Functional Effects of Systemically Administered Agonists and Antagonists of \(\mu\), \(\delta\), and \(\kappa\) Opioid Receptor Subtypes on Body Temperature in Mice

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ABSTRACT
We have investigated the roles of peripheral and central \(\mu\), \(\delta\), and \(\kappa\) opioid receptors and their subtypes in opioid-induced hypothermia in mice. Measuring rectal temperature after i.p. injection, opioid agonists [morphine, fentanyl, SNC80 \((\pm)-4-((\alpha R)-\alpha-(2S,5R)-4-allyl-2,5-dimethyl-1-piperaziny1)3-methoxybenzyl]-N,N-diethylbenzamide], U50,488H \([(trans)-(3,4-dichloro-N-methyl-N-[2-(1-pyrroloidinyl)cyclohexyl]-benzeneacetamide)], and loperamide] were tested alone or with opioid antagonists [naloxone, \(\beta\)-funaltrexamine, naloxonazine, naltirindole, 7-benzylidenenaltrexone (BNTX), naltriben, nor-binaltorphimine, 2-(3,4-dichlorophenyl)-N-methyl-N-[(1S)-1-(3-isothiocyanatophenyl)2-(1-pyrrolidinyl)ethyl]acetamide (DIPPA), and methyl-naltrexone] given 15 min after the agonist. All agonists produced dose-related hypothermia, although at low doses, morphine and U50,488H produced hyperthermia. The effects of morphine and fentanyl were antagonized by naloxone and by the \(\mu_1\) antagonist naloxonazine. The \(\delta_2\) antagonist naltriben potentiated the hypothermic effect of \(\mu\) agonists. SNC80-induced hypothermia was blocked by the \(\delta\) antagonist naltirindole but not by the \(\delta_1\) antagonist BNTX. Depending on the dose, the \(\delta_2\) antagonist naltriben produced either a potentiation or an attenuation of the effect of SNC80. U50,488H-induced hypothermia was antagonized by the \(\kappa\) antagonist nor-binaltorphimine but not by acute treatment with the irreversible \(\kappa\) antagonist DIPPA. The peripherally acting opioid loperamide produced hypothermia that could be blocked by several \(\mu_1\), \(\delta\), or \(\kappa\)-selective antagonists as well as by the peripherally acting antagonist methyl-naltrexone. Methyl-naltrexone produced a weak potentiation of morphine-, fentanyl-, and U50,488H-induced hypothermia, whereas a significant attenuation of SNC80-induced hypothermia was observed. In conclusion, at high doses, morphine- and fentanyl-induced hypothermia may involve composite action on \(\mu\), \(\kappa\), and possibly \(\delta\) opioid receptors after initial activation. In the mediation of \(\delta\) opioid-induced hypothermia, no clear selectivity between the \(\delta_1\) and \(\delta_2\) subtypes was defined. The studies provide new evidence that maintenance of the initial effects of agonist/receptor activation vary with the agonist and the receptor. The existence of both central and peripheral components of opioid-induced hypothermia is also emphasized.

It has long been recognized that opioids such as morphine can produce a range of effects on body temperature in a number of species including man (Su et al., 1987; Adler et al., 1988; Wheelahan et al., 1998). The available data indicate that the species and strain, the ambient temperature, the level of restraint, the dose, and the specific opioid used may greatly affect thermoregulatory responses to opioids. In particular, the distinct roles of the \(\mu\), \(\delta\), and \(\kappa\) receptor types may be important in understanding the mechanisms of opioid-induced effects on body temperature, and these receptors may also play specific roles in thermoregulation (Spencer et al., 1988, 1990; Handler et al., 1992, 1994; Wilson and Howard, 1996).

Opioid receptor subtypes have been suggested on the basis of binding and pharmacological studies, although their relevance to the field of thermoregulation has not been fully evaluated. The existence of \(\mu_1\) and \(\mu_2\) receptor subtypes was proposed to explain the presence of a high- and a low-affinity \(\mu\) receptor site using the \(\mu_1\) antagonist naloxonazine (Pasternak et al., 1980; Wolozin and Pasternak, 1981). The subdivision of the \(\delta\) opioid receptor was proposed after several observations including differential antagonism of the effects of \(\delta\) agonists by BNTX and naltriben (Sofuoglu et al., 1991, 1993), which are \(\delta_1\) and \(\delta_2\) antagonists, respectively. Subdivision of the \(\kappa\) receptor is not well supported by in vivo evidence due to the lack of subtype-selective antagonists.

The effects on body temperature of morphine and other narcotic analgesics, which act primarily on the \(\mu\) opioid receptor, are biphasic in rats and mice, with low doses produc-
ing hyperthermia and higher doses resulting in hypothermia at thermoneutral ambient temperatures (Rosow et al., 1980; Geller et al., 1983). In both of these species, however, higher and lower environmental temperature can profoundly affect body temperature responses to morphine and similar opioids (Rosow et al., 1980; Handler et al., 1994). At cool ambient temperatures, dose-related hypothermia was measured, whereas warm ambient temperatures resulted in dose-related hyperthermia. This has been demonstrated using partially restrained mice at ambient temperatures of 20, 25, or 30°C following subcutaneous injection of several narcotic analgesics from different chemical classes (Rosow et al., 1980). Comparable findings were obtained in rats at ambient temperatures of 5, 24, or 32°C following intraperitoneal or intracerebral morphine (Paolino and Bertrand, 1968). In addition, i.c.v. administration of the μ-selective agonist PL-017 in rats resulted in hypothermia at an ambient temperature of 5°C but hyperthermia at 30°C (Handler et al., 1994). These changes are due to alterations in oxygen consumption and heat loss (Adler et al., 1988). Morphine-induced hyperthermia and hypothermia in mice can be antagonized by naloxone, which provides further evidence for the involvement of opioid receptors (Rosow et al., 1982).

