κ - and δ -Opioids Block Sympathetically Dependent Hyperalgesia

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Direct hyperalgesia induced by prostaglandin E₂ (PGE₂) can be blocked by μ - but not δ - or κ -opioids. However, there is evidence that κ - and δ -opioid receptors are located on sympathetic postganglionic neuron (SPGN) terminals, which mediate bradykinin (BK) hyperalgesia via SPGN-terminal-dependent production of PGE₂. Therefore, we evaluated the antinociceptive effect of δ - and κ -opioids on BK hyperalgesia. We demonstrate that the mechanical hyperalgesia induced by intradermal injection of BK can be blocked by the κ -opioid agonist trans-3,4-dichloro-N-methyl-N[2-(pyrrolidinyl)cyclohexyl] benzeneacetamide (U50,488H) and by the δ -opioid agonist (D-Pen^{2,5})-enkephalin (DPDPE), as well as the μ -opioid agonist Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAMGO). Pertussis toxin prevented the inhibition of BK-induced hyperalgesia by U50,488H, DPDPE, or DAMGO. We conclude that the observed peripheral analgesic effects of κ - and δ -opioid agonists result from actions upon SPGN terminals and that these effects are mediated by inhibitory G-proteins.

Prostaglandin E₂ (PGE₂) produces hyperalgesia directly by an action on primary afferent nociceptors (Patermicelakis and Rood, 1982; Taiwo et al., 1987; Taiwo and Levine, 1989). In previous studies, we have demonstrated that the hyperalgesia induced by intradermal injection of PGE₂ can be blocked by μ - but not by δ - and κ -opioids (Levine and Taiwo, 1989) injected intradermally. The failure of the δ - and κ -agonists to block PGE₂ hyperalgesia was unexpected, not only because μ -, κ -, and δ -opioid-induced antinociception have been demonstrated in inflamed tissue (Hargreaves et al., 1988; Stein et al., 1989), but also because δ - and κ -opioid agonists, as well as μ -agonists, reduce somatic Ca²⁺-dependent action potentials in cultured dorsal root ganglion cells (Werz and Macdonald, 1982, 1985). In addition, opioid binding sites are likely to be present on peripheral processes of afferents because they have been demonstrated by competitive binding on dorsal roots and their spinal terminals (Hiller et al., 1978; Gamse et al., 1979; Fields et al., 1980).

There is, however, evidence that inflammatory agents produce hyperalgesia not only by a direct action on peripheral terminals of nociceptive primary afferents (Patermicelakis and

Rood, 1982; Levine et al., 1986; Taiwo and Levine, 1989), but also indirectly, by stimulating postganglionic sympathetic neurons (SPGNs) to release substances that can then act on the primary afferent (Lembeck et al., 1976; Levine et al., 1986). Because κ - and δ -receptors are located on SPGN terminals (Illes, 1980; Hughes, 1981; Wuster et al., 1981; Illes et al., 1985; Berzetei et al., 1987, 1988) and because the hyperalgesia induced by bradykinin (BK) and norepinephrine has been demonstrated to be dependent on SPGN terminal production of hyperalgesic prostaglandins (Levine et al., 1986), it was of interest to evaluate the hypothesis that modulation of hyperalgesia by δ - and κ -opioids is produced through an indirect action. We report that BK hyperalgesia is, in fact, blocked by κ - and δ - (as well as μ -) opioids. The production of prostaglandins by BK has been demonstrated to be mediated by a stimulatory G-protein (G_s)-linked activation of phospholipase A₂ to release arachidonic acid (Burch et al., 1986; Burch and Axelrod, 1987; Axelrod et al., 1988). Therefore, we evaluated the hypothesis that opioid modulation of BK hyperalgesia involves the activation of an inhibitory G-protein (G_i; Childers and LaRiviere, 1984; Holz et al., 1986; Makman et al., 1988), thereby antagonizing the G_s-mediated release of arachidonic acid.

