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The presynaptic modulation of corticostriatal afferents by μ-opioids is mediated by K^+ conductances

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Abstract

Population spikes associated with the paired pulse ratio protocol were used to measure the presynaptic inhibition of corticostriatal transmission caused by μ-opioid receptor activation. A 1 μM of [D-Ala_2, *N*-$MePhe_4$, Gly-ol_5]-enkephalin (DAMGO), a selective μ-opioid receptor agonist, enhanced paired pulse facilitation by 44 ± 8%. This effect was completely blocked by 2 nM of the selective μ-receptor antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-NH (CTOP). Antagonists of N- and P/Q-type Ca^{2+} channels inhibited, whereas antagonists of potassium channels enhanced, synaptic transmission. A 1 μM of ω-conotoxin GVIA, a blocker of N-type Ca^{2+} channels, had no effect on the action of DAMGO, but 400 nM ω-agatoxin TK, a blocker of P/Q-type Ca^{2+}-channels, partially blocked the action of this opioid. However, 5 mM Cs^{2+} and 400 μM Ba^{2+}, unselective antagonists of potassium conductances, completely prevented the action of DAMGO on corticostriatal transmission. These data suggest that presynaptic inhibition of corticostriatal afferents by μ-opioids is mediated by the modulation of K^+ conductances in corticostriatal afferents.

Keywords: μ-Opioid receptor; Opioid, presynaptic inhibition; K^+ channel; Striatum; Corticostriatal afferent

1. Introduction

The striatum contains one of the highest concentrations of opioid peptides in the brain (Graybiel et al., 1981; Graybiel, 1990). The density of μ-opioid receptors in the striatum is not uniform: they exist in higher density in the striosomal patches than in the matrix (Graybiel et al., 1981, Herkenham and Pert, 1981; Wang et al., 1996). Terminals containing leu-enkephalin can be seen making contacts at spines, where most corticostriatal glutamatergic afferents make their contacts (Wang and Pickel, 1998). The activation of opioid receptors modulates transmitter release presynaptically in many areas of the brain (Cherubini and North, 1985; Jiang and North, 1992; Capogna et al., 1993; Johnson and North, 1993; Schlösser et al., 1995; Ries et al., 1996; Stanford and Cooper, 1999). Accordingly, it has been found that, in corticostriatal synapses, μ- and δ-opioid receptors produce presynaptic inhibition (Jiang and North, 1992).

μ-Opioids decrease Ca^{2+} currents belonging to the N- (α_{1B}) and to the P/Q- (α_{1A}) type of Ca^{2+} channels (Cherubini and North, 1985; Rhim and Miller, 1994; Stefani et al., 1994; Twitchell and Rane, 1994; Soldo and Moises, 1997; Rusin and Moises, 1998; Connor et al., 1999; Endo and Yawo, 2000). N- (α_{1B}) type Ca^{2+} channels are modulated by opioids at some nerve terminals (Endo and Yawo, 2000). Therefore, opioids may regulate transmitter release by modulating Ca^{2+} influx at synaptic terminals (Endo and Yawo, 2000; Ostermeier et al., 2000). Both N- and P/Q-type Ca^{2+} channels are present at corticostriatal terminals (Lovinger et al., 1994; Bargas et al., 1998; Barral et al., 2001). Thus, a first question is whether presynaptic inhibition by μ-opioids is mediated by N- or P/Q-type Ca^{2+} channels at corticostriatal afferents.

μ-Opioids also modulate K^+ conductances (Cherubini and North, 1985; Chen and Yu, 1994; North et al., 1987; Rhim et al., 1993; Fan and Crain, 1995; Kovoor et al., 1995; Kobayashi et al., 1996; Muller et al., 1999; Piros et al., 2000). Among them are inward rectifying K^+ channels of the GIRK type (i.e., modulated by G proteins or $K_{IR}3$) (North et al.,

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1987; Kobayashi et al., 1996; Brunton and Charpak, 1998; Svodova and Lupica, 1998), Ca^{2+}-activated K^+ channels (Twitchell and Rane, 1994), cationic inward rectifiers (Svodova and Lupica, 1998) and voltage-activated K^+ channels (Zoltay and Cooper, 1993; Muller et al., 1999). There is evidence that, in some cases, presynaptic modulation of transmitter release occurs through K^+ channel facilitation (Miller, 1998; Meir et al., 1999). Therefore, another possibility is that μ-opioids exert their presynaptic effects through the modulation of K^+ channels. Although these two possibilities (Ca^{2+} and K^+) do not necessarily exclude each other, the present experiments were aimed to find the main conductance, Ca^{2+} or K^+, involved in the presynaptic inhibition by μ-opioids.

