The Role of Dopaminergic Systems in Opioid Receptor Desensitization in Nucleus Accumbens and Caudate Putamen of Rat After Chronic Morphine Treatment

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ABSTRACT

Morphine treatment of rats (60–70 mg/kg/day, 7 days) reduced δ opioid receptor-mediated inhibition of adenylyl cyclase activity in caudate putamen without any change in regulation by µ receptors. Earlier studies suggested that dopamine D2 and µ opioid receptors that regulate adenylyl cyclase are expressed preferentially by striato-nigral neurons, whereas adenosine A2a and δ opioid receptors are expressed preferentially by striato-pallidal neurons. Chronic morphine treatment also resulted in a reduction of dopamine D2 receptor-mediated inhibition of A2a receptor-stimulated adenylyl cyclase. Treatment with a D2 receptor antagonist (eticlopride; 1 mg/kg/day) for 7 days reduced D2 receptor stimulation of adenylyl cyclase. In contrast, chronic treatment with a D1 receptor antagonist R(+)-7-chloro-8-dihydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine HCl (SCH 23390; 2.5 mg/kg/day) resulted in a reduction of δ1 and δ2 opioid inhibition of adenylyl cyclase, with no change in the inhibitory activity of a µ agonist. The inhibitory activity of the D2 agonist quinelorane against adenosine A2a-activated enzyme was also reduced by this treatment. Thus chronic D1 blockade, like chronic morphine treatment, appears to cause a selective impairment of the regulation of adenylyl cyclase in A2a receptor-expressing striato-pallidal neurons. D2 receptor activation appears to play an important role in the desensitization of δ receptors, because concurrent administration of the D2 antagonist eticlopride with morphine prevented the desensitization of δ and D2 receptors. Similar results were obtained in nucleus accumbens, which suggests a role for D2 receptor desensitization in the adaptive response of this brain region to chronic morphine.

Opioids inhibit adenylyl cyclase via the activation of a pertussis toxin-sensitive G protein, Gt;GI (reviewed by Childers, 1991). Many G proteins-linked receptors have been found to exhibit a diminished responsiveness (desensitization or tolerance) in the continued presence of agonists (Law et al., 1983; Benovic et al., 1986; Puttfarken et al., 1988; Lefkowitz et al., 1990; Simmons et al., 1990). Chronic morphine treatment induces adaptations in G-proteins and cAMP system in numerous brain regions known to be involved in the chronic and acute effects of opiates (Duman et al., 1988; Nestler and Tallman, 1988; Nestler et al., 1989; Tertullinger et al., 1991; Harris and Williams, 1991; Matsuoka et al., 1994).

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ABBREVIATIONS: ANOVA, analysis of variance; CGS 21680, 2-p-(2-carboxyethyl)phenethylamino-5’-N-ethylcarboxyamidoadenosine HCl; DT-II, [D-Ala2]deltorphin II; EGTA, ethylene glycol-bis(β-aminoethyl ether) N,N,N’,N’-tetraacetic acid; GABA, γ-aminobutyric acid; SKF 38393, (±)-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine HCl; SCH 23390, R(+)-7-chloro-8-dihydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine HCl; TOT, total or maximum activity under an assay condition (in figures); VTA, ventral tegmental area.

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system in mediating selective desensitization of δ opioid receptors after chronic morphine treatment in the caudate putamen and the nucleus accumbens. In striatum, adenyl cyclase activity is subject to regulation by agonist with high selectivity for both μ and δ receptors (Izenwasser et al., 1993). Pharmacological analysis suggests that the activity of this enzyme in striatal membranes is inhibited by agonist acting through either the δ1 or the δ2 subtype of opioid receptor (Buzás et al., 1994; Noble and Cox, 1995), although the molecular basis for the observed differences in ligand selectivities in functional assays for these δ receptor subtypes is not yet established. Furthermore, the various opioid receptors that regulate adenyl cyclase in caudate putamen show an apparent differential neuronal location that corresponds to the differential location of D1 and D2 dopamine receptors on striato-nigral and striato-pallidial efferent neurons (Gerfen et al., 1990; Le Moine et al., 1990; Noble and Cox, 1995). These observations suggest a neuroanatomical explanation of the effects induced by chronic morphine treatment.