Materials and Methods

The experiments were performed on 120 male Sprague-Dawley rats (250–300 gm; Bantin and Kingman, Fremont, CA). The threshold for the nociceptive paw-withdrawal flexion reflex was quantified with a Basile Analgesymeter (Stoelting, Chicago, IL). This instrument exerts a force that increases at a constant rate. The force was applied by a dome-tipped plinth (0.7 mm in radius, with a curvature of 36°) to the dorsum of the rat's hindpaw. The paw rested on a Teflon platform, which had a low coefficient of friction. Thus, when the rat withdrew its paw, the paw slid easily off the platform, without injury. The mechanical nociceptive threshold was defined as the force, in grams, at which the rat withdrew its paw. The site on the paw at which the nociceptive threshold was measured was marked with a felt-tip pen. This was the site at which test agents were subsequently injected. No pain-associated behaviors occurred after the intradermal injection of bradykinin, other than during testing in the paw-withdrawal test. Furthermore, because the hyperalgesic agent, bradykinin, was injected into the dorsal surface of the hindpaw, no signs of discomfort were observed when the rats ambulated. The protocol for this study was approved by the University of California at San Francisco Institutional Review Board.

During the week before the experiments, rats were trained in the paw-withdrawal reflex at 5-min intervals for a period of 2 hr each day. This adaptation procedure produces a stable baseline threshold measurement and enhances the detectability of agents that modulate hyperalgesic mechanisms (Taiwo et al., 1989). On the day of the experiment, the rats were exposed to the test stimulus at 5-min intervals for 2 hr. The baseline threshold was defined as the mean of the last 6 determinations before injection of the test agent. The baseline threshold in the rats used in this study was 136.8 ± 1.2 gm ($n = 240$). After measuring the baseline threshold, test agents were injected intradermally, in a volume of 2.5 μ l, in each hindpaw. Thresholds for withdrawal were then remeasured

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at 10, 15, and 20 min. The effect of each test agent, at each dose, was calculated as the average percentage change from baseline threshold of these 3 consecutive determinations. Increasing doses of hyperalgesic agents, alone or in the presence of a fixed dose of selective opioid agonists, antagonists, or pertussis toxin, were injected at 25-min intervals. We have previously demonstrated that the hyperalgesia produced by bradykinin injected this way has a latency of onset of 5.2 ± 0.7 min (Taiwo et al., 1987) and a duration of 110 ± 10 min (Taiwo and Levine, 1988).

The contribution of the inhibitory guanine nucleotide regulatory protein (G_i) to opioid-induced analgesia was evaluated by co-injecting bradykinin in the presence of pertussis toxin, which irreversibly binds to and blocks activation of G_i (Holz et al., 1986).

The test agents used in this study were the μ -agonist Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAMGO; Handa et al., 1981), the δ -agonist [D-Pen^{2,5}] enkephalin (DPDPE; Mosberg et al., 1983; Peninsula Labs, Belmont, CA), the κ -agonist trans-3,4-dichloro-N-methyl-N[2-(pyrrolidinyl)cyclohexyl] benzeneacetamide (U50,488H; VonVoightlander et al., 1983; courtesy of Dr. Robert Lahti, Upjohn Co., Kalamazoo, MI), the opioid antagonist naloxone hydrochloride, bradykinin, and pertussis toxin (Sigma, St. Louis, MO). All the agents were dissolved in saline.

Dose-response curves for bradykinin were compared to dose-response curves in the presence of different opioid agonists or antagonists, or pertussis toxin, by 2-way repeated-measures ANOVA.

Results

Intradermal injection of BK produced a dose-dependent decrease in mechanical nociceptive threshold [$F(3, 33) = 27.6$; $p < 0.001$; Fig. 1A]. Although none of the opioid agonists or antagonists (DAMGO, U50,488H, DPDPE, and naloxone) alone had a significant effect on mechanical nociceptive threshold (Levine and Taiwo, 1989), all 3 opioid agonists produced a dose-dependent antagonism of BK-induced hyperalgesia [$F(5, 42) = 8.5$, $p < 0.001$; $F(5, 42) = 8.43$, $p < 0.001$; $F(5, 42) = 9.49$, $p < 0.001$; Fig. 1A–C, respectively].

