Kappa Opioid Receptor Tolerance in the Guinea Pig Hippocampus

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ABSTRACT

We investigated whether chronic, in vivo administration of U50,488H, a kappa-1 opioid agonist, caused the development of tolerance to both the electrophysiological effects of applied kappa opioids and endogenously released dynorphins. In hippocampal slices from drug-naive guinea pigs, application of U69,593, a kappa-1 agonist, produced a concentration-dependent inhibition (EC50 = 20 nM) of the amplitude of the granule cell population response in the dentate gyrus. In slices from chronically U50,488H-treated animals, the concentration-response curve for U69,593 was shifted 3-fold to the right (EC50 = 59 nM), with a significant decrease in the maximal effect of U69,593. We also found that the effects of endogenously released dynorphins were significantly attenuated by chronic U50,488H treatment. There was no cross-tolerance between kappa and mu opioid receptor agonists as measured with the in vitro electrophysiological assay, and the noncompetitive N-methyl-D-aspartate receptor antagonist MK801 did not prevent the development of tolerance to either the electrophysiological effects or the hypothermic effects of kappa opioids. Our study demonstrates that receptor-selective tolerance to the kappa opioid actions in the guinea pig hippocampus does develop after chronic U50,488H treatment; but, unlike the mechanisms reported to underlie tolerance to kappa opioid analgesia, the inhibitory effects in the hippocampus did not depend on activation of N-methyl-D-aspartate receptors.

Opioid tolerance and dependence are important facets of drug abuse and limit the effective use of opioid analgesics. Opioid peptides and their receptors are abundant in the hippocampus (McLean et al., 1987; Wagner et al., 1990, 1991), and the pharmacological effects of opiates in the hippocampus are well defined (see Simmons and Chavkin, 1996). Previous studies demonstrated that activation of the kappa-1 opioid receptor by either exogenous (U69,593, a selective kappa-1 agonist) or endogenously released opioids presynaptically inhibits the release of excitatory amino acids from perforant path afferents and blocks induction of long-term potentiation in the guinea pig dentate gyrus (Wagner et al., 1993; Simmons et al., 1994; Terman et al., 1994). This inhibitory action of kappa opioids at the perforant path-granule cell synapse, a major excitatory input to the hippocampal formation, seems to play an important role in modulating excitatory activity of the hippocampus, and the kappa opioid system may influence spatial learning, memory and epileptogenesis (Jiang et al., 1989; Tortella et al., 1989, 1990; Decker and McGaugh, 1991). However, it is not known how the actions of kappa opioids and endogenously released opioids are affected by chronic opioid exposure.

The analgesic actions of kappa opioid agonists are more resistant than mu opioids to the development of tolerance, and the withdrawal reaction is less severe (Cowan and Murray, 1990; Bhargava, 1994). Nevertheless, it has been demonstrated that chronic administration of U50,488H produces tolerance to the analgesic, neuroendocrine and hypothermic effects of this opioid agonist (Bhargava et al., 1989a,b; Milan’es et al., 1991). Although the neuronal and molecular mechanisms that underlie kappa opioid tolerance remain unclear, pharmacodynamic changes are unlikely because kappa opioid tolerance is not caused by enhanced drug metabolism (Vonvoigtlander et al., 1984). The tolerance may be caused by a reduction in receptor-effector coupling efficacy (i.e., agonist intrinsic efficacy), because there is no consistent evidence of changes in kappa receptor binding site density or binding affinity in the brain after chronic U50,488H treatment (Bhargava et al., 1989a; Ho and Takemori, 1989). Recently, extensive evidence indicated that NMDA receptor antagonists and interactions among different opioid receptors alter the opioid tolerance process (Trujillo and Akil, 1991; Tanganelli et al., 1991; Sofuoglu et al., 1992; Bhargava and Thorat, 1994). For example, antagonism of central NMDA receptors significantly attenuated the development of morphine tolerance (Trujillo and Akil, 1991; Marek et al., 1991; Ben et al., 1992). It has also been demonstrated that NMDA receptor antagonists prevent the development of the tolerance to analgesic effects of kappa opioids (Bhargava and

ABBREVIATIONS: nBNI, norbinaltorphimine; DAMGO, [d-Ala2,NMePhe4, glyol5]enkephalin; NMDA, N-methyl-D-aspartate; LTP, long term potentiation; s.c., subcutaneous; S1/2, stimulus intensity that evoked a half-maximal response.