2. Materials and methods

Brain slices (400 μm) were obtained from male Wistar rats (100–120 g) as previously described (Barral et al., 1999). In our institution, animal treatment adheres to the European community guidelines for the use of experimental animals. Sagittal neostriatal slices were cut on a vibratome and incubated at room temperature in saline containing (in mM): 125 NaCl; 3 KCl; 1 $MgCl_2$; 2 $CaCl_2$; 25 $NaHCO_3$, 0.2 thiourea, 0.2 (−)-ascorbic acid and 11 glucose (saturated with 95% O_2 and 5% CO_2; 298 mosM/l; pH: 7.40). Individual slices were transferred to a submerged chamber at 32–34 °C. Superfusion rate was adjusted to 1–2 ml/min. Extracellular field potentials were obtained with micropipettes filled with 0.9% NaCl (2–4 MΩ) and records were obtained with an AC amplifier. Extracellular recordings were digitized and saved on VHS tapes at 40 kHz to be analysed off-line. All recordings were filtered at 1–3 kHz and digitized simultaneously by a digitizer (Instrutech, Great Neck, NY) and an AT-MIO-16E10 National Instruments (NI-DAQ) board in a PC clone using customized programs made at the laboratory in the Labview environment (National Instruments, Austin, TX). The digitizer was used to save the experiments on tape, while the NI-DAQ board was used to save the data as ASCII or binary files in the computer hard disk for further off-line analysis. Taped data could be displayed any number of times and sent to the computer through the NI-DAQ interface using the customized programs.

Field stimulation was done with concentric bipolar electrodes at the cortical white matter. Stimuli were a pair of brief square voltage pulses (4–40 V; 100–200 μs; 0.06–0.4 Hz) delivered with a stimulator. The time interval between the pair of stimuli was 15 to 50 ms. A total of 10 μM bicuculline was added to the bathing medium to eliminate the inhibitory component of the synaptic response due to $GABA_A$ receptor activation (Nisenbaum et al., 1992; Bargas et al., 1998).

With the paired pulse protocol, presynaptic inhibition is expected to enhance the paired pulse ratio (Barral et al., 2001), or S_2/S_1, of the responses to two stimuli of equal strength expressed as a percentage. S_2 is the second and S_1 is the first orthodromic response:

$$\%\mathrm{PPR} = 100 \times \left(\frac{S_2}{S_1} - 1\right) \quad (1)$$

Control stimuli were adjusted to see little or no paired pulse facilitation (PPR≈ S_2/S_1≈ 1). This made it possible to detect the paired pulse facilitation (PPF or PPR>1) produced by the μ-opioid receptor agonist [D-Ala_2, *N*-$MePhe_4$, Gly-ol_5]-enkephalin (DAMGO), as well as the effects of Ca^{2+} or K^+ channel antagonists. S_2/S_1 ratio was measured every 5 min and averaged. When the S_2/S_1 ratio was stable in the presence of a channel antagonist, the stimulus strength could be adjusted to recruit more afferents and restore, as much as possible, the control S_2/S_1 ratio. The rationale was that if a Ca^{2+} or K^+ conductance is necessary for the presynaptic action of DAMGO, then the blockage of this conductance should prevent or greatly reduce the action of DAMGO on PPR.

2.1. Drugs

D-Phe-Cys-Tyr-D-Trp-Orn-Thr-NH_2 (CTOP), [D-Ala_2, *N*-$MePhe_4$, Gly-ol_5]-enkephalin (DAMGO), bicuculline (RBI, Natick, MA), ω-conotoxin GVIA and ω-agatoxin-TK (Peptides Int., Louisville, KY) were all dissolved from freshly prepared stock solutions into the superfusion saline.