Changes in rodent body temperatures induced by δ opioid agonists have been investigated mainly through the use of peptide agonists. Administration of i.c.v. DPDPDE to rats resulted in marked hypothermia followed by a rebound hyperthermic effect at high doses (Spencer et al., 1988). In a similar study, a hypothermic effect was produced at an ambient temperature of 5°C whereas no effect was measured at 30°C (Handler et al., 1994). In addition, ambient temperatures of 4, 22, and 34°C were shown to affect responses to i.c.v. [d-Ala²]deltorphin II such that hypothermic potency was increased by lowering ambient temperature (Broccardo and Imparuta, 1992). These responses were also significantly reduced by naltrindole. The recently introduced nonpeptide δ agonist SNC80 has been shown to produce hypothermia in rats after intraperitoneal administration (Pohorecky et al., 1999).

Many authors have reported hypothermia in rodents after systemic or central administration of κ agonists, especially the selective nonpeptide agent U50,488H (Geller et al., 1983, 1986; Spencer et al., 1990; Handler et al., 1994). In addition, central administration of U50,488H in rats produced hypothermia at an environmental temperature of 20°C but no effect at 29°C (Mandenoff et al., 1991). Rebound hyperthermia following initial hypothermia has been reported after high i.c.v. doses of U50,488H in rats (Spencer et al., 1988). The hypothermic effect of a more potent κ agonist TRK-820, was shown to be blocked by the selective κ antagonist nor-binaltorphimine but not by naloxone or naltrindole (Endoh et al., 1999), indicating the presence of a specific κ-mediated mechanism.

Pretreatment with specific antagonists has been used as a method for providing evidence for the role of given receptors in mediating an effect. In contrast to this, administering antagonists after agonists can determine the importance of maintaining the initial receptor activation as well as uncover changes in the role of receptor systems during the progression of the event. Such information about the role of opioid receptors in body temperature could help in understanding the dynamics of opioid-induced hypothermia and prove useful in using body temperature as a means of characterizing opioids. Therefore, the ability of different opioid antagonists to alter hypothermia in opioid-pretreated mice, with respect to the μ, δ and κ receptors and their subtypes, was compared in the present study. To do so, antagonists were administered when hypothermia had started to develop, after a fixed duration following opioid agonist pretreatment. Thus, evidence was obtained about the importance of the agonist/receptor activity in maintaining the initial role of that receptor. Morphine and fentanyl were selected as classic narcotic analgesics with strong μ opioid activity, SNC80 as a selective δ agonist, and U50,488H as a selective κ agonist (Corbett et al., 1999). A range of antagonists with different receptor-selectivity profiles were used: naloxone (μ), β-funtalreminaxine (irreversible μ), naloaxonazine (μ₁), naltrindole (δ), BNTX (δ₁), naltiben (δ₂), nor-binaltorphimine, (κ), and DIPPA (irreversible κ) (Corbett et al., 1999). To evaluate the peripherally mediated effect of opioids, we also selected the opioid agonist loperamide and the opioid antagonist methyl-naltrexone, since these agents penetrate poorly into the central nervous system (Van Nueten et al., 1979; Yuan and Foss, 1999). We selected a controlled laboratory ambient temperature of 22°C and used unrestrained animals to avoid stress-related effects that may influence thermoregulation (del Rio-Garcia et al., 1985; Adler et al., 1988) as well as after endogenous opioid systems (Vaswani et al., 1988; Pohorecky et al., 1999).

Materials and Methods

Animals

Approval from the Institutional Animal Care and Use Committee was obtained prior to performing the described experiments. Male NMRI mice weighing 30 to 40 g were used. Mice were housed for 2 weeks in colonial stock cages following arrival from Inn Credo (L’Arbresle, France). Animals were transferred to individual housing on the day prior to testing to allow overnight habituation. Food and water were available ad libitum at all times. The environmental temperature was controlled (22 ± 1°C), and the laboratory was maintained on a 12-h light/dark cycle (AM/PM). All experiments were carried out during the light phase.

Temperature Test Method

All temperatures were measured using a Comark C9001 thermometer (Comark, Sheffield, UK) and probe (length, 2.5 cm; diameter, 1 mm). Mice were unrestrained; they were individually removed from their housing for each temperature measurement and returned immediately afterward. Animals were held only by index finger and thumb at the base of the tail with all paws resting on the workbench. This allowed insertion of the probe without risk of tissue damage and without obvious distress. The probe was manually inserted 2.5 cm into the rectum and allowed to equilibrate for 5 s.

Procedure

Experiment 1: Effects of Agonists. Different doses of morphine, fentanyl, SNC80, U50,488H, or loperamide were selected from the metric series: 0.16, 0.63, 2.5, 10, and 40 mg/kg (saline control group, n = 20; opioid test groups, n = 10 per dose). Intraperitoneal injection of agonists immediately followed a preliminary temperature measurement. Rectal body temperatures were then measured every 30 min until 150 min after agonist treatment.