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Thorst, 1994; Bhargava et al., 1995; Kolesnikov et al., 1993). Interestingly, kappa opioid agonists also inhibit analgesic tolerance to morphine, which suggests an interaction between kappa and mu opioid receptor systems (Takahashi et al., 1991). Understanding the mechanism of interaction between mu and kappa receptors and the NMDA receptor may lead to the development of novel therapies for pain relief and drug abuse. In the present study, we examined whether chronic administration of U50,488H affected the inhibitory actions of exogenous and endogenous kappa opioids in the dentate gyrus of the hippocampal formation and whether that tolerance was affected by NMDA receptor antagonism.

Methods and Materials

**Chronic U50,488H treatment.** Male Hartley guinea pigs (175–250 g) were injected s.c. with U50,488H twice a day (10- to 12-h intervals) in an ascending dosage schedule: day 1, 10 and 15 mg/kg; day 2, 25 and 30 mg/kg; day 3, 50 and 60 mg/kg; day 4, 70 and 75 mg/kg (Milanés et al., 1991). On day 5, the animals were injected with U50,488H (25 mg/kg) and were sacrificed 30 min after the injection. Control animals received the same volume of saline (0.2 mL/100g) on the same schedule. Rectal temperatures were recorded with a digital thermometer (Yellow Springs Instruments, Yellow Springs, OH) immediately before and 1 h after injection of either saline or 10 mg/kg of U50,488H. The rest of the scheduled dose of U50,488H was given after the temperature measurement. U50,488H was used in vitro because it penetrates the blood-brain barrier significantly better than does U69,593 (Bianchi, 1989).

To prepare morphine-tolerant hippocampal slices, guinea pigs were implanted subcutaneously with morphine pellets under light anesthesia (75 mg of base each, three on day 1 and five on day 3). The morphine-tolerant animals were implanted subcutaneously with morphine pellets under light anesthesia (75 mg of base each, three on day 1 and five on day 3). This method has been previously shown to induce morphine tolerance (Goldstein and Schulz, 1973). The morphine-tolerant animals were sacrificed on day 6.

**Hippocampal slice preparation.** Guinea pigs were decapitated, and the brains quickly removed, cooled, blocked and cut with a Vibratome (Staelting Inst. Wood Dale, IL) into 500- and 200-μm sections. Slices were transferred to a warmed (34°C) submerged tissue recording chamber perfused at 1 ml/min with modified artificial cerebrospinal fluid (in mM): NaCl, 126; KCl, 3.5; CaCl2, 4; MgCl2, 4; NaH2PO4, 1.25; NaHCO3, 26; glucose, 10; and 10 μM bicuculline saturated with 95% O2/5% CO2 (pH 7.4). For paired-pulse experiments, artificial cerebrospinal fluid did not contain bicuculline, and the concentrations of CaCl2 and MgCl2 were decreased to 2.5 mM and 1.3 mM, respectively.

**Electrophysiology.** After an hour of equilibration in the recording chamber, a glass recording electrode (1–2 μm tip diameter) was filled with 3 M NaCl and placed in the granule cell layer. A 100-μm concentric bipolar stimulating electrode (SNE-100, Rhodes Medical Supply, Woodlands Hills, CA) was placed in the outer molecular layer at the apex of the dentate gyrus to stimulate the perforant path. Population responses of granule cells (fig. 1, inset) were measured with a digitizing oscilloscope (SD10 Tektronix, Beaverton, OR). Stimulation usually consisted of single square wave pulses of 0.3-msec duration at 25 to 300 μA. The S1/2 was given at 1-min intervals throughout the experimental period. Stimulation to produce perforant path LTP consisted of three 100-Hz trains of 0.3-msec 100-μA pulses, given one train every 10 sec (train duration varied as a function of the particular experiment). LTP was operationally defined as the mean change from baseline population response amplitude from 26 to 30 min after perforant path tetanic stimulation. For paired-pulse stimulation of the dentate granule cells, the pulses were delivered at an interpulse interval of 20 msec. All drugs were applied by perfusion in the modified artificial cerebrospinal fluid. Effects of the drug were measured from 10 to 20 min after drug addition, at which time changes in the responses were found to be stable.

We confirmed that hippocampal slices prepared from animals pretreated with U50,488H were washed free of residual drug at the time the electrophysiological measures were made with use of a nBNI test. nBNI (100 nM) rapidly reverses the effects of applied U50,488H or U69,593 when drugs are applied in vitro. nBNI applied alone had no effect on the amplitude of the dentate population response evoked under the stimulation parameters used in this study. Similarly, nBNI added to slices prepared from a guinea pig receiving 25 mg/kg U50,488H 30 min before decapitation did not cause any change in electrophysiological response amplitude after nBNI (100 nM) challenge, 60 min after the slices were placed in the recording chamber.