Materials and Methods

Animals and surgery. The experiments reported herein were conducted according to the principles set forth in the Guide for Care and Use of Laboratory Animals (Institute of Animal Resources, National Research Council, NIH publication 85-23). Male Sprague-Dawley rats (Taconic, Germantown, NY), weighing 220 to 250 g at the start of the experiment, were used. Rats were group-housed in standard laboratory cages and kept in a temperature- and humidity-controlled colony room for at least 1 week before the surgery. Food and water were available ad libitum.

Osmotic pumps (2ML1, Alza Corporation, Palo Alto, CA) that delivered 10 μl/hr were used to administer saline or drug by continuous s.c. infusion. The pumps were filled with 64 mg/ml morphine in saline, with 2.5 mg/ml SCH 23390, with 1.04 mg/ml eticlopride, with a combination of two drugs, or with saline alone. The pumps were surgically implanted s.c., caudal to the dorsum of the neck, under halothane anesthesia. After recovery, the rats were housed in single-animal cages with free access to food and water. These experiments were conducted as part of a series of studies of the effects of chronic morphine treatment. Results from some of the treatment groups are reported in Noble and Cox (1996).

Membrane preparation. On the seventh day after implantation of the osmotic pump, rats were killed by decapitation. Their brains were rapidly removed. Caudate putamen and nucleus accumbens were obtained by gross dissection. Dissected tissues from isolated brain regions of one rat were homogenized separately and diluted into buffer (20 mM Tris-HCl (pH 7.4), 2 mM EGTA, 1 mM MgCl2, and 250 mM sucrose) and centrifuged at 27,000 g for 15 min at 4°C. The pellets were resuspended in fresh buffer and centrifuged again for 15 min. The supernatants were discarded, and the pellets were homogenized in 30% w/v ice-cold buffer (2 mM Tris-HCl (pH 7.4) and 2 mM EGTA) for determination of adenyl cyclase activity.

Determination of adenyl cyclase activity. Tissue homogenate (15–30 μg of protein in 10 μl) was added on ice to assay tubes (final volume 60 μl) containing 80 mM Tris-HCl (pH 7.4), 10 mM theophylline (or 150 μM papaverine in experiments performed in the presence of CGS 21680), 1 mM MgSO4, 0.8 mM EGTA, 30 mM NaCl, 0.25 mM ATP, 0.01 mM GTP and either the drug being tested or water (all drugs tested in this assay were soluble in water at the concentrations used). Triplicate samples for each treatment were incubated at 30°C for 5 min. Adenyl cyclase activity was terminated by placing the tubes into boiling water for 2 min. The amount of cAMP formed was determined by a [3H]cAMP protein binding assay (Brown et al., 1971). [3H]cAMP (final concentration 4 nM) in citrate-phosphate buffer (pH 5.0) and then binding protein prepared from bovine adrenal glands were added to each sample. Additional samples were prepared, without tissue, containing known amounts of cAMP; these served as standards for quantification. The binding reaction was allowed to reach equilibrium by incubation for 90 min at 4°C, and the assay was terminated by the addition of charcoal and centrifugation (1000 g for 10 min, at 4°C) to separate the free tritiated cAMP from that which was bound to the binding protein. Aliquots from the supernatant containing bound cAMP were placed into scintillation vials to which Beckman Ready Value Scintillation Cocktail was added, and radioactivity was determined by liquid scintillation spectrometry. Radioactivity was converted to picomoles of cAMP by comparison to the curve derived from the standards. Protein values were determined by a modification of the Lowry procedure, using bovine serum albumin as standard (Peterson, 1977). Results are expressed as percentage of the respective basal or stimulated activity (i.e., naive or morphine-dependent rats) measured in the absence of opioid.