Experiment 2: Interactions between Agonists and Antagonists. A single fixed dose of each agonist was selected from experiment 1, based on the presence of a strong hypothermic effect and reversal to baseline over time. The selected doses were 40 mg/kg morphine, 2.5 mg/kg fentanyl, 40 mg/kg SNC80, 40 mg/kg U50,488H,
and 2.5 mg/kg loperamide. Fixed doses of the antagonists (0.63, 2.5, and 10 mg/kg) were tested against each agonist (saline control group, n = 40; agonist controls, n = 20 per dose; agonist versus antagonist, n = 10 per dose). To do so, 15 min prior to injection of the antagonist, preliminary body temperatures were measured, followed immediately by agonist pretreatment. Successive temperatures were then measured every 15 min until 45 min after antagonist treatment, which is 60 min after the agonist treatment. The effects on rectal temperature of the above selected doses of antagonists were assessed by taking a preliminary temperature measurement followed by injecting the test dose and then measuring every 15 min for 60 min (n = 10 per group).

Drugs

Morphine-HCl was purchased from Belgopvia SA (Louvain-La-Neuve, Belgium). Fentanyl-HCl and loperamide-HCl were obtained from Janssen Pharmaceutica (Beerse, Belgium). U50,488-HCl (trans-)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneaceticamide], naloxone-HCl, naloxonazine-2HCl, naltrindole-HCl, naltriben mesylate, nor-binaltorphimine-2HCl, methyl-naltrexone-2HCl, SNC80, DIPPA, and BNTX were purchased from Tocris Cookson Ltd. (Bristol, UK). All drugs were freshly prepared as aqueous solutions prior to experimentation. Drugs were administered intraperitoneally in 10 ml/kg volumes per injection. All doses refer to base equivalents. Control animals received an equivalent volume of sterile physiological saline (0.9% NaCl; Baxter, McGaw Park, IL). All injections were given intraperitoneally.

Data Analysis

All data values are expressed as mean ± S.E.M. Differences with regard to pre- and postinjection measurements were evaluated using a Wilcoxon signed rank test (two-tailed). Differences between experimental conditions were evaluated using a Mann-Whitney U test (Siegel, 1956). Asterisks indicate statistical significance: *P < 0.05; **P < 0.1; ***P < 0.001.

Based on the large number of statistical tests carried out between agonist/antagonist groups and agonist/vehicle groups, criteria were set for determination of the presence of interactions (Table 1).

Results

Experiment 1: Effects of Agonists (Fig. 1). Morphine, fentanyl, SNC80, U50,488H, and loperamide all produced changes in rectal body temperature compared with saline (Fig. 1). The mean preinjection body temperature of the saline control group (n = 20) was 37.32 ± 0.11°C. Subsequent measurements until 120 min after the saline injection were greater than the preliminary value, with the highest mean body temperature of 37.63 ± 0.12°C occurring 90 min after injection (**, P = 0.0054).

Morphine and U50,488H produced dual responses with low doses resulting in hyperthermia and higher doses resulting in hypothermia. Lasting hyperthermia, reaching a maximum of 0.86 ± 0.18°C was measured 150 min after 2.5 mg/kg morphine. Higher doses of morphine produced hypothermia, which returned to baseline, with a maximal effect of −2.21 ± 0.57°C measured 60 min after the 40 mg/kg dose. At a dose of 10 mg/kg, U50,488H produced a maximal hyperthermic effect of 0.97 ± 0.14°C measured 30 min after injection, before returning to baseline after 90 min. U50,488H produced a marked decrease in body temperature of −2.41 ± 0.42°C that occurred 30 min after the 40 mg/kg dose, returning to baseline between 60 and 90 min followed by a continued rise to a maximum of 1.02 ± 0.17°C after 120 min.

Fentanyl, SNC80, and loperamide all produced dose-dependent hypothermia. Fentanyl produced long-lasting hypothermia at the 2.5 mg/kg dose level, which reached a maximum of −2.39 ± 0.14°C at 30 min after injection. The hypothermic effect of 40 mg/kg SNC80 was also long lasting, with a maximal decrease of −4.13 ± 0.20°C at 30 min after injection. Loperamide at 2.5 mg/kg produced a drop in body temperature of −3.06 ± 0.36°C at 30 min after injection, which returned to baseline after 90 min.

Morphine (from 2.5 mg/kg) and fentanyl (from 0.16 mg/kg) also produced μ opioid behavioral changes including arched back, Straub-tail, and motoric excitation. These effects were not observed with loperamide at the doses tested, which instead produced an observed decrease in the activity of the animals from 2.5 mg/kg upward. SNC80 and U50,488H produced an observed decrease in the activity of the animals only at the 40 mg/kg dose level.

