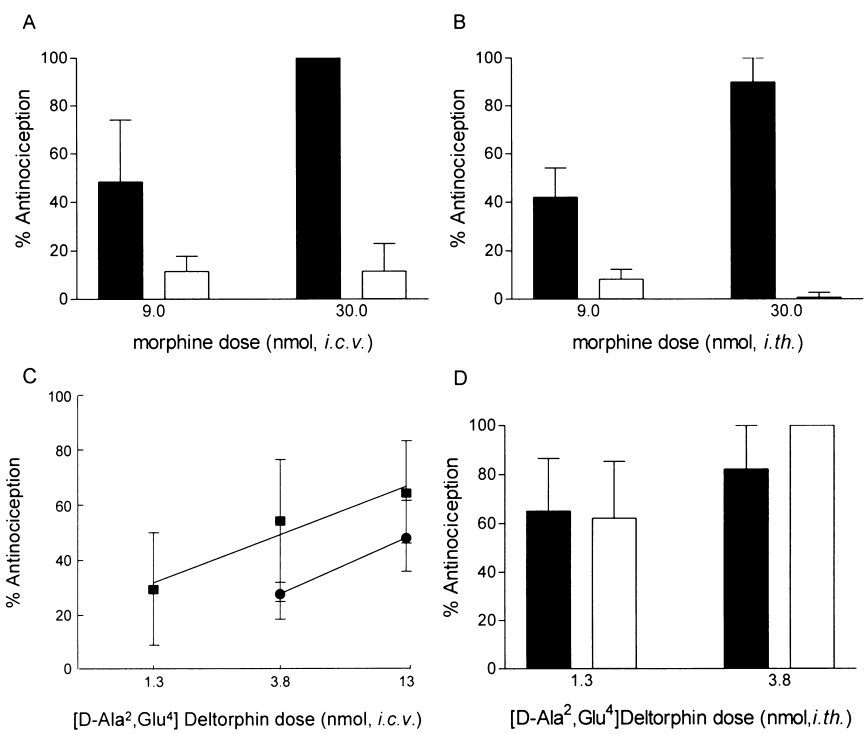

Fig. 3. Morphine given i.c.v. (A) or i.th. (B) produced antinociception in the 55°C hot water tail flick test in control mice (filled bar). Morphine was without effect after injection into −/− mice (open bar). The i.c.v. (C) injection of DELT produced antinociception in the 55°C hot water tail flick test in both control (●) and −/− (■) mice. The antinociceptive potency of DELT in control mice was not significantly different from that of DELT in −/− mice after i.c.v. administration. (D) No significant differences in i.th. DELT-mediated antinociception were observed comparing control (filled bar) or −/− (open bar) animals at either concentration tested. Data represent mean ± S.E.M. as % antinociception. $N = 4$ to 7 mice per dosage group.

tion did not differ significantly at either concentration of DELT tested (1.3 or 3.8 nmol, Fig. 3D). DELT (i.th., 1.3 nmol) produced 65 and 62% antinociception in control and −/− mice, respectively, whereas DELT (i.th., 3.8 nmol) produced 82 and 100% antinociception, respectively. Baseline tail flick latencies were not altered in the −/− as compared to control mice (data not shown).

In contrast, while i.c.v. administration of DPDPE produced a robust antinociceptive response in both control and −/− groups (Fig. 4A), the potency of this compound was significantly reduced in the −/− animals; the A_{50} values were 11 (6.6–17.2) and 42 (31.3–56.5) nmol, respectively, indicating a significant ($P < 0.05$) 4-fold potency decrease when comparing the control and −/− groups. I.th. administration of DPDPE also showed antinociceptive response in both groups but significantly decreased potency in the −/− mice (Fig. 4B). The i.th. A_{50} values for DPDPE were 4 (1.8–8.7) and 38 (27.9–51.1) nmol in control and −/− mice, respectively, indicating a significant ($P < 0.05$) 9-fold potency decrease in the −/− group.

Systemic administration of SNC80 (60 mg/kg, i.p.) showed an antinociceptive response which did not differ significantly between control and −/− groups; this dose of SNC80 produced responses of 85 ± 9.5 and 100 ± 0% maximal antinociception, respectively, as measured by the 55°C tail flick assay in control and −/− mice (data not shown).

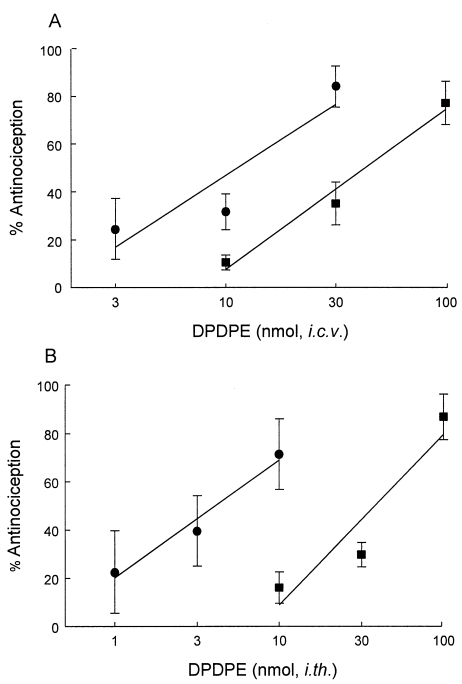

Fig. 4. The i.c.v. (A) or i.th. (B) injection of DPDPE produced dose-dependent antinociception in the 55°C hot water tail flick test in both control (●) and −/− (■) mice. The antinociceptive potency of DPDPE in control mice was significantly ($p < 0.05$) greater than that of DPDPE in −/− mice after either route of administration. Data represent mean ± S.E.M. as % antinociception. $N = 4$ to 7 mice per dosage group.

