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Bremazocine is an agonist at κ-opioid receptors and an antagonist at μ-opioid receptors in the guinea-pig myenteric plexus

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1 The agonist and antagonist activity of bremazocine at opioid receptors in the guinea-pig myenteric plexus preparation was determined in untreated tissues and in tissues in which either μ- or κ-opioid receptors were blocked preferentially.

2 After pretreatment of the tissue with β-funaltrexamine for 90 min followed by washing out, the IC_{50} value of the selective μ-ligand [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was increased 67 fold whereas the IC_{50} values of the selective κ-ligand U-69,593 and of the non-selective κ-ligand bremazocine were not significantly changed. In this experimental design bremazocine acted only on κ-receptors.

3 After pretreatment of the tissue with β-chlornaltrexamine and 10 μM of the μ-ligand for 30 min followed by washout, the IC_{50} value of the μ-ligand was increased 2 fold whereas the IC_{50} value of the selective κ-ligand was increased 32 fold and that of bremazocine 62 fold. Under these experimental conditions, it was shown that bremazocine is an antagonist against [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin at the μ-receptor (K_e = 1.6 nM). The residual agonist activity of bremazocine is at the κ-receptor.

4 In naive myenteric plexus preparations the μ-antagonist activity of bremazocine cannot be demonstrated because its potency at the κ-receptor is very high. This dual action may be of importance for the responses of bremazocine in other peripheral and central tissues.

Introduction

Although widely used as a κ-opioid agonist, bremazocine has roughly equal affinities at the μ-, δ- and κ-binding sites (Magnan *et al.*, 1982). It has been shown that it is a pure antagonist at the δ-receptors in the hamster vas deferens (McKnight *et al.*, 1985) and that it is a pure antagonist in the isolated vas deferens of the rat, probably at μ-receptors (Gillan *et al.*, 1981). We have determined the antagonist action of bremazocine at the μ-opioid receptors in the guinea-pig myenteric plexus-longitudinal muscle preparation by the use of the non-selective opioid alkylating agent, β-chlornaltrexamine (Portoghese *et al.*, 1979; Goldstein & James, 1984). In our experiments κ-receptors were blocked by β-chlornaltrexamine while μ-receptors were protected by a high concentration of the selective μ-ligand [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin. Some of the results have been reported to the British Pharmacological Society (Corbett *et al.*, 1985a).

Methods

Bioassays

The myenteric plexus-longitudinal muscle preparation of the guinea-pig small intestine was set up for field stimulation as described previously (Corbett *et al.*, 1984). The potencies of agonists were obtained from dose-response curves with the exception that, after β-chlornaltrexamine pretreatment, the agonist IC_{50} values of bremazocine were determined by the 'single-dose' method (Kosterlitz & Watt, 1968). The antagonist equilibrium dissociation constant (K_e, nM) of bremazocine against [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was determined by this 'single-dose' method. The K_e values of naloxone were obtained by the method of Arunlakshana & Schild (1959).

Pre-incubation of myenteric plexus preparations with β-chlornaltrexamine or β-funaltrexamine

Myenteric plexus preparations were incubated in Krebs solution with β-chlornaltrexamine (30 nM) for

30 min and then washed with drug-free solution for 90 min; to prevent alkylation of μ-receptors, the selective μ-agonist [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (10 μM) was added to the organ bath 5 min before the addition of β-chlornaltrexamine. To block the μ-receptors irreversibly, preparations were incubated with β-funaltrexamine (100 nM) for 90 min followed by a 90 min wash with drug-free Krebs solution (Corbett *et al.*, 1985b). Agonist potencies were determined before and after β-chlornaltrexamine or β-funaltrexamine treatment.

Drugs

The following drugs were used: [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin and (−)-bremazocine hydrochloride (Dr D. Römer, Sandoz); U-69,593 (5α,7α,8β)-(−)-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl) benzeneacetamide (Dr R. Lahti, The Upjohn Company); β-chlornaltrexamine and β-funaltrexamine (Research Biochemicals Incorporated); naloxone hydrochloride (Endo Laboratories).