**Data analysis.** Concentration-response curves to opioids were normalized as percentage of basal population response or basal paired-pulse basal ratio. Paired-pulse ratios were calculated by dividing the amplitude of the second population spike by that of the first response evoked by a stimulus amplitude sufficient to produce one half the maximal effect in the first response (S1/2). To calculate EC50, opioid responses were normalized to a maximum inhibition value; then fit to the equation: Y = 100/[1 + (C/K)] by Sigma Plot software, where Y is the normalized percent inhibition, C represents the concentration of opioid agonist, K is the drug concentration producing 50% effect (EC50) and n is the Hill coefficient. Statistical analysis was performed by analysis of variance and least significant difference test for appropriate post hoc comparisons. P < .05 was used as the criterion for statistical significance.

**Materials.** U69,593 (Research Biochemicals, Natick, MA) was dissolved in 50% ethanol at a stock concentration >10–6 M and then diluted more than 1000-fold in artificial cerebrospinal fluid before slice perfusion. U50,488H (Research Biochemicals), nBNI (Research Biochemicals), bicuculline methiodide (Sigma Chemical Co., St. Louis, MO) and DAMGO (Peninsula Laboratories, Belmont, CA) were dissolved in water at a concentration 1000-fold higher than the final concentration.

**Results**

Injection of guinea pigs with U50,488H (s.c.) for 4 days induced a progressive decrease in the hypothermic effect of the drug (fig. 1A), consistent with previous reports (Bhargava et al., 1989a; Milanés et al., 1991). Initial injection of U50,488H (10 mg/kg, test dose) produced a profound hypothermic effect with an average decrease in rectal temperature of 2.54 ± 0.17°C (n = 6). After repeated injections of U50,488H, the hypothermic effect was significantly (P < .05) reduced on day 3 (1.28 ± 0.07°C, n = 5), day 4 (0.97 ± 0.12, n = 5) and day 5 (0.76 ± 0.07, n = 6), which showed the development of tolerance to the hypothermic effects of U50,488H. Repeated injections of saline did not produce a change in guinea pig body temperature (fig. 1A). Hippocampal slices were prepared from the animals on day 5 for electrophysiological analysis. Evoked population responses of granule cells to perforant path stimulation were recorded (fig. 1B, inset). The average of the amplitude of the basal population responses was 1.42 ± 0.08 mV (S1/2 = 89 ± 4 μA, n = 52). As observed previously, dentate granule cell population response amplitudes were reduced by U69,593 in a concentration-dependent manner in the slices from drug-naive animals (fig. 1B). The dose of U69,593 which produced a half-maximal effect (EC50) was 20 nM (geometric mean, 16–24 nM, 95% confidence interval, n = 5, independent experiments). The basal population responses in slices from U50,488H-tolerant animals were 1.5 ± 0.13 mV (S1/2 = 4 ± 0.05 μA, n = 32), which is not significantly different from drug-naive animals. However, the concentration-response curve for U69,593 was shifted to the right with an increase in the EC50 to 59 nM (55–63 nM, 95% confidence interval, n = 6, independent experiments) (fig. 1B). Furthermore, the maximal inhibition of U69,593 (1 μM) in slices from the tolerant animals was significantly reduced from 43 ± 5% to 28 ± 3% (P < .05). This reduction in apparent efficacy of U69,593 in the tolerant animals suggests a reduction in functional kappa-1 opioid receptor reserve.
We demonstrated previously that endogenous dynorphins, released from granule cells after tetanic stimulation, inhibit LTP of the perforant path-granule cell synapse (Wagner et al., 1993; Terman et al., 1994). In the present study, we used tetanic stimulation to induce the release of endogenous dynorphins, and then examined whether the effects of these endogenous opioids were also affected by chronic U50,488H treatment. As previously reported (Terman et al., 1994), tetanic stimulation (500 msec) of the perforant path in slices from drug-naive animals produced a long-lasting increase in the granule cell population response amplitude (69.7 ± 10.4%, n = 15). This LTP produced was significantly enhanced in the presence of nBNI (161 ± 20%, n = 7), which implied that endogenous kappa opioids released by perforant path tetanic stimulation normally inhibit LTP induction (Terman et al., 1994). Tetanic stimulation (500 msec) of the perforant path in slices from kappa opioid-tolerant animals induced significantly more LTP (105.7 ± 10.4%) than induced in slices from drug-naive animals (69.7 ± 10.4%, P < .05, fig. 2). These data indicate that the inhibitory effects of endogenously released kappa opioids were reduced in the kappa opioid-tolerant guinea pigs. Our previous study had shown that the LTP induced by short-duration stimulation (21 msec) was not affected by nBNI, and thus not modulated by endogenous dynorphins (Terman et al., 1994). In the present study, we found that there was no significant difference in the LTP induced by the short-duration stimulation in slices from drug-naive and kappa-tolerant animals (fig. 2). This finding indicates that chronic kappa opioid treatment specifically alters the function of endogenous dynorphins rather than nonspecifically affecting LTP-induction mechanisms.