### Table 1

Summary of agonist/antagonist interactions

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Morphine</th>
<th>Fentanyl</th>
<th>SNC80</th>
<th>U50,488H</th>
<th>Loperamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naloxone</td>
<td>AAA</td>
<td>AAA</td>
<td>AA</td>
<td>–</td>
<td>AAA</td>
</tr>
<tr>
<td>β-Funaltrexamine</td>
<td>–</td>
<td>–</td>
<td>AA</td>
<td>–</td>
<td>AAA</td>
</tr>
<tr>
<td>Naloxonazine</td>
<td>AAA</td>
<td>A</td>
<td>–</td>
<td>–</td>
<td>AAA</td>
</tr>
<tr>
<td>Naltrindole</td>
<td>P</td>
<td>–</td>
<td>AAA</td>
<td>–</td>
<td>AAA</td>
</tr>
<tr>
<td>BNTX</td>
<td>A</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>AAA</td>
</tr>
<tr>
<td>Naltrexine</td>
<td>PPP</td>
<td>PPP</td>
<td>AA/P</td>
<td>–</td>
<td>AAA</td>
</tr>
<tr>
<td>Nor-binaltorphimine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>AAA</td>
</tr>
<tr>
<td>DIPPA</td>
<td>A</td>
<td>–</td>
<td>–</td>
<td>PPP</td>
<td>–</td>
</tr>
<tr>
<td>Methyl-naltrexone</td>
<td>P</td>
<td>P</td>
<td>AAA</td>
<td>P</td>
<td>AAA</td>
</tr>
</tbody>
</table>

The symbols indicate that antagonist co-administration produced the following changes in agonist effects: A, weak antagonism; AA, antagonism; AAA, marked antagonism; P, weak potentiation; PP, potentiation; PPP, marked potentiation; –, no effect.

† Naltrexin produced an antagonism at 0.63 mg/kg and a weak potentiation at 10 mg/kg.

‡ No effect except for in the 2.5 mg/kg naltrindole group, where U50,488H pretreatment caused a smaller hypothermia than controls.
Experiment 2: Interactions between Agonists and Antagonists (Figs. 2–7; Table 1). In the saline/saline group \((n = 40)\), the preinjection rectal temperature was \(37.39 \pm 0.10^\circ C\), rising to \(37.88 \pm 0.14^\circ C\) after 15 min following the first saline injection (**\(P < 0.0012\)). Rectal temperatures were found to increase steadily over time, reaching a maximum of \(38.20 \pm 0.10^\circ C\) measured 45 min after the second injection.

Pretreatment of the different agonists \((n = 20)\) 15 min prior to a saline injection produced hypothermic effects lasting over the duration of the experiment that were comparable to those measured in experiment 1. All agonists produced their maximal decreases in body temperature at 30 min after treatment: 40 mg/kg morphine \((-3.66 \pm 0.25^\circ C)\), 2.5 mg/kg fentanyl \((-2.26 \pm 0.36^\circ C)\), 40 mg/kg SNC80 \((-4.98 \pm 0.20^\circ C)\), 40 mg/kg U50,488H \((-3.08 \pm 0.37^\circ C)\), and 2.5 mg/kg loperamide \((-2.98 \pm 0.25^\circ C)\) (Figs. 3–7). The 15-min duration between the agonist pretreatment and the saline or antagonist injection allowed comparisons to be made between the effects of the pretreatment in the agonist/saline group against those in the agonist/antagonist groups. In only a single experiment was a significant difference found: U50,488H/saline \((-1.62 \pm 0.17^\circ C)\) was significantly different than the U50,488H/naltriben 2.5 mg/kg group \((-0.71 \pm 0.30^\circ C; P = 0.0145)\) (Fig. 6).

Of the antagonists tested for intrinsic effects on body temperature, β-funaltrexamine and naltriben produced small decreases in body temperature at the highest dose tested (10 mg/kg) (Fig. 2). Maximal effects of \(-0.18 \pm 0.23^\circ C\) and \(-0.26 \pm 0.17^\circ C\), respectively, were measured 60 min after injection. BNTX at a dose of 10 mg/kg produced a hyperthermia of \(1.38 \pm 0.16^\circ C\) measured 60 min after injection (Fig. 2). DIPPA also produced hyperthermia with a maximal effect of \(1.38 \pm 0.18^\circ C\) measured 15 min after injection of a 2.5 mg/kg dose (Fig. 2). Naloxone, naloxonazine, naltirindole, nor-binaltorphimine, and methyl-naltrexone produced no effects on body temperature at the doses tested.

Morphine-induced hypothermia was antagonized strongly between the effects of the pretreatment in the agonist/saline group against those in the agonist/antagonist groups. In only a single experiment was a significant difference found: U50,488H/saline \((-1.62 \pm 0.17^\circ C)\) was significantly different than the U50,488H/naltriben 2.5 mg/kg group \((-0.71 \pm 0.30^\circ C; P = 0.0145)\) (Fig. 6).
by doses of 0.63, 2.5, and 10 mg/kg of both naloxone and naloxonazine and weakly by 0.63 and 2.5 mg/kg DIPPA (Fig. 3). The strongest antagonism was observed with 2.5 mg/kg naloxone, which reduced the effect of morphine after 45 min to $-0.45 \pm 0.22^\circ C$ compared with $-3.22 \pm 0.24^\circ C$ for the morphine control group. A weak potentiation of morphine-induced hypothermia was measured 30 and 45 min after 10 mg/kg DIPPA, reaching a maximum at 45 min ($-5.19 \pm 0.66^\circ C$) (Fig. 3). Weak potentiation of morphine-induced hypothermia was measured 30 min after 0.63 mg/kg naltrindole ($-4.14 \pm 0.25^\circ C$) and 2.5 mg/kg methyl-naltrexone ($-4.23 \pm 0.27$) compared with $-3.38 \pm 0.24^\circ C$ for the morphine control group (Fig. 3).