Chemicals. The following drugs and chemicals were used in this study: DAMGO, DPDPDE, DT-II, Tyr-D-Phe-D-Pen, ATP (disodium salt), GTP (lithium salt), cAMP (sodium salt), theophylline, EGTA (Sigma Chemicals Co., St. Louis, MO), SCH 23390, eticlopride, SKF 38393, CGS 21680, papaverine HCl, quinelorane (Research Biochemicals Inc., Natick, MA), morphine sulfate (Merck Chemical Div., Rahway, NJ) and [3H]cyclic AMP (ammonium salt; specific activity 28.1 Ci/mmol) (Du Pont NEN Research Products, Boston, MA).

Statistical analysis. Dose-response curves from adenyl cyclase assays were analyzed with a two-way ANOVA. If a significant effect was observed, a one-way ANOVA was used, followed by a Newman-Keuls’ test, to determine the significance at each concentration. The level of significance was set at P < .05.

Results

Effects of chronic morphine treatment on inhibition of adenyl cyclase activity induced by opioid agonists and dopamine agonists in the caudate putamen. We have previously reported (Noble and Cox, 1996) the selective impairment of δ opioid receptor-mediated inhibition of basal adenyl cyclase activity in the rat caudate putamen after morphine treatment. We now show that this morphine treatment does not affect the ability of either the D2-selective dopamine agonist SKF 38393 or the A2a-selective adenosine agonist CGS 21680 to stimulate adenyl cyclase activity in the rat caudate putamen (table 1) [F(1,17) = 0.205, P > .05 and F(1,17) = 0.068, P > .05, respectively]. The inhibitory effects of the δ2 receptor agonist DT-II were tested against adenyl cyclase activity stimulated by SKF 38393 or CGS 21680 (fig. 1). The chronic morphine pretreatment attenuated the ability of DT-II to inhibit CGS 21680 (0.1 μM)-stimulated adenyl cyclase (when compared with the inhibitory effects of DT-II in caudate putamen from saline-treated rats) [P(1,24) = 22.722, P < .001] but had no effect on the efficacy or potency of DT-II in inhibiting SKF 38393 (10 μM)-stimulated adenyl cyclase activity [F(1,18) = 1.091, P > .05].

As a measure of the function of D2/D3 receptors after chronic drug treatments, the inhibitory activity of the D2/D3-selective dopamine agonist quinolone against CGS 21680-stimulated adenyl cyclase activity was tested, because other studies have shown that adenosine A2a and dopamine D2 receptors are co-localized on striato-pallidal neurons (Le Moine et al., 1990; Gerfen, 1992; Augood and Emson, 1994)). Quinolone inhibited CGS 21680-stimulated adenyl cyclase in a dose-dependent manner in saline-treated rats [fig.
2; F(3,11) = 11.08, P < .001]. This inhibition was antagonized by the D2 dopamine antagonist eticlopride (100 nM; data not shown). A rightward shift of the quinelorane dose-response curve as an inhibitor of CGS 21680-stimulated adenylyl cyclase activity was observed after chronic morphine treatment (fig. 2). Two-way ANOVA analysis revealed a significant effect [F(1,23) = 14.21, P < .001]; thus chronic morphine treatment resulted in a reduced inhibition of adenylyl cyclase by D2/D3 receptor activation in caudate putamen. Results are expressed in percentages of SKF 38393-stimulated enzyme.

Effects of chronic dopamine antagonist treatments on inhibition of adenylyl cyclase activity induced by opioid agonists and dopamine agonists in the caudate putamen. Chronic treatment with the D1 dopamine agonist SCH 23390 (0.6 mg/rat/day, for 7 days) induced a desensitization of δ opioid receptors in the caudate putamen (fig. 3). The δ1 opioid agonist DPDPE and the δ2 opioid agonist DT-II appeared unable to inhibit basal adenylyl cyclase activity in SCH 23390-treated rats as compared with saline-treated rats [F(1,22) = 22.856, P < .001 and F(1,20) = 72.818, P < .001, respectively]. In contrast, no modification of ability of the μ opioid agonist DAMGO to inhibit basal adenylyl cyclase activity was observed in SCH 23390-treated rats as compared with saline-treated rats [F(1,20) = 2.891, P > .05] (data not shown).