2.2. Data analysis

Statistical significance of PPR change was examined in each experiment, with the same tissue being its own control before DAMGO addition. The statistic used in this case was a paired non-parametric test (Wilcoxon's *T*; Systat, Evanston IL). The PPR change in percentage, of all samples, was thereafter compared with each other using analysis of variance (ANOVA; Systat).

3. Results

3.1. Presynaptic modulation by μ-opioid receptor activation

Fig. 1 illustrates the action of the μ-opioid receptor agonist, DAMGO (1 μM), on striatal field potentials evoked by cortical white matter stimulation. An initial application of the μ-opioid receptor antagonist, CTOP (2 nM), produced a small decrease (<10%) in control PPR or turned it into paired pulse depression (Fig. 1). This suggested a constitutive activation of μ-opioid receptors in the control condition. Thereafter, the addition of DAMGO (1 μM) in the presence of the antagonist CTOP did not produce a significant change in PPR. However, when the μ-opioid receptor antagonist was removed from the superfusion medium, the action of DAMGO could be seen as a rapid increase in PPF up to

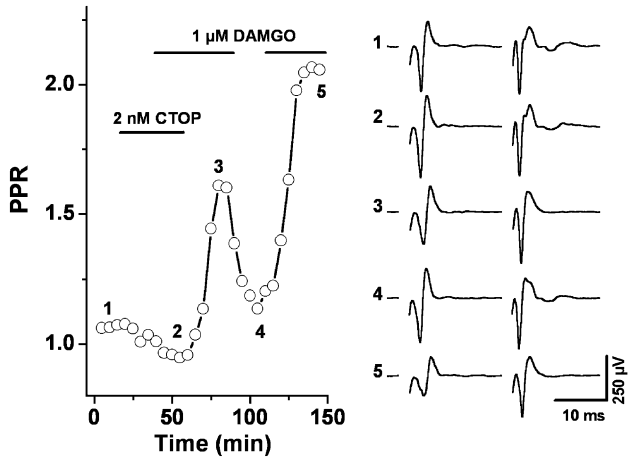

Fig. 1. Presynaptic inhibition produced by μ-opioid receptor activation. Left: Time course of a representative experiment. Right: Sample records from experiment depicted on the left. Numbers correspond to recording time. Horizontal bars indicate times of drug superfusion. Stimulus strength was adjusted to obtain PPR ≈ 1. Addition of CTOP (2 nM) produced a small decrease in PPR. Subsequent addition of DAMGO (1 μM) in the presence of CTOP had no effect. However, when the μ-opioid receptor antagonist, CTOP, was removed, the μ-opioid action could be observed. The effect could be washed out. Re-application of DAMGO produced the action again. Note that in addition to PPF, there was a decrease in the amplitude of both orthodromic (S_1 and S_2) responses.

(mean ± S.E.M.) 44 ± 8%, median = 35%, $n = 14$, $P < 0.001$ (Wilcoxon's T) (Fig. 1). This was accompanied by a reduction in the amplitude of both orthodromic components. The effect of DAMGO was reversible and it could be restored by re-application of the drug (Fig. 1). However, continuous application of DAMGO led to desensitization, seen as a gradual decrease in the response (Fig. 2; $n = 7$). The recovery from this fast desensitization was very slow (not shown) and precluded the construction of dose–response plots in the same preparation. These Results confirmed that opioids inhibit corticostriatal transmission presynaptically (Jiang and North, 1992). They also show that this action may occur through the activation of μ-opioid receptors and that the response is subject to desensitization.

3.2. Blockage of P/Q- but not N-type Ca^{2+} channels partially occludes μ-opiate modulation

N- and P/Q-type Ca^{2+} channels are involved in glutamate release from neostriatal afferents (Turner et al., 1993; Lovinger et al., 1994; Bargas et al., 1998; Hill and Brotchie, 1999; Barral et al., 2001). Thus, experiments were carried out with specific Ca^{2+} channel antagonists in order to see if any of them occluded the action of DAMGO. Thus, ω-conotoxin GVIA (1 μM) (Fig. 3A) and ω-agatoxin TK (400 nM) (Fig. 3B) were administered to block N- and P/Q-type Ca^{2+} channels, respectively (Teramoto et al., 1995; Wu and Saggau, 1997; Barral et al., 2001). Notice that the actions of both ω-conotoxin GVIA and ω-agatoxin TK were confirmed by PPR enhancement when administered before the opioid receptor agonist (Fig. 3A,B) (Bargas et al., 1998; Barral et al., 2001).