Fentanyl-induced hypothermia was antagonized by 10 mg/kg naloxone as evidenced by significantly higher temperatures occurring 15, 30, and 45 min after naloxone compared with the fentanyl control group (Fig. 4). The effect of fentanyl was reduced to $0.35 \pm 0.33^\circ C$ measured 30 min after 10 mg/kg naloxone compared with $-1.89 \pm 0.39^\circ C$ for the fentanyl control group. In addition, a weak antagonism was observed 15 min after 10 mg/kg naloxonazine, reducing the fentanyl hypothermia to $-0.98 \pm 0.24^\circ C$ compared with $-2.26 \pm 0.36^\circ C$ for the fentanyl control group (Fig. 4). Potentiation of fentanyl-induced hypothermia was observed 30 and 45 min after 10 mg/kg naltriben in a manner similar to that seen with morphine-induced hypothermia (Figs. 3 and 4). This effect reached $-3.57 \pm 0.60^\circ C$ measured 30 min after 10 mg/kg naltriben. Fentanyl-induced hypothermia was also weakly potentiated 30 and 45 min after 0.63 mg/kg methyl-naltrexone with a reduction in body temperature to $-3.30 \pm 0.38^\circ C$ after 30 min (Fig. 4).

SNC80-induced hypothermia was dose-dependently antagonized by naltrindole. Significant antagonism was also observed with methyl-naltrexone. In addition, antagonism was measured after naloxone and $\beta$-funaltrexamine. Following 10 mg/kg naltriben, the effect of SNC80 was reduced at all of the time points measured, with the greatest differences from the SNC80 control group occurring after 15 and 30 min (Fig. 5). These values were $1.61 \pm 0.39$ and $-1.10 \pm 0.38^\circ C$, respectively, compared with $-4.98 \pm 0.2$ and $-4.42 \pm 0.32^\circ C$ for the SNC80 control group. Methyl-naltrexone reduced the effect of SNC80 measured 30 and 45 min after doses of 0.63, 2.5, and 10 mg/kg (Fig. 5). The greatest effect of methyl-naltrexone was measured 45 min after the 10 mg/kg dose, which reduced the decrease in rectal temperature to $-1.62 \pm 0.32^\circ C$ compared with the SNC80 control group value of $-3.41 \pm 0.36^\circ C$. After 15 min following 10 mg/kg naloxone, SNC80-induced hypothermia was reduced to $-3.82 \pm 0.32^\circ C$ (Fig. 5). A similar reduction to $-3.56 \pm 0.27^\circ C$ was measured 15 min after 0.63 mg/kg $\beta$-funaltrexamine (Fig. 5). Mixed effects on SNC80-induced hypothermia occurred in response to naltriben, with antagonism occurring 15 and 30 min following 2.5 mg/kg naltriben and a potentiation occurring 45 min after the 10 mg/kg dose (Fig. 5).

Only nor-binaltorphimine produced a clear antagonism of U50,488H-induced hypothermia (Fig. 6). The greatest reduction was observed with 10 mg/kg nor-binaltorphimine, causing reductions to $-0.14 \pm 0.22$ and $0.23 \pm 0.22^\circ C$ after 30 and 45 min, respectively, compared with $-2.12 \pm 0.26$ and $-1.32 \pm 0.24^\circ C$ for the U50,488H control group. Rectal temperatures 15, 30, and 45 min following 2.5 mg/kg naltriben were also significantly higher than the U50,488H control group, although as mentioned above, pretreatment of U50,488H in this test group produced a smaller hypothermia than the control group (Fig. 6). Potentiation of U50,488H-induced hypothermia was observed 45 min following 0.63 (to $-2.44 \pm 0.37^\circ C$) and 10 mg/kg (to $-4.17 \pm 0.88^\circ C$) DIPPA (Fig. 6). Methyl-naltrexone at a dose of 10 mg/kg produced...
Fig. 3. Effects of different opioid antagonists on morphine-induced hypothermia. Given are the mean ± S.E.M. changes in rectal body temperature. Fifteen minutes prior to injection of the antagonist, preliminary body temperatures were measured, animals were pre-treated with morphine (40 mg/kg i.p.), and a baseline control group received saline (○), n = 40. At 0 min, the morphine-pretreated animals received an antagonist [0.63 (△), 2.5 (∇), or 10 (■) mg/kg i.p, n = 10 for each group], whereas the morphine control group (●), n = 20 and the baseline control group received saline. Differences between morphine/antagonist groups and the morphine control group at the various time points were evaluated using a Mann-Whitney U test (two-tailed). The asterisks indicate statistical significance (*, P < 0.05; **, P < 0.01; ***, P < 0.001).

Fig. 4. Effects of different opioid antagonists on fentanyl-induced hypothermia. Given are the mean ± S.E.M. changes in rectal body temperature. Fifteen minutes prior to injection of the antagonist, preliminary body temperatures were measured, animals were pre-treated with fentanyl (2.5 mg/kg i.p.), and a baseline control group received saline (○), n = 40. At 0 min, the fentanyl-pretreated animals received an antagonist [0.63 (△), 2.5 (∇), or 10 (■) mg/kg i.p, n = 10 for each group], whereas the fentanyl control group (●), n = 20 and the baseline control group received saline. Differences between fentanyl/antagonist groups and the fentanyl control group at the various time points were evaluated using a Mann-Whitney U test (two-tailed). The asterisks indicate statistical significance (*, P < 0.05; **, P < 0.01; ***, P < 0.001).
weak potentiation (to $-2.75 \pm 0.36^\circ$C) of the hypothermic effect of U50,488H after 45 min (Fig. 6).