Chronic treatment with the D1 dopamine receptor antagonist SCH 23390 did not significantly affect the stimulation of adenylyl cyclase by the D1 agonist SKF 38393 or the adenosine A2a receptor agonist CGS 21680 (table 1). However, chronic treatment with SCH 23390 resulted in a reduced inhibitory potency of the selective D2/D3 receptor agonist quinelorane as an inhibitor of CGS 21680-stimulated adenylyl cyclase; a rightward shift of the dose-response curve obtained with quinelorane was observed [F(1,19) = 27.992, P < .001] (fig. 3). Chronic treatment with SCH 23390 had no effect on the efficacy of DT-II as an inhibitor of SKF 38393-stimulated adenylyl cyclase activity [F(1,16) = 0.337, P > .05] (fig. 4), but, like chronic morphine treatment, chronic SCH 23390 blocked the ability of DT-II to inhibit CGS 21680-stimulated adenylyl cyclase [F(1,19) = 53.59, P < .001] without changing the inhibitory activity of DT-II against SKF 38393-stimulated enzyme.

Chronic treatment with the D2 dopamine antagonist eticlopride (0.25 mg/rat/day, for 7 days) induced a desensitization of D1 dopamine receptors. Thus the D1 dopamine agonist SKF 38393 displayed a lower ability to stimulate adenylyl cyclase activity in eticlopride-treated rats than in saline-treated rats [F(1,20) = 38.144, P < .001] (fig. 5). In contrast, no modification in the ability of μ (DAMGO), δ1 (DPDPE) or δ2 (DT-II) opioid agonists, or of the D2 dopamine agonist

### Table 1

<table>
<thead>
<tr>
<th>Chronic Treatments</th>
<th>Basal Activity</th>
<th>SKF 38393-Stimulated Activity (10 μM)</th>
<th>CGS 21680-Stimulated Activity (10 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>123 ± 19</td>
<td>194 ± 9 (158 ± 11%)</td>
<td>173 ± 11 (140 ± 9%)</td>
</tr>
<tr>
<td>Morphine</td>
<td>155 ± 7</td>
<td>247 ± 19 (160 ± 12%)</td>
<td>226 ± 19 (146 ± 12%)</td>
</tr>
<tr>
<td>SCH 23390</td>
<td>140 ± 8</td>
<td>200 ± 7 (144 ± 5%)</td>
<td>218 ± 10 (155 ± 7%)</td>
</tr>
<tr>
<td>Eticlopride</td>
<td>143 ± 6</td>
<td>175 ± 8 (122 ± 5%)</td>
<td>225 ± 19 (156 ± 13%)</td>
</tr>
<tr>
<td>Eticlopride and</td>
<td>152 ± 6</td>
<td>180 ± 7 (118 ± 2%)</td>
<td>252 ± 20 (166 ± 14%)</td>
</tr>
</tbody>
</table>

*See "Materials and Methods."*

*Significantly less stimulation of cAMP production by SKF 38393 after chronic eticlopride compared with saline treatment (P < .05).

*Significantly less stimulation of cAMP production by SKF 38393 after chronic eticlopride and morphine compared with morphine treatment (P < .01).
quinelorane, to inhibit basal adenylyl cyclase activity was observed after chronic eticlopride treatment (data not shown).

However, the desensitizing effects of chronic morphine treatment on \( \delta_1 \) and \( \delta_2 \) opioid receptor regulation of adenylyl cyclase activity were prevented by the concurrent administration of eticlopride. Two-way ANOVA analysis revealed no significant differences between control rats and animals treated with morphine and eticlopride in the ability of DPDPE \[ F(1,24) = 0.369, P > .05 \] or DT-II \[ F(1,19) = 0.840, P > .05 \] to inhibit adenylyl cyclase activity in caudate putamen. In contrast, a significant difference was observed between rats treated with morphine and eticlopride and animals chronically treated with morphine alone in the inhibition of adenylyl cyclase activity by the \( \delta_1 \) opioid agonist DPDPE \[ F(1,27) = 55.467, P < .001 \] or the \( \delta_2 \) opioid agonist DT-II \[ F(1,24) = 12.001, P < .01 \] (fig. 6). Similarly, eticlopride treatment prevented the morphine-induced loss of quinelorane inhibition of adenylyl cyclase activity \[ F(1,23) = 1.454, P > .05 \], comparing control with treatment with morphine and eticlopride; \( F(1,23) = 11.720, P < .01 \), comparing control with treatment with morphine alone (fig. 7).