Fig. 3A shows that when the opioid receptor agonist DAMGO (1 μM) was applied in the presence of ω-CgTx GVIA (1 μM), an increase in PPR of 49 ± 4% was detected (median = 46%, $n = 8$, $P < 0.02$; Wilcoxon's T). This PPR increase was significant, indicating that ω-conotoxin GVIA did not prevent the action of DAMGO. Moreover, this modulation was not significantly different to that obtained in the absence of ω-conotoxin GVIA (see below and Fig. 5), suggesting that N-type Ca^{2+} channel blockade does not interfere with the actions of the opioid. It was therefore concluded that N-type Ca^{2+} channels do not mediate the effects of μ-opiates on corticostriatal transmission.

Fig. 3B shows a similar experiment in the presence of the P/Q-type Ca^{2+} channel blocker, ω-agatoxin TK (400 nM). This concentration of ω-agatoxin TK is saturating in this system (Barral et al., 2001). In this case, the action of DAMGO was partly occluded. Fig. 3B illustrates a representative case. Thus, PPR was increased an average of 17 ± 3% (median = 17%; $n = 6$; $P < 0.03$; Wilcoxon's T). This PPR increase, although small, was significant. This would not be the case if ω-agatoxin TK had blocked completely the action of DAMGO. Previous work validating the present protocol had shown that both cholinergic–muscarinic and GABAergic-$GABA_B$ receptor modulation can be completely blocked by ω-agatoxin TK (Barral et al., 1999, 2000). However, in the present case, only a partial occlusion was documented. These results suggested that P/Q-type Ca^{2+} channels might mediate an indirect effect (e.g., through Ca^{2+}-activated K^+ channels), that Ca^{2+} channels are not the main conductances involved in opioid actions, or finally, that another type of Ca^{2+} channel different from the N- or P/Q-types is also involved (e.g., R-type). Therefore, in the next experiments we chose to test the first two possibilities.

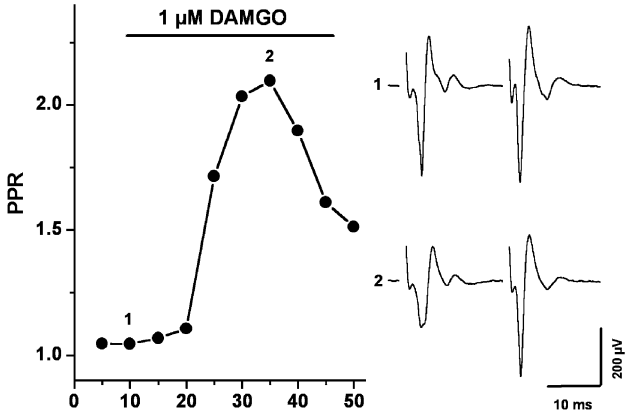

Fig. 2. Desensitization of presynaptic inhibition produced by μ-opioids. Left: Time course of a representative experiment. Right: Sample records from experiment depicted on the left. Numbers correspond to recording time. Horizontal bar indicates time of DAMGO application. Note that PPR change could not be maintained upon continuous DAMGO (1 μM) administration.