After 45 min, naloxone (10 mg/kg), naltrindole (10 mg/kg), BNTX (2.5 mg/kg), and nor-binaltorphimine (2.5 mg/kg) reduced the hypothermic effect of loperamide ($-1.73 \pm 0.24^\circ$C) to above 0°C, representing a marked antagonism (Fig. 7). Similarly, $\beta$-funaltrexamine (10 mg/kg), naloxonazine (2.5 and 10 mg/kg) and methyl-naltrexone (0.63, 2.5 and 10 mg/kg) reduced loperamide-induced hypothermia to within $-0.35^\circ$C after 45 min (Fig. 7). The effect of naltridene was somewhat weaker with the greatest antagonism to $-0.72 \pm 0.40^\circ$C measured 45 min after a 10 mg/kg dose (Fig. 7). Of the nine antagonists tested, only DIPPA produced no effect on loperamide-induced hypothermia (Fig. 7).

**Discussion**

Decreases in rectal body temperature in mice were brought about by systemic administration of several different opioid agonists that act on $\mu$, $\delta$ or $\kappa$ opioid receptors. Morphine and U50,488H were also shown here to produce hyperthermia at low doses. The effects shown here with morphine, fentanyl, SNC80, and U50,488H are comparable to those reported in the current literature (see Introduction).

However, certain observations with single compounds warrant further discussion. First, hyperthermia can be expected from $\mu$ agonists although no hyperthermia was observed with fentanyl, which is far more selective for $\mu$ receptors than morphine (Maguire et al., 1992). At a dose of 0.16 mg/kg fentanyl, no effects on body temperature were seen, although this dose readily produces marked and lasting antinociception in mice (Niemegeers et al., 1976). It is possible that 0.16 mg/kg fentanyl represents the balance point of a dual dose-response curve mediated by different opioid receptor types, as previously demonstrated for morphine (Chen et al., 1996). In addition, the dose of fentanyl required to produce hypothermia was relatively high at 0.63 mg/kg, indicating that $\delta$ and $\kappa$ receptors could have been activated.

With regard to the role of the $\kappa$ receptor, hyperthermia resulted 30 min after 10 mg/kg U50,488H, and at 40 mg/kg, hypothermia followed by rebound hyperthermia was seen. Similarly, rebound hyperthermia was measured following an initial hypothermia after i.c.v. administration of U50,488H in unrestrained rats (Spencer et al., 1988). Hyperthermia directly following administration of a $\kappa$ agonist has not been reported previously. However, since temperatures were determined every 30 min in the characterization of the dose-response curve, the possibility that an initial short-lasting hypothermia was missed cannot be ruled out.

In addition to the findings with centrally penetrating agents, we have also found that loperamide produced a dose-dependent decrease in rectal body temperature in mice. Since loperamide does not cross the blood-brain barrier (Van Nueten et al., 1979), this finding indicates that opioid receptors in the periphery can be stimulated to produce hyperthermia in mice. The opioid antagonist methyl-naltrexone does not cross the blood-brain barrier (Van Nueten et al., 1979), this finding indicates that opioid receptors in the periphery can be stimulated to produce hyperthermia in mice. The opioid receptor, hyperthermia followed by rebound hyperthermia was seen. Similarly, rebound hyperthermia was measured following an initial hypothermia after i.c.v. administration of U50,488H in unrestrained rats (Spencer et al., 1988). Hyperthermia directly following administration of a $\kappa$ agonist has not been reported previously. However, since temperatures were determined every 30 min in the characterization of the dose-response curve, the possibility that an initial short-lasting hypothermia was missed cannot be ruled out.

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Effects of different opioid antagonists on U50,488H-induced hypothermia. Given are the mean ± S.E.M. changes in rectal body temperature. Fifteen minutes prior to injection of the antagonist, preliminary body temperatures were measured, animals were pretreated with U50,488H (40 mg/kg i.p.), and a baseline control group received saline (○), n = 40. At 0 min, the U50,488H-pretreated animals received an antagonist [0.63 (▲), 2.5 (▼), or 10 (■) mg/kg i.p, n = 10 for each group], whereas the U50,488H control group (◆), n = 20 and the baseline control group received saline. Differences between U50,488H/antagonist groups and the U50,488H control group at the various time points were evaluated using a Mann-Whitney U test (two-tailed). The asterisks indicate statistical significance (*, P < 0.05; **, P < 0.01; ***, P < 0.001).
Fig. 7. Effects of different opioid antagonists on loperamide-induced hypothermia. Given are the mean ± S.E.M. changes in rectal body temperature. Fifteen minutes prior to injection of the antagonist, preliminary body temperatures were measured, animals were pretreated with loperamide (2.5 mg/kg i.p.), and a baseline control group received saline (○), n = 40. At 0 min, the loperamide-pretreated animals received an antagonist (0.63 (▲), 2.5 (●), or 10 (■) mg/kg i.p, n = 10 for each group), whereas the loperamide control group (○), n = 20 and the baseline control group received saline. Differences between loperamide/antagonist groups and the loperamide control group at the various time points were evaluated using a Mann-Whitney U test (two-tailed). The asterisks indicate statistical significance (*, P < 0.05; **, P < 0.01; *** P < 0.001).
an opioid agonist only in the periphery, centrally penetrating agents bind to opioid receptors at both the peripheral and central levels, thus activating both mechanisms. Furthermore, it is possible that the hypothermic effects of peripheral opioid receptor activation may not simply be additive to the effects of central activation if both occur together, as evidenced by the weak potentiation of morphine-, fentanyl-, and U50,488H-induced hypothermia caused by methyl-naltrexone. For the opioids mentioned, central mechanisms may predominate. By contrast, hypothermia induced by the δ agonist SNC80, was significantly reduced by methyl-naltrexone, indicating a significant peripherally mediated mechanism of action for systemic δ opioids.