Effects of chronic \( D_2 \) dopamine receptor antagonist treatment on inhibition of adenylyl cyclase activity induced by opioid agonists and dopamine agonists in the nucleus accumbens. As reported previously (Noble and Cox, 1996), chronic morphine treatment induced a selective impairment of \( \delta \) opioid receptors (\( \delta_1 \) and \( \delta_2 \)) but had no effect on the ability of the \( \mu \) opioid agonist to inhibit basal adenylyl cyclase activity (fig. 8). Two-way ANOVA analysis revealed a significant difference in the ability of the \( \delta_1 \) opioid agonist DPDPE to inhibit adenylyl cyclase activity in morphine-treated rats as compared with saline-treated rats \[ F(1,30) = 28.529, P < .001 \], as well as in the ability of the \( \delta_2 \) opioid agonist DT-II to inhibit the enzyme \[ F(1,31) = 28.749, P < .001 \], whereas no difference could be observed in the inhibi-

![Fig. 2. Effects of quinelorane on \( \Lambda_{\alpha} \) adenosine receptor-stimulated adenylyl cyclase activity in saline-treated rats (■) or morphine-treated rats (●) in the caudate putamen. Results are expressed in percentages of CGS 21680 (0.1 M)-stimulated adenylyl cyclase activity, as means ± S.E.M. from three or more independent experiments, each performed in triplicate. ★ indicates significant differences between treatments (P < .05; Newman-Keuls’ test).](image1)

![Fig. 3. Effects of DPDPE and DT-II on basal adenylyl cyclase activity or of quinelorane on CGS 21680-stimulated adenylyl cyclase activity in saline-treated rats (■) or SCH 23390-treated rats (●) in the caudate putamen. Results are expressed in percentages of basal adenylyl cyclase activity or in percentages of CGS 21680 (0.1 M)-stimulated adenylyl cyclase activity, as means ± S.E.M. from three or more independent experiments, each performed in triplicate. ★★ P < .01 and ★★ P < .01 indicate significant differences between treatments (Newman-Keuls’ test).](image2)
tion induced by the μ opioid agonist DAMGO \([F(1,35) = 0.001, P > .05]\).

Chronic treatment of morphine combined with chronic eticlopride treatment, which alone did not induce any modification in the ability of opioid agonists to inhibit adenylyl cyclase activity (data not shown), blocked the morphine-induced desensitization of δ opioid receptors in nucleus accum-bens (fig. 8). Two-way ANOVA analysis revealed no significant difference between control rats and animals treated with morphine and eticlopride in the ability of DPDPE \([F(1,29) = 1.239, P > .05]\) or DT-II \([F(1,27) = 2.050, P > .05]\) to inhibit adenylyl cyclase activity. In contrast, a significant difference could be observed between morphine-treated rats and animals chronically treated with both morphine and eticlopride in the ability of the δ₁ opioid agonist \([F(1,23) =

**Fig. 4.** Effects of DT-II on D₁ dopamine receptor-stimulated adenylyl cyclase activity or A₂a adenosine receptor-stimulated adenylyl cyclase activity in saline-treated rats (□) or SCH 23390-treated rats (●) in the caudate putamen. Results are expressed in percentages of SKF 38393 (10 μM)-stimulated adenylyl cyclase activity or in percentages of CGS 21680 (0.1 μM)-stimulated adenylyl cyclase activity, as means ± S.E.M. from three or more independent experiments, each performed in triplicate. ★ P < .05 indicates significant differences between treatments (Newman-Keuls' test).

**Fig. 5.** Effects of SKF 38393 on basal adenylyl cyclase activity in saline-treated rats (□) or eticlopride-treated rats (●) in the caudate putamen. Results are expressed in percentage of basal adenylyl cyclase activity as means ± S.E.M. from three or more independent experiments, each performed in triplicate. ★★ P < .01 and ★★★ P < .001 indicate significant differences between treatments (Newman-Keuls' test).