Naloxone (10 ng to 10 μ g) did not significantly alter BK hyperalgesia [$F(12, 93) = 0.78$; $p > 0.05$]. However, the inhibition of BK hyperalgesia by 1 μ g DAMGO, U50,488H, and DPDPE was dose-dependently antagonized by co-injection of these doses of naloxone [significant dose (naloxone) $\times$ dose (BK) interaction, $F(3, 75) = 48.4$, $p < 0.001$ (DAMGO); $F(3, 75) = 8.39$, $p < 0.001$ (U50,488H); $F(3, 75) = 25.67$, $p < 0.001$ (DPDPE); Fig. 2A–C, respectively].

Intradermal injection of pertussis toxin (1 μ g) by itself did not significantly affect mechanical nociceptive threshold (-3.36 ± 2.31 ; $n = 18$). This dose of pertussis toxin, however, did significantly enhance BK hyperalgesia [$F(1,16) = 14.60$; $p < 0.01$; Fig. 3A]. The same dose of pertussis toxin also prevented the inhibition of BK-induced hyperalgesia that had been observed with a 1- μ g dose of each opioid: DAMGO [$F(1,10) = 27.45$; $p < 0.001$], U50,488H [$F(1,10) = 28.03$; $p < 0.001$], and DPDPE [$F(1,10) = 44.98$; $p < 0.001$; Fig. 3A–C, respectively].

Discussion

We studied the analgesic effects of selective μ -, κ -, and δ -receptor opioid agonists on the hyperalgesia induced by BK, a hyperalgesia that is dependent on SPGN terminals (Levine et al., 1986). We observed that intradermal injection of the μ -agonist DAMGO, the κ -agonist U50,488H, or the δ -agonist DPDPE each prevented the cutaneous mechanical hyperalgesia induced by BK. Because naloxone antagonized the effects of all opioids, we conclude that these peripheral analgesic effects are opioid-receptor mediated.

Because BK-induced hyperalgesia is mediated by an SPGN-terminal-dependent production of PGE₂ (Lembeck et al., 1976; Levine et al., 1986; Taiwo and Levine, 1988) and because

Figure 1. Dose-dependence relationship for the effect of intradermally injected BK ($n = 12$) on mechanical nociceptive threshold, alone (○) or when co-injected with 1 ng (▼), 10 ng (◆), 100 ng (▲), 1 μ g (●), or 10 μ g (■) of various opioid agonists: DAMGO (A; $n = 6$), U50,488H (B; $n = 6$), and DPDPE (C; $n = 6$). In B and C, and in subsequent figures, the dose-response curve for BK (broken line) is repeated for ease of comparison. In this and subsequent figures, each point represents the mean $\pm$ SEM of 6 or 12 experiments.

μ -opioids inhibit PGE₂ hyperalgesia (Levine and Taiwo, 1989), the inhibition of BK-induced hyperalgesia by the μ -opioid agonist DAMGO was expected. Because of this direct effect of μ -opioids, it is difficult to determine if μ -opioids also have an antinociceptive action on the SPGN terminal. In this regard, however, it is of note that DAMGO appears to more potently

Figure 2. Dose-dependence relationship for the effect of BK on nociceptive threshold, alone ($\square$; $n = 12$) or in the presence of $1 \mu\text{g}$ of the various opioid agonists: DAMGO (*A*; $n = 6$), U50,488H (*B*; $n = 6$), and DPDPE (*C*; $n = 6$) in the absence ($\circ$) and presence of 10 ng ($\bullet$), 100 ng ($\blacktriangle$), $1 \mu\text{g}$ ($\blacktriangledown$) and $10 \mu\text{g}$ ($\blacksquare$; $n = 6$ for all) of the opioid antagonist naloxone. The control BK curve (broken line) and the dose-response curve in the presence of opioid agonists alone ($\circ$) are repeated from Figure 1, for ease of comparison.