To further understand the mechanism of kappa opioid tolerance, we tested whether there was cross-tolerance between kappa and mu opioid receptors in the guinea pig hippocampus. In contrast to the effects of kappa opioid receptors, activation of mu opioid receptor facilitates granule cell excitability. As shown in figure 3A DAMGO (a mu opioid agonist) significantly enhanced the second response of a paired-pulse stimulation (interpulse interval, 20 msec) and therefore increased paired-pulse ratio (amplitude of the second population spike/amplitude of the first response). As shown in figure 3B, DAMGO produced a facilitatory response in a concentration-dependent manner in slices from opioid-naive animals. However, the facilitatory effects of DAMGO on the population response were not reduced in U50,488H-tolerant animals, which indicated a lack of cross-tolerance between mu and kappa opioid receptors on modulation of dentate gyrus granule cell excitability. As expected, the concentration-response curve of DAMGO was shifted to the right with a reduction in the maximal effect, from 158 ± 36% to 64 ± 13% (P < .05) in slices from animals treated chronically with morphine. Interestingly, we noticed that the facilitating effect of DAMGO, especially in the low-concentration range, appeared to be significantly potentiated in the slices from U50,488H-tolerant animals (fig. 3B). DAMGO (30 nM) induced 23 ± 7% facilitation in drug-naive animals (n = 9), whereas it produced a 54 ± 6% facilitation in slices from U50,488H treated animals (n = 5; P < .05).
We further examined whether chronic morphine treatment affected the response to kappa-1 opioid receptor activation in the dentate gyrus. In animals chronically treated with morphine pellets for 6 days, the inhibitory effect of U69,593 on the granule cell response to perforant path stimulation was clearly not attenuated (fig. 3D). The hypothermic effect of U50,488H was also not affected in morphine-tolerant animals compared with control animals (fig. 3C). Similar to mu opioid response being potentiated by chronic kappa treatment, the maximal inhibitory effects of U69,593 (1–10 μM) were significantly enhanced from 39 ± 4.5% to 52 ± 3.8% in morphine-tolerant animals (n = 12, P < .05, fig. 3D). These results demonstrate that the treatment conditions used to generate opioid tolerance did not produce cross-tolerance between mu and kappa opioid receptors in the dentate gyrus. Conversely, tolerance to either mu or kappa opioid receptor in the hippocampus enhanced the effects of the other opioid receptor agonist, which suggested a negative interaction between the two opioid receptor systems.

NMDA receptor antagonists have been shown to prevent the development of tolerance to the analgesic effects of morphine and U50,488H in mice and rats (Trujillo and Akil, 1991; Bhargava, 1995; Bhargava and Thorat, 1994). To test whether NMDA receptors were also required for the development of tolerance to the inhibitory effects of kappa opioids in the hippocampus, MK801, a noncompetitive NMDA receptor antagonist, was used at the dose previously found to affect morphine and U50,488H analgesic tolerance (0.1 mg/kg). Following the same protocol, we pretreated guinea pigs with MK801 (0.1 mg/kg) 15 min before every injection of U50,488H. As shown in figure 4A, pretreatment of MK801 did not prevent the development of tolerance to the hypothermic effect of U50,488H, consistent with a previous report (Bhargava et al., 1995; Bhargava, 1995). Moreover, tolerance to the inhibitory effects of U69,593 in the hippocampal dentate gyrus was not prevented by pretreatment with MK801 (fig. 4B). These data suggest that the development of tolerance to the hypothermic and the inhibitory effects of kappa opioid in the hippocampal dentate gyrus do not require activation of NMDA receptors.