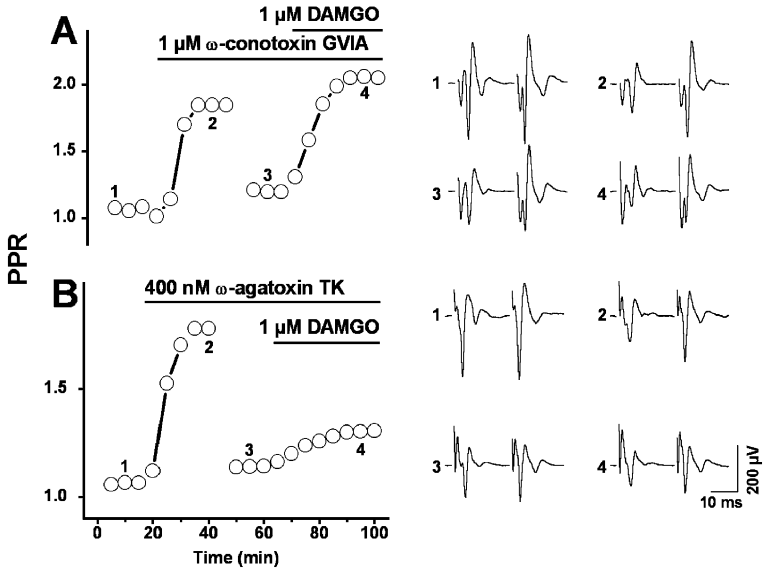

Fig. 3. Calcium channels antagonists do not completely prevent presynaptic inhibition produced by μ-opioid receptor activation. Left: Time courses of representative experiments. Right: Sample records from experiments depicted on the left. Numbers correspond to recording time. Horizontal bars indicate times of DAMGO and Ca^{2+}-channel antagonist application. (A) PPR (S_2/S_1 ratio) was enhanced by 1 μM of ω-conotoxin GVIA, indicating that N-type Ca^{2+} channels participate in glutamate release. After stable PPR was achieved, the stimulus strength was increased in order to recruit ω-conotoxin GVIA resistant terminals and to restore, as much is possible, the initial PPR. Addition of 1 μM DAMGO was able to enhance PPR in the presence of ω-conotoxin GVIA ($n=8$). (B) A similar representative experiment in the presence of 400 nM ω-agatoxin TK (P/Q-type Ca^{2+} channel blocker) shows the involvement of this channel type in glutamate release, i.e., increase in PPR ($n=6$). The action of DAMGO persisted after P/Q channel blockage. However, there was a partial inhibition.

3.3. Participation of K^+ channels in the presynaptic modulation by μ-opiates

Several classes of potassium channels are used by nerve terminals (Meir et al., 1999). With the possible exception of the fast A-type K^+ channel, many, if not all, K^+ channels types can be blocked by Cs^+ or barium Ba^{2+} when added to extracellular saline (Meir et al., 1999; Hille, 2001). Therefore, as a first step we investigated the effects of Cs^+ (5 mM) and Ba^{2+} (400 μM) on field potential PPR to see if K^+-conduc-

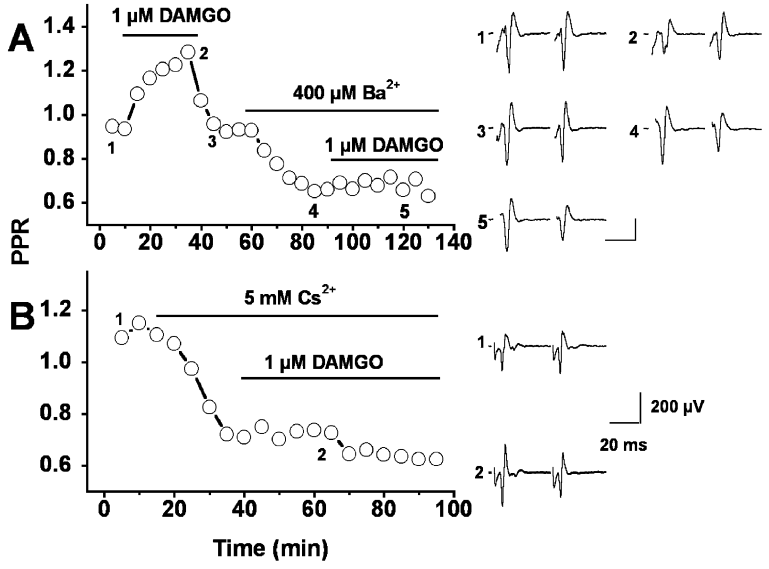

Fig. 4. K^+ channel antagonists occlude presynaptic inhibition produced by μ-opioid receptor activation. Left: Time courses of representative experiments. Right: Sample records from experiments depicted on the left. Numbers correspond to recording time. Horizontal bars indicate times of application of DAMGO and K^+ channel blockers. (A) Time course of one representative experiment shows first that 1 μM DAMGO produced usual PPF. DAMGO was washed out and followed by the addition of 400 μM Ba^{2+}, which produced paired pulse depression. Subsequent application of DAMGO, in the presence of Ba^{2+} action, had no effect. (B) A 5 mM of Cs^+ produced paired pulse depression. Addition of 1 μM DAMGO during Cs^+ action was unable to produce PPF ($n=4$). With the probable exception of fast A-type K^+ channels, Ba^{2+} and Cs^+ block most K^+ channel types (Hille, 2001).