Figure 3. Dose-dependence relationship for the effect of BK on the nociceptive threshold in the presence of pertussis toxin alone ($1 \mu\text{g}$; $n = 6$; $\square$) and pertussis toxin ($1 \mu\text{g}$) plus DAMGO (*A*; $1 \mu\text{g}$; $n = 6$), U50,488H (*B*; $1 \mu\text{g}$; $n = 6$), and DPDPE (*C*; $1 \mu\text{g}$; $n = 6$; $\blacklozenge$). The dose-dependence relationship for the effect of BK alone ($n = 12$; $\circ$) and in the presence of the opioid agonists ($\bullet$; $n = 6$ each) are redrawn from Figure 1, for ease of comparison.

antagonize BK- than PGE_2 -induced hyperalgesia, where complete attenuation is produced only by at least a 10-fold-higher dose of DAMGO ($1 \mu\text{g}$; Levine and Taiwo, 1989). This apparent greater potency against BK-induced hyperalgesia may reflect an additional site of action at the SPGN terminal (Lord et al., 1977; Illes et al., 1980b; Schulz et al., 1980; Shaw et al., 1982; Ward

et al., 1982), where it may have a greater potency in inhibiting the production of hyperalgesic prostaglandins.

PGE_2 hyperalgesia was *not* affected by δ - and κ -opioids, indicating that their effects on BK were at a site of action prior to the production of PGE_2 . Taken together with the observation that κ - and δ -opioid receptors have been reported on SPGN

terminals (Illes et al., 1980a, 1985; Hughes, 1981; Wuster et al., 1981; Berzetei et al., 1987, 1988), we suggest that an SPGN site of action is involved in the peripheral antinociception of κ - and δ -opioid agonists. The hyperalgesia present in certain other inflammatory states (e.g., Freund's adjuvant and carrageenan) is also blocked by all 3 opioid agonists (Stein et al., 1989) and is also likely to be associated with a κ - and δ -opioid-sensitive release of PGE₂ from the SPGN terminal.

The neurogenic inflammatory responses produced by antidromic peripheral nerve stimulation or by local application of chemical irritants are also blocked by peripheral opiate injection. These opioid inhibitory effects have been predominantly μ -opioid effects (Bartho and Szolcsanyi, 1981; Lembeck et al., 1982; Lembeck and Donnerer, 1985; Russell et al., 1985; Frossard and Barnes, 1987; Belvisi et al., 1988), though δ - (Yaksh, 1988) and κ -effects (Russell et al., 1987) have also been reported. Although it is hypothesized, in these studies, that the opioids block the peripheral release of neuropeptides, such as substance P, by an action on primary afferents, there is no a priori reason to believe that δ - and κ -opioids should also produce antihyperalgesic effects by an action on primary afferents.

BK produces many of its effects via G-protein-coupled mechanisms (Conklin et al., 1988). In fact, BK-stimulated PGE₂ production in Swiss 3T3 fibroblasts is dependent on a G_i-linked activation of phospholipase A₂ (Burch and Axelrod, 1987). Because opioids activate inhibitory G-proteins (Childers and LaRiviere, 1984; Holz et al., 1986; Makman et al., 1988), we tested, in a somewhat indirect fashion, the role of G-proteins in the opioid-induced reduction of BK hyperalgesia. We found that pertussis toxin prevented the analgesic effects of all opioid agonists. This suggests that a pertussis toxin-sensitive G_i modulates κ - and δ - as well as μ -peripheral antinociceptive effects (Levine and Taiwo, 1989), as has been previously demonstrated for other actions of opioids, such as their depressant effects on dorsal horn network responses in spinal cord-ganglia cultures (Crain et al., 1987) and their regulation of neuronal voltage-dependent calcium channels (Hescheler et al., 1987), as well as their intrathecal antinociceptive effects (Hoehn et al., 1988).

In summary, these data suggest that the peripheral analgesic effects of κ - and δ -opioid agonists on BK hyperalgesia are due to actions at SPGN terminals and that G_i proteins are involved.

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