**Discussion**

The principal finding in this study was that repeated administration of kappa opioids results in the development of tolerance to the inhibitory effects of kappa opioid receptor activation in the guinea pig dentate gyrus. Moreover, the effects of endogenous dynorphin opioids in this region were also significantly reduced by chronic kappa opioid treatment. Under the treatment conditions used, there was no evidence of cross-tolerance between mu and kappa-1 opioids in this brain region. Instead, chronic kappa opioid treatment appeared to potentiate the effects of mu opioids on perforant path to granule cell neurotransmission, and chronic mu opioid treatment produced a similar potentiation of kappa opioid effects. MK801 did not prevent tolerance to kappa-1 agonist hypothermic or electrophysiological effects. Thus, unlike tolerance to kappa analgesia, tolerance to the hypothermic and the inhibitory effects in the hippocampus did not depend on activation of NMDA receptors. These data suggest that there are mechanistic differences between the development of tol-
The mechanisms underlying the development of kappa opioid tolerance are unclear. It has been shown that there is no change in U50,488H metabolism after chronic U50,488H administration, which indicates that disposition factors do not account for the kappa opioid tolerance (Vonvoigtlander et al., 1984). Based on the binding studies, it has been shown that chronic U50,488H treatment did not reduce maximum kappa opioid receptor binding sites in the brain and spinal cord (Ho and Takemori, 1989). Therefore, functional uncoupling between receptor and G proteins (through phosphorylation or dephosphorylation) and role of intracellular messenger pathway has been suggested for the mechanism of opioid tolerance. Reduction in spare opioid receptors has been demonstrated following chronic opioid treatment in the hippocampal CA1 region, mouse vas deferens, guinea pig ileum and NG108–15 cells (Fantozzi et al., 1981; Cox and Chavkin, 1983; Chavkin and Goldstein, 1984; Wimpey et al., 1989; Kennedy and Henderson, 1991). The reduction in the spare receptors may be caused by either the receptor functional uncoupling or a decrease in the expression of functional receptors. In present study, we show that the maximal inhibition of U69,593 (10 μM) was significantly reduced in the tolerant animals, which indicates that reduction of functional receptor reserve may be involved in the kappa opioid tolerance.

The lack of cross-tolerance between mu and kappa opioid receptors observed in this study is consistent with the observations that these receptors are on different neurons in the hippocampus. In the dentate gyrus, kappa opioids act on presynaptic kappa-1 receptors to inhibit excitatory amino acid release from perforant path terminals, and mu opioids act on interneurons to inhibit γ-aminobutyric acid release (see Simmons and Chavkin, 1996).

In the present studies, not only was there a lack of cross-tolerance between mu and kappa opioid receptors, but chronic treatment with either mu or kappa opioid agonists resulted in an enhanced sensitivity to the other opioid agonist in the guinea pig hippocampus. Interactions between the two opioid receptor types have been reported previously in opioid tolerance studies. For mice, chronic morphine treatment results in selective up-regulation of kappa opioid receptors in several brain regions (Gulati and Bhargava, 1988). Chronic antagonism of mu opioid receptors enhanced kappa opioid agonist analgesic effects (Walker et al., 1991). Conversely, tolerance to U50,488H increased mu binding sites in the brain, and activation of kappa opioid receptor attenuates the development of morphine tolerance (Takahashi et al., 1991; Thorat et al., 1993). Therefore, up-regulation of the nontolerant type of opioid receptor expression after chronic opioid treatment may explain the supersensitivity observed in the present study.

Compelling evidence indicates that activation of NMDA receptors plays a crucial role in the development of tolerance to the analgesic effects of opioids (Marek et al., 1991; Trujillo and Akil, 1991; Ben et al., 1992). Coadministration of mor-
phine or U50,488H with MK801, a noncompetitive NMDA receptor antagonist, effectively prevents the development of tolerance to morphine or U50,488H analgesic effects in several animal models including rats, mice and guinea pigs (Trujillo and Akil, 1991; Bhargava, 1995; Tanganelli et al., 1991; Bhargava andThorat, 1994). In the present study, we demonstrated that pretreatment with MK801 could not prevent the development of tolerance to the kappa opioid inhibitory effect in the hippocampus. Consistent with a previous report by Bhargava et al. (1995), we also did not see prevention of the development of tolerance to the hypothermic effect of U50,488H by pretreatment with MK801. It has been suggested that development of analgesic tolerance is a consequence of a series of cellular events caused by opioids somehow initiating and/or enhancing the activation of NMDA receptors within the spinal cord (Mao et al., 1995). This process may not occur in the hippocampus or other regions of the brain. Our results indicate that unlike the development of tolerance to kappa opioid analgesia, the tolerance to kappa opioid hypothermic and inhibitory effects in the hippocampal dentate gyrus does not require activation of NMDA receptors. Understanding the mechanisms underlying opioid tolerance may allow the future regulation of this process.

References


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