In the preoptic anterior hypothalamus, there is convincing evidence that μ receptors mediate hyperthermia, whereas κ receptors mediate hypothermia in unrestrained rats (Xin et al., 1997). In addition, antisense oligonucleotides against μ opioid receptors but not κ receptors significantly attenuated the hyperthermia induced by low doses of systemic morphine, whereas the reverse was true for the hypothermia induced by high doses (Chen et al., 1996). Such results provide clear evidence for the roles of central μ and κ opioid receptors in unrestrained rats and further demonstrate that the dominant site of action of morphine-induced hypothermia is within the brain, not the periphery. Our findings relating to μ and κ receptor tonic balance must therefore be closely addressed. In particular, no effect on morphine- or fentanyl-induced hypothermia was evident with the specific antagonist SNC80, which clearly contrasted with previous work where animals were pretreated with antagonists (Chen et al., 1996; Xin et al., 1997). Second, the reversal of morphine- and fentanyl-induced hypothermia observed after naloxone is unlikely to be due solely to κ antagonism, as the more μ-selective agent naltrexone produced similar effects. The finding that morphine and fentanyl produced hyperthermia only at relatively high and perhaps nonselective doses may be important. However, in the presence of an established hypothermia induced by these agents, κ antagonism has been found to be insufficient to reduce hypothermia. If a μ-hyperthermia/κ-hypothermia model is accepted, then these results are the first to show that the modulatory roles of the μ and κ opioid receptors change as a result of continued activation. These changes may be part of a dynamic balance of opioid receptor occupation and the effect on receptor function of body temperature itself. Failure of κ antagonism but the success of μ antagonism to reverse morphine- and fentanyl-induced hypothermia could also be considered consequences of sudden receptor blockade producing rebound effects and upsetting this balance. Systemic blockade of μ receptors after even a short duration of morphine exposure is known to trigger a withdrawal-like stress response (Houshyar et al., 2001), which could potentially alter the opioid control of body temperature. Naloxonazine has been proposed to bind selectively to a high-affinity μ₁ site, and this could indicate that the role of the μ receptor is limited to the μ₁ receptor type. The role of the μ receptor may be specific to this agent, since although naloxone produced similar results, this antagonist is relatively unselective and so may have produced its effects via a different mechanism. The administration of antagonists during the development of hypothermia rather than as a pretreatment, as well as the specific antagonists used, most likely account for the effects of the μ receptor in the present work.

The possible modulatory role of peripheral opioid receptors has not previously been investigated. Giving antisense oligonucleotide against the κ receptor showed a large but not fully complete blockade of hypothermia after 30 mg/kg subcutaneous morphine (Chen et al., 1996). Residual κ receptor activity was suggested, but this could equally be due to the continued peripheral activity of morphine. The peripheral occupation of opioid receptors may be trivial during concomitant central occupation, but as loperamide demonstrates, the activation of peripheral opioid receptors on their own is highly significant. Little is known about the peripheral mechanism of opioid-induced hypothermia and the consequences of sudden blockade. The importance of this could be tested by giving i.c.v. naloxonazine after high doses of i.c.v. and systemic morphine.

Further considerations relate to the use of different species and strains with regard to thermoregulation at the specific laboratory temperature or stress-related differences. Evidence that highly selective μ agonists can produce hypothermia in certain settings is a further consideration. First, PL-017 produced hypothermia in rats exposed to an environmental temperature of 5°C (Handler et al., 1994). Before the μ specificity of this hypothermia was shown, it had been demonstrated previously that morphine lowers body temperature by suppression of central thermogenic responses to the low environmental temperature (Lotti et al., 1966). There may exist differences in the extent to which different strains respond to environmental temperatures. Second, DAMGO was shown to produce dose-dependent hypothermia in restrained rats (Spencer et al., 1988). The effect of restraint as a physiological stressor, which may induce release of endogenous opioids, was used as an explanation for this finding. Restraint diminishes morphine-induced hyperthermia and enhances high-dose hypothermia (Adler et al., 1988). This shift may also be indicative of a change in the role of the μ receptor during stress.