17.261, P < .001) on the δ₂ opioid agonist \([F(1,28) = 14.027, P < .001]\) to inhibit adenylyl cyclase activity.

**Discussion**

A selective impairment of δ opioid receptor function in the caudate putamen and the nucleus accumbens after chronic morphine treatment was confirmed in the present study. Moreover, the results obtained demonstrate that the D₂ dopamine antagonist eticlopride, administered chronically throughout the chronic morphine treatment, abolished this effect in both structures.

Several laboratories have obtained evidence supporting the hypothesis of heterogeneity of δ opioid receptors (Jiang et al., 1991; Mattia et al., 1991; Sofuoglu et al., 1991; Büzész et al., 1994; Noble and Cox, 1995). Furthermore, in the caudate putamen it has been shown that D₁ dopamine and A₂a adenosine receptors, which both increase adenylyl cyclase activity by stimulating Gₛ (Stiles, 1992; reviewed by Angulo and McEwen, 1994), are preferentially expressed by striato-nigral neurons and striato-pallidal neurons, respectively (Gerfen and Young, 1988; Gerfen et al., 1990; Le Moine et al., 1990; Schiffmann et al., 1991; reviewed by Gerfen, 1992; Augood and Emson, 1994). We have measured the ability of opioid agonists to inhibit selectively adenylyl cyclase activity measured in the presence of one activator of adenylyl cyclase but not in the presence of the other activator, and we suggest that such selective inhibitory activity is consistent with the hypothesis that the inhibitory receptor is located on the same cell membranes (and therefore on the same neuron population) as the activating receptor. Using this approach, differential neuronal locations in the caudate putamen of μ, δ₁ and δ₂ opioid receptors have been proposed (Noble and Cox, 1995). Thus μ opioid receptors that regulate adenylyl cyclase in caudate putamen appear to be preferentially expressed by striato-nigral neurons and δ₁ opioid re-
ceptors by striato-pallidal neurons, whereas δ1 opioid receptors appear to be expressed by both of these striatal efferent neuron populations. It is important to note that this differential distribution of μ and δ1 receptors may apply only to the opioid receptor types that regulate adenylyl cyclase activity. If separate subsets of μ and δ1 receptors mediate opioid regulation of ion channel function, then these receptor subsets may not share the same neuronal distribution. For this reason, immunocytochemical localization of opioid receptor types may not confirm the proposed distribution of the subsets of receptors that regulate adenylyl cyclase.

A reduced ability of the δ1 opioid agonist DPDPE (Mosberg et al., 1983; Jiang et al., 1991; Mattia et al., 1991; Sofuoglu et al., 1991) and of the δ2 opioid agonist DT-II (Erspamer et al., 1989; Jiang et al., 1991; Mattia et al., 1991; Sofuoglu et al., 1991) to inhibit basal adenylyl cyclase activity was observed in the caudate putamen and the nucleus accumbens after morphine treatment as compared with control animals (Noble and Cox, 1996). Because δ1 opioid receptors that regulate adenylyl cyclase appear to be present on both striato-nigral neurons and striato-pallidal neurons (Noble and Cox, 1995), it was interesting to determine whether this desensitization was unique to one of these populations by evaluating the effects of DT-II after selective stimulation of adenylyl cyclase with the D1 dopamine agonist SKF 38393 (striato-nigral neurons) or the A2a adenosine receptor agonist CGS 21680 (striato-pallidal neurons). After chronic morphine treatment, DT-II inhibited SKF 38393-stimulated adenylyl cyclase activity in the same intensity and concentration range as in control rats, whereas a reduced ability of the δ2 opioid agonist to inhibit CGS 21680-stimulated adenylyl cyclase activity was observed in tissues from the morphine-treated animals. These results indicate that after chronic morphine treatment, there is a selective impairment of δ1 opioid receptors localized on striato-pallidal neurons without alteration of δ2 opioid receptors expressed by striato-nigral neurons.