tances are involved in regulating glutamate release at corticostriatal afferents. As shown in Fig. 4, both K^+ channel blockers decreased PPR and produced paired pulse depression. Cs^+ decreased PPR by 53 ± 12% (median = 53%, $n = 6$, $P < 0.03$; Wilcoxon's T), and Ba^{2+} decreased PPR to 33 ± 4% (median = 31%, $n = 5$, $P < 0.05$; Wilcoxon's T) of the control. In both cases, the effects were the expected ones: the blockage of K^+ conductances in the corticostriatal terminals increased the probability of transmitter release, thus producing paired pulse depression (Barral et al., 2001). Future work will determine which types of K^+ conductances are involved.

To test if K^+-conductances at corticostriatal terminals mediate μ-opioid receptor activation, 1 μM DAMGO was added once the effects of Cs^+ or Ba^{2+} (Fig. 4) had reached the steady state. In some experiments, such as that illustrated in Fig. 4A, the opioid receptor ligand was tested first to see its activity. Thereafter, it was washed out and then the K^+ channel blocker was added (e.g., 400 μM Ba^{2+} in Fig. 4A). In this condition, a second application of the opioid was without effect (Fig. 3; $n = 6$). Cs^+ (5 mM) also produced PPD and occluded the action of DAMGO (Fig. 3B; $n = 4$) (see Fig. 5). It was concluded that K^+ conductance blockade had more effect than Ca^{2+} conductance blockade in preventing the effect of DAMGO. Therefore, the results suggest that K^+-conductances at corticostriatal terminals are involved in the actions of DAMGO at these terminals.

Fig. 5 summarizes the results of these experiments. These are plotted as the percent increase in PPR after the addition of DAMGO to the bath saline in the absence or presence of several agents: ω-conotoxin GVIA, ω-agatoxin TK, Ba^{2+} and Cs^+. The analysis of variance between these groups (ANOVA), followed by a post-hoc Tukey test, showed that (a) DAMGO in the presence of ω-conotoxin GVIA (N-type Ca^{2+} channel block) had no effect ($P = 0.9$), (b) DAMGO in the presence of ω-agatoxin TK (P/Q-type Ca^{2+} channel block) had a borderline effect ($P = 0.07$), (c) DAMGO in the presence of Ba^{2+} (K^+ channel block) had a significant effect ($P < 0.003$), and (d) DAMGO in the presence of Cs^+ (K^+ channel block) had a significant effect ($P < 0.002$) (Fig. 4).

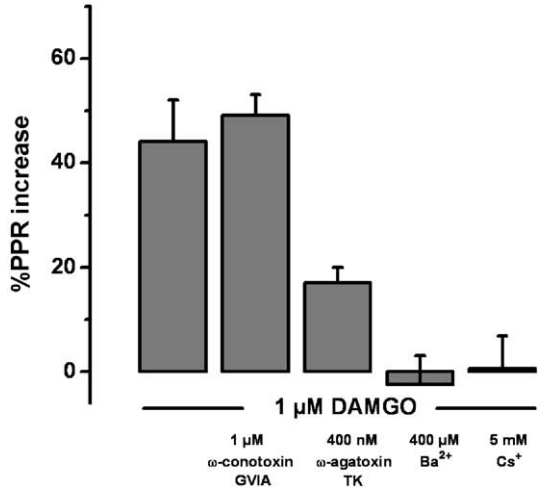

Fig. 5. Summary of μ-opioid actions in the presence of different channel antagonists. The histogram shows that in the presence of Ca^{2+} channel antagonists, the action of the μ-opioid receptor agonist, DAMGO, is not completely prevented. However, the blockage of K^+ channels with Ba^{2+} or Cs^+ occluded the effect of DAMGO on corticostriatal PPR.