There are several principle mechanisms by which drugs can alter body temperature. The preoptic anterior hypothalamus serves as the controlling thermoregulatory set point (Adler et al., 1988). Changes in set point, as opposed to loss of heat control systems, can be demonstrated using different environmental temperatures through which animals are free to move (Cox et al., 1976). It has further been demonstrated that a rise in thermoregulatory set point causes vasoconstriction, which reduces heat loss (Adler et al., 1988). After observations from several species, morphine-induced hypothermia has been primarily attributed to a decrease in oxygen consumption rather than increased heat loss (Lotti et al., 1966; Lin et al., 1980). These observations indicate that there are several central and peripheral sites at which drugs, and possibly opioids, can alter the control of body temperature. A decrease in activity of animals treated with loperamide was observed in the present work. Similar results have been obtained from place preference and locomotor studies (Bechara and van der Kooy, 1985; Bedingfield et al., 1999). However, it has been suggested that the body temperature effects of opioids do not correlate well with motoric activity (Adler et al., 1988), and this effect alone cannot account for the dramatic hypothermia seen in mice following loperamide. In addition, we have observed increased motoric activity in
mice after morphine and fentanyl at doses that induced hypothermia. Loperamide does not directly affect central control of body temperature in the same way as centrally penetrating agents, and therefore the observed hypothermia is more likely to be due to increased heat loss rather than decreased oxygen consumption and/or decreased metabolic heat production. Further experiments to determine oxygen consumption and warm/cold place preference after loperamide would provide valuable insight into the mechanism of action of this agent.

The results presented here in mice show that several different opioid antagonists from different chemical classes and exhibiting different selectivity profiles antagonize the hypothermic effects of loperamide. If this agent produced its effects by increasing heat loss, the results may suggest that this mechanism is more readily reversible than the induced decrease in heat production produced by centrally penetrating opioids.

The irreversible μ antagonist β-funtaltrexamine did not significantly alter either morphine or fentanyl-induced hypothermia. However, the receptor binding properties of β-funtaltrexamine are noncompetitive (Corbett et al., 1985), and due to the study design, which focused on acute interactions, full antagonist activity may not have developed. The pharmacological profile of this agent is somewhat different to the other μ antagonists, however, since it possesses an initial κ agonist activity and has been shown to produce analgesia in mice (Ward et al., 1982; Qi et al., 1990). In addition, we have shown here that a 10 mg/kg dose of β-funtaltrexamine can induce hypothermia in mice, which is probably due to κ receptor stimulation.

The δ antagonist BNTX produced weak antagonism of morphine-induced hypothermia. However, this agent produced a small but significant increase in body temperature when given alone. The δ2 antagonist naltrindole at the 10 mg/kg dose level produced a marked potentiation of both morphine- and fentanyl-induced hypothermia. However, 10 mg/kg naltrindole was found to produce a small decrease in body temperature by itself. In addition, the nonsubtype-selective δ antagonist naltrindole produced a weak potentiation of the effects of morphine. It has been suggested that μ and δ receptors may be complexed, and pharmacological studies have provided evidence for specific interactions. For example, modulation of morphine antinociception by δ agonists was found to be sensitive to β-funtaltrexamine but not μ1, naloxonazine, providing evidence for the functional role of complexed and noncomplexed μ and δ receptors (Heyman et al., 1989). In addition, locomotor stimulation and place preference effects of morphine are reduced by naltrindole (Narita et al., 1993; Kamei et al., 1997).

SNC80-induced hypothermia was markedly antagonized by the δ opioid antagonist naltrindole and also by the peripheral antagonist methyl-naltrexone. However, the degree of antagonism with methyl-naltrexone was less than with naltrindole. The failure of the δ subtype-selective agents to produce a strong antagonism of the effects of SNC80 may indicate a lack of δ/δ subtype specificity with regard to δ opioid-induced hypothermia. However, the δ-selective antagonist naltrindole at the 2.5 mg/kg dose level significantly reduced SNC80-induced hypothermia. In contrast, 10 mg/kg naltrindole, a dose that produces hypothermia by itself, produced a significant increase in SNC80-induced hypothermia. It was also at this dose that naltrindole potentiated the effects of morphine and fentanyl. It may be possible therefore that naltrindole exhibits biphasic dose-response characteristics. We have also shown that naloxone and β-funtaltrexamine significantly reduced the effect of SNC80, whereas naloxonazine produced no effect. Together, these observations provide further support for the existence of a functional pharmacological role for a μ/δ complex.

The κ opioid antagonist nor-binaltorphimine produced inhibition of U50,488H-induced hypothermia. In contrast, the irreversible κ antagonist DIPPA produced an increase in U50,488H-induced hypothermia from 2.5 mg/kg. It remains to be determined whether pretreatment with DIPPA would abolish hypothermia induced by a subsequent injection of U50,488H.

In summary, we have used a body temperature assay in mice to show that opioid-induced hypothermia can be modulated by multiple opioid receptor systems, which have the ability to interact with each other. At the relatively high doses used, morphine- and fentanyl-induced hypothermia may involve composite action on μ, κ, and possibly δ opioid receptors. The consequences of sudden blockade of opioid receptors after their initial activation may be significant in understanding the reversibility of morphine- and fentanyl-induced hypothermia. We have also clearly emphasized the existence of both central and peripheral components of opioid-induced hypothermia in mice. In the mediation of δ opioid-induced hypothermia, no clear selectivity between the δ1/δ2 subtypes was defined. In addition, further evidence has been provided for the existence of specific μ/δ interactions at the in vivo pharmacological level.

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