In situ hybridization studies have also demonstrated that D2 dopamine receptors, negatively coupled to adenylyl cyclase via G/Go proteins, are also preferentially expressed by striato-pallidal neurons but not by striato-nigral neurons (Le Moine et al., 1990; Gerfen et al., 1990; reviewed by Gerfen, 1992). In good agreement with this observation, the D2 dopamine agonist quinelorane was able to inhibit CGS 21680-stimulated adenylyl cyclase activity in control animals. This inhibitory effect was selectively antagonized by the D2 dopamine antagonist eticlopride. Quinelorane was significantly less effective in inhibiting adenylyl cyclase activity in morphine-treated animals than in control rats, which suggests that dopamine D2 receptor function in striato-pallidal neurons is impaired after chronic morphine treatment.
Because morphine is a μ-selective agonist (Matthes et al., 1996), the heterologous desensitization of the adenylyl cyclase-coupled receptors (i.e., of δ opioid receptors and D<sub>2</sub> dopamine receptors) in striato-pallidal neurons is probably the result of an indirect mechanism that involves other neurotransmitters. In previous experiments, it has been reported that chronic treatment with cocaine, an indirect dopamine agonist, induced a selective impairment of δ opioid receptors in the caudate putamen (Unterwald et al., 1993), a result that suggests involvement of the dopaminergic system in the effects observed in the present study. To examine the possible involvement of the dopaminergic system in these effects, we determined the effects of chronic D<sub>1</sub> dopamine antagonist (SCH 23390) and chronic D<sub>2</sub> dopamine antagonist (eticlopride) treatments on the adaptations in the regulation of adenylyl cyclase induced by chronic morphine treatment.

Chronic treatment with the D<sub>1</sub> antagonist SCH 23390 resulted in a desensitization of D<sub>2</sub> dopamine receptors and of δ<sub>1</sub> and δ<sub>2</sub> opioid receptors. The desensitization of the δ<sub>2</sub> opioid receptors was shown to be uniquely related to adenosine A<sub>2a</sub>-activated adenylyl cyclase, which indicated that the δ<sub>2</sub> receptor desensitization was specific for the δ<sub>2</sub> receptors expressed by striato-pallidal neurons. (Previous studies have suggested that δ<sub>1</sub> receptors that regulate adenylyl cyclase in caudate putamen are selectively localized on striato-pallidal neurons; Noble and Cox, 1995). Thus it appears that the effects of chronic morphine closely resemble those of chronic D<sub>1</sub> antagonist treatment: a selective impairment of receptors negatively coupled to adenylyl cyclase in striato-pallidal neurons without modification of those localized on striato-nigral neurons (fig. 9). In contrast, chronic treatment with the D<sub>2</sub> antagonist eticlopride did not change D<sub>2</sub> or opioid receptor regulation of adenylyl cyclase in caudate putamen but resulted in a partial desensitization of D<sub>1</sub>-stimulated adenylyl cyclase activity.

It has previously been shown that activation of μ receptors hyperpolarizes and thus reduces the release of transmitters from GABA neurons in the VTA (Johnson and North, 1992).

**Fig. 8.** Effects of DT-II, DPDPE or DAMGO on basal adenylyl cyclase activity in the nucleus accumbens of rats treated with saline (□), morphine (●) or morphine and eticlopride (○). Enzyme activity in the presence of inhibitor is expressed as a percent of basal adenylyl cyclase activity. Values are means ± S.E.M. from three or more independent experiments, each performed in triplicate. *P < .05 as compared with saline group; †P < .05 as compared with morphine/eticlopride group (Newman-Keuls’ test). The data points connected by dashed lines have previously been reported (Noble and Cox, 1996).