4. Discussion

The present experiments confirmed the presynaptic modulation by opioids of corticostriatal afferents. They showed that this modulation can be mediated by μ-opioid receptors since it can be blocked by a selective receptor antagonist (CTOP) and induced by a selective receptor agonist (DAMGO) (Jiang and North 1992). Next, it was shown that Ca^{2+}-channel antagonists for N-type Ca^{2+} channels did not prevent the actions of the μ-opiod receptor agonist, demonstrating that presynaptic N-type Ca^{2+} channels do not mediate the actions of μ-opioids in corticostriatal afferents. However, a partial block of μ-opioid receptor activation was seen with ω-agatoxin TK. Finally, the present results showed that the blockage of K^+-conductances occluded the modulation of glutamate release at corticostriatal afferents induced by μ-opioids. In fact, the blockage of K^+ conductances produced paired pulse depression, i.e., enhanced release probability. Therefore, the results of the present work indicate that K^+ conductances mediate the presynaptic modulation of corticostriatal synapses by μ-opiates.

4.1. Presynaptic inhibition by μ-opioids

The confirmation of the presynaptic modulation by opiates of corticostriatal afferents, determined using the PPR protocol with field potentials, adds to the long list of presynaptic effects accurately measured with this technique. In several cases, independent confirmation with other techniques and protocols has been done (see refs. in Barral et al., 1999, 2000). In the present case, there is a previous report using intracellular recording (Jiang and North, 1992). Thus, the PPR protocol with field potentials is sufficiently robust to screen the presynaptic actions of modulators (Bargas et al., 1998; Barral et al., 1999, 2000, 2001).

Significant differences between the actions of the selective μ-opioid receptor agonist, DAMGO, and other presynaptic modulators of the same afferents became evident during the present study. For example, a dose–response study using several opioid concentrations on the same preparation (e.g., Barral et al., 1999, 2000, 2001) was not possible due to fast-desensitization of the opioid effects. Nonetheless, the concentration of DAMGO used was probably saturating since it was in the micromolar range (Calabrese, 2001). In spite of this, the maximal effect obtained was between 40% and 50% of the PPR increase. For comparison, the response was 250% with muscarine (Barral et al., 1999) and 450% with baclofen (Barral et al., 2000),

using the same preparation, technique and experimental protocol. The effect, then, seems comparatively small. One explanation for this difference is that DAMGO may not affect transmitter release in all recorded terminals, but only in some of them (e.g., Flores-Hernández et al., 1997). In agreement with this interpretation stands the fact that striatal opioid receptors are preferentially located in the striosomes (Graybiel, 1990; Herkenham and Pert, 1981; Wang et al., 1996). Hence, if mainly the terminals located on the striosomes contribute to the effect in the present recordings, this would explain why the PPR change was only about 50%, much less than the effect produced by more widely distributed receptors. In summary, fast desensitization and a quantitatively smaller effect than that elicited by other transmitters were characteristic of μ-opioid modulation.

4.2. Presynaptic modulation by μ-opioids preferentially involves the regulation of K^+-conductances

Another difference between the presynaptic modulation by opioids as compared to modulation by other transmitters is that μ-opioid receptor activation preferentially involves K^+ and not Ca^{2+} conductances. Opioid receptor ligands reduce Ca^{2+} (Cherubini and North, 1985; Rhim and Miller, 1994; Stefani et al., 1994; Twitchell and Rane, 1994; Soldo and Moises, 1997; Rusin and Moises, 1998; Connor et al., 1999; Endo and Yawo, 2000) and enhance K^+ conductances (Cherubini and North, 1985; Chen and Yu, 1994; North et al., 1987; Rhim et al., 1993; Fan and Crain, 1995; Kovoor et al., 1995; Kobayashi et al., 1996; Muller et al., 1999; Piros et al., 2000). Either of these two actions would inhibit transmitter release (Cherubini and North, 1985; Miller, 1998; Meir et al., 1999). In the present case, opioid effects were not occluded after N-type Ca^{2+} channel blockade, and only partially after P/Q-type Ca^{2+}-channel blockade (e.g., it could be mediated by indirect effects). This is in contrast with findings with other transmitters in which P/Q-type Ca^{2+} channel blockade virtually prevented all modulation by muscarine or baclofen (Barral et al., 1999, 2000). It is also different from that of other presynaptic terminals, where opioids modulate N-type Ca^{2+} channels (Endo and Yawo, 2000). Thus, the present work shows that blockade of K^+-conductances occluded all presynaptic effects of the μ-opiod receptor agonist DAMGO, suggesting that K^+ conductances mediate the effects of μ-opioids at corticostriatal afferents (Cherubini and North, 1985; Fredholm, 1990). Future work will determine the specific K^+ conductances involved.