4. Discussion

Recent reports using μ-opioid receptor knockout mice have suggested that δ-opioid receptor-mediated antinociception is dependent upon the μ receptor (Matthes et al., 1996 and 1998; Sora et al., 1997a). Such findings were unexpected in light of an extensive literature supporting antinociception mediated by the δ-opioid receptor. We thus compared δ-opioid receptor-mediated antinociception and G protein activation in control and −/− mice using several δ-selective agonists. The objectives of this investigation were twofold: (1) to determine whether δ-opioid receptor function was altered in the μ-opioid receptor knockout mice and (2) to determine if the knockout had differential effects on antinociception and G protein activation mediated by structurally distinct δ-selective agonists.

A critical control in this study was to verify that μ-opioid receptor function was absent in the −/− animals. The μ-selective agonist DAMGO did not stimulate G protein activity (Fig. 1A) and morphine demonstrated nonsignificant antinociceptive effect (Fig. 3A and 3B) in the −/− mice thus validating the functional knockout of the μ-opioid receptor. We compared SNC80- and DELT-stimulated G protein activity in whole brain membranes prepared from control and −/− mice. G protein activation mediated by these drugs was unaltered in the −/− membranes as compared to controls (Fig. 1D and 1E, Table 1). DELT was a partial agonist as compared to SNC80, consistent with the findings of other investigators (Clark et al., 1997). As previous studies have indicated that the μ-opioid receptor may influence antinociception mediated by i.th. administered δ-selective agonists (Porreca et al., 1987), we examined G protein activation by SNC80 in spinal cord membranes (Fig. 2A, Table 2). There were no significant differences in the E_{max} and EC_{50} of the SNC80 response comparing control and −/− spinal cord membranes. Taken together, these findings demonstrate that the functional coupling of the δ-opioid receptor to G proteins is unaltered in the knockout animals.

In an earlier report it was observed that DPDPE did not mediate antinociception in the μ-opioid receptor knockout mice used in these studies (Sora et al., 1997a). As δ-selective drugs stimulate G protein activation in these mice (Fig. 1 and Table 1), we reasoned that some compensatory change may have occurred during the knockout of the opioid μ receptor that blocks δ-mediated antinociception at a step distal to G protein activation. We thus examined DELT-mediated antinociception (i.c.v. and i.th.) in control and −/− mice (Fig. 3). DELT stimulated antinociception in both control and −/− mice. There were no statistically significant changes in DELT-mediated antinociception after i.c.v. or i.th. injection when comparing control

and −/− mice. Intraperitoneal injection of SNC80 also mediated maximal antinociception in both control and −/− mice. These data strongly argue that δ-opioid selective pathways leading to antinociception are not significantly altered in the −/− mice.

In contrast to the results described above, pCl-DPDPE-stimulated G protein activity as determined by the E_{max} was reduced in −/− mouse brain membranes as compared to controls (Fig. 1, Table 1); DPDPE did not stimulate sufficient G protein activation for statistical analysis in the −/− spinal cord membranes (Table 2). Considering (1) the low potency of DPDPE in the control spinal cord membranes (8 ± 12 μM), (2) the poor stimulation of G proteins by DPDPE in −/− spinal cord membranes and (3) that pCl-DPDPE-stimulated G protein activation in control and −/− brain membranes deviates only at the highest drug concentrations (> 1 μM) tested, these results are consistent with the interpretation that reduced G protein stimulation by DPDPE and pCl-DPDPE in −/− membranes is due to the loss of a low affinity interaction of these drugs with the μ-opioid receptor. Consideration of these data together with the decrease in potency of DPDPE-induced antinociception after i.c.v or i.th. injection in the −/− mice (Fig. 4) suggest the possibility that DPDPE has a component of its antinociceptive activity mediated via μ-opioid receptors. An alternative possibility is that occupation of μ-opioid receptors may result in a modulation of DPDPE-stimulated antinociception. If we assume that: (1) DPDPE interacts at the μ-opioid receptor with low affinity and (2) the DPDPE antinociception concentration response curves in −/− mice reflect drug interaction primarily with the δ-opioid receptor, then the contribution of DPDPE-bound μ-opioid receptor to antinociception must occur at low receptor occupancy in the control mouse. Similar results have been previously reported involving opioid receptors. Vaught et al. (1982) reported that [Leu^5]enkephalin and [D-Ala^2, D-Leu^5]enkephalin increased the antinociceptive potency of morphine. We later reported that sub-analgesic concentrations of DPDPE increased the efficacy and potency of morphine-mediated antinociception (Jiang et al., 1990). Such data demonstrate that minimal activation of a receptor capable of mediating antinociception can potentiate antinociception mediated through other opioid receptors (Welch and Stevens, 1992).

In summary, our data show that δ-opioid receptors are functionally coupled to G protein activation and antinociception in μ-opioid receptor knockout mice. Our data, along with earlier reports, suggest that a component of the antinociceptive potency of DPDPE may be mediated through μ-opioid receptors. Alternatively, the greater antinociceptive potency of DPDPE in the control vs. the −/− mice may be due to a synergistic effect between the δ-opioid receptor and a minimally activated μ-opioid receptor. However, these findings with DPDPE do not detract from the fact that DELT mediated full antinociception efficacy and potency at both supraspinal and spinal sites in control and in −/− mice. Such findings provide evidence that a functional μ-opioid receptor is not required for δ-opioid receptor-mediated antinociception or stimulation of intracellular second messenger pathways.

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