**Fig. 9.** Schematic representation of the effects of chronic morphine and SCH 23390 treatment on the regulation of adenylyl cyclase activity (AC) by stimulatory (D<sub>1</sub>, A<sub>2a</sub>) and inhibitory (D<sub>2</sub>, μ, δ<sub>1</sub>, δ<sub>2</sub>) receptors in caudate putamen. It is assumed that dopamine D<sub>1</sub> receptors selectively activate AC in striato-nigral neurons, whereas adenosine A<sub>2a</sub> receptors selectively activate AC in striato-pallidal neurons (Noble and Cox, 1995).
and other brain regions (Nicoll et al., 1980). In contrast, activation of D1 dopamine receptors facilitates GABA release in the VTA (Cameron and Williams, 1993) and in primary cultures of embryonic rat striatal neurons (Phillips and Cox, in preparation). We therefore propose that treatment with either the \( \mu \) agonist morphine or the D1 antagonist SCH 23390 results in reduced GABA neuron-mediated inhibition of the substantia nigra dopamine neurons and thus induces an increased release of dopamine in caudate putamen. The increased levels of dopamine have little consequence for the striato-nigral neurons, because either their D1 receptors are blocked by SCH 23390 or the neurons are inhibited by morphine acting on \( \mu \) receptors. During chronic morphine treatment, it is possible that coincident activation of G\(_i/o\) through D1 receptors counteracts adaptive changes induced by prolonged activation of G\(_i/o\) through \( \mu \) receptors in the striato-nigral neurons. In contrast, in the striato-pallidal neurons, the increased level of released dopamine after chronic morphine or chronic D1 antagonist treatment may result in increased activation of the D2 receptors, because these neurons are not directly regulated by either \( \mu \) agonists or D1 antagonists (Noble and Cox, 1995). In the striato-pallidal neurons, D2 and \( \delta \) receptor activation have similar consequences: an inhibition of adenyl cyclase activity via G\(_i\) activation. Treatments that result in enhanced activation of the striato-pallidal D2 receptors (e.g., chronic morphine or D1 antagonist) result in a heterologous desensitization in inhibitory receptor regulation of AC in this pathway. Mechanisms that might account for the heterologous desensitization of D2 receptors include a reduction in the activity or levels of G\(_i\) in these neurons (Childers, 1991; Izenwasser et al., 1993; Noble and Cox, 1995). However, direct measurement of G\(_i\) subunits in striatum after chronic morphine treatment failed to demonstrate any change in their amounts (Dr. T. E. Cote, personal communication). Another possibility is a D2 receptor-mediated sensitization of AC to activation through G\(_i\) (Watts and Neve, 1996), although this mechanism seems less likely because GCS 21680-stimulated AC activity was not changed by morphine or SCH 23390 treatment in the present study. The role of an initial activation of D2 receptors in the desensitization of \( \delta \) opioid receptors is confirmed by our observation that blockade of D2 dopamine receptors prevented the \( \delta \) opioid receptor desensitization normally observed after morphine treatment.

Chronic treatment of rats with cocaine or morphine also leads to long-term biochemical and functional changes in the nucleus accumbens, a brain region implicated in mediating the reinforcing effects of drugs of abuse (Goeders et al., 1984; Bozarth, 1986; Terwilliger et al., 1991; Unterwald et al., 1993; Izenwasser et al., 1996). Chronic morphine treatment also induced a selective impairment of \( \delta \) opioid receptors that regulate adenyl cyclase activity in this brain structure (Noble and Cox, 1996). In nucleus accumbens, as in caudate putamen, chronic eticlopride treatment administered concurrently with the chronic morphine treatment blocked the selective desensitization of \( \delta_1 \) and \( \delta_2 \) opioid receptors induced by morphine treatment. This result is consistent with previous reports that indicated an involvement of the mesolimbic dopamine system in mediating the motivational effects of opioids (Hand and Franklin, 1985; Smith et al., 1985; Shippenberg and Herz, 1988; Cadot et al., 1991). Our results indicate a critical role for dopamine in the mediation of morphine-induced opioid receptor desensitization in the nucleus accumbens.

In conclusion, it appears that chronic morphine treatment induced a selective desensitization of \( \delta \) opioid receptors in the caudate putamen and the nucleus accumbens via an indirect mechanism that involves the dopaminergic system. It is possible that under more intense chronic morphine treatments than those used in the present study, a direct desensitization of \( \mu \) opioid receptors might also be observed in both structures.

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