K^+ conductance block is often used to increase release probability, which makes presynaptic inhibition produced by transmitters more evident (Fredholm, 1990; Ries et al., 1996; Flores-Hernández et al., 1997; Hill and Brotchie, 1999; Cepeda et al., 2001; Lambe and Aghajanian, 2001). Therefore, when K^+ conductance block prevents a presynaptic action, there is little doubt left about K^+ conductance involvement (Cherubini and North, 1985; Fredholm, 1990). K^+ conductances may control transmitter release (e.g., Augustine, 1990; Zoltay and Cooper, 1991; Robitaille and Charlton, 1992; Chen and Yu, 1994; Moreno et al., 1995; Roeper et al., 1997; Coetzee et al., 1999; Meir et al., 1999; Miralles and Solsona, 1996) by regulating action potential duration (Wheeler et al., 1996; Sabatini and Regher, 1997; Qian and Saggau, 1999). The variety of K^+ conductances involved in corticostriatal terminals is potentially great. Thus, future studies are needed to determine which channels are modulated. Presynaptic actions of opiates have been related before to K^+ conductance modulation at several presynaptic terminals (Cherubini and North, 1985; Fredholm, 1990; Rhim et al., 1993; Zoltay and Cooper, 1993; Ingram et al., 1998; Muller et al., 1999). Particularly interesting is the case for GIRK-type (Kir3) inward rectifier channels. These channels are subject to fast desensitization by opiate receptor ligands when expressed in Xenopus oocytes together with μ-receptors (Chen and Yu, 1994; Kovoor et al., 1995). Moreover, GIRK channels are present at some glutamatergic terminals, e.g., thalamo-cortical (Ponce et al., 1996), and are also present in pyramidal cells that project to the striatum (Karschin et al., 1996; Ostermeier et al., 2000). Therefore, an important question for future studies is whether this channel protein (GIRK) mediates opioid-induced fast-desensitizing presynaptic inhibition at corticostriatal terminals.

4.3. Functional and pharmacological implications

Opioid receptors are preferentially located on striosomes (Graybiel, 1990; Herkenham and Pert, 1981; Wang et al., 1996). Since we found only a partial regulation, indicating that only a few afferents were being modulated, it is logical to conclude that this modulation is perhaps mainly exerted on the afferents coming from temporolimbic cortices (Ragsdale and Graybiel, 1988; White and Hiroi, 1998). Striosomes project to dopaminergic cells in the substantia nigra compacta and ventral tegmental area (Jimenez-Castellanos and Graybiel, 1989; Gerfen, 1992; Nakano, 2000). Therefore, the receptors studied here may be a part of the "reward pathway". The motor "approach" behavior to environmental stimuli that represent reward is one of the basic mechanisms that animals have in order to adapt to their environment (Hollerman et al., 2000; Suri et al., 2001). The striatum selects motor programs for goal-directed activity based on the motivational significance of the outcome (Hikosaka, 1998). Motivational significance is coded by the dopaminergic system (Hollerman et al., 2000; Suri et al., 2001). The dopaminergic system can be controlled by excitatory inputs from the prefrontal cortex and the subthalamic nucleus (Sesack and Pickel, 1992; Benazzouz et al., 2000) and inhibitory entries from GABAergic interneurons and spiny neurons from the striosomes (Gerfen, 1992; Johnson and North, 1992). The present mechanism could be a part of the striosomal control. It would decrease the phasic

inhibition produced on dopamine cells by regulating the cortical input of striosomes. By disinhibiting dopamine cells, opiates may increase dopamine tone preceding motor behavior (Johnson and North, 1993; Hollerman et al., 2000). Probably, this mechanism is under the opposite regulation of dopaminergic and cholinergic activated protein kinase C and protein kinase A (Chen and Yu, 1994).

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