Results

Agonist action of bremazocine in bioassays

Bremazocine is a potent agonist in the myenteric plexus of the guinea-pig ileum which contains both μ- and κ-opioid receptors (Hutchinson *et al.*, 1975; Chavkin & Goldstein, 1981); the IC_{50} value is 0.16 ± 0.05 nM (Figure 1a; Table 1). The highly selective κ-compound U-69,593 (Corbett *et al.*, 1985a) was less potent with an IC_{50} value of 1.95 ± 0.28 nM ($n = 4$). A high concentration of naloxone (100 nM) was required to antagonize the agonist actions of bremazocine and of U-69,593. A concentration of 10 nM naloxone caused no significant antagonism of either agonist compound but an equiactive concentration of the μ-selective agonist [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (Corbett *et al.*, 1984) was completely antagonized by this concentration of naloxone (Figure 1a). The equilibrium dissociation constant (K_e) of naloxone was 14.2 ± 1.9 nM ($n = 4$) against bremazocine, 17.8 ± 3.8 nM ($n = 4$) against U-69,593 and 1.9 ± 0.3 nM ($n = 3$) against [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin. The slopes of the Schild plots were not significantly different from unity.

Blockade of the μ-receptors by pre-incubation of the myenteric plexus with β-funaltrexamine did not alter the agonist potency of bremazocine; the IC_{50} values were 0.14 ± 0.06 nM ($n = 4$) before pretreatment and 0.10 ± 0.03 nM afterwards. Following pre-incubation with β-funaltrexamine the K_e of naloxone against bremazocine was 13.2 ± 1.3 nM ($n = 4$). The agonist potency of U-69,593 also was unchanged, whereas the IC_{50} value of the μ-ligand [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was increased 67 fold, from 12.3 ± 1.47 nM to 820 ± 260 nM ($n = 6$).

Effects of pre-incubation with β-chlornaltrexamine on the activity of bremazocine in the guinea-pig myenteric plexus

After pre-incubation of the myenteric plexus with β-chlornaltrexamine in the presence of the protecting μ-ligand [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (10 μM), 15 nM of the κ-selective agonist U-69,593 inhibited the electrically-evoked contractions by 30% in the example shown whereas, in preparations not pretreated with β-chlornaltrexamine, 2 nM of U-69,593 caused a 60% depression of the contractions (Figure 1a and b). In contrast, the potency of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was little affected since the μ-receptors had been protected. The ratios, IC_{50} after pretreatment with β-chlornaltrexamine to IC_{50} before pretreatment, were 32 for U-69,593 and 2 for [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (Table 1). When the μ-receptors were not protected with [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin during pre-incubation with β-chlornaltrexamine, the dose-ratio for U-69,593 was 28 ($n = 5$) and that for [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was 18 ($n = 4$).

In untreated preparations or in preparations pretreated with β-funaltrexamine, bremazocine was an agonist without antagonist activity. When the μ-receptors in the myenteric plexus were protected during the pre-incubation with β-chlornaltrexamine, the agonist potency of bremazocine was markedly reduced, the IC_{50} values increasing from 0.16 to 10 nM (Figure 1c; Table 1); now bremazocine (5 nM) caused a reduction in the agonist activity of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (Figure 1c). The K_e value for this antagonist effect was 1.63 ± 0.31 nM ($n = 4$). Neither before nor after β-chlornaltrexamine pretreatment did U-69,593 display any antagonist activity.

After pre-incubation of the myenteric plexus with β-chlornaltrexamine and protection of the μ-receptors, the residual agonist actions of bremazocine and U-69,593 were not antagonized by 10 nM naloxone which is sufficient for antagonism at μ-receptors, whereas 100 nM gave complete antagonism (Figure 1c).

Discussion

In the guinea-pig myenteric plexus preparation the agonist action of bremazocine is on κ-opioid receptors. This view is based on the high K_e value of naloxone (13 nM) against the agonist action of bremazocine which is indicative of an action at κ-receptors. This K_e value is similar to that found for naloxone against the selective κ-agonist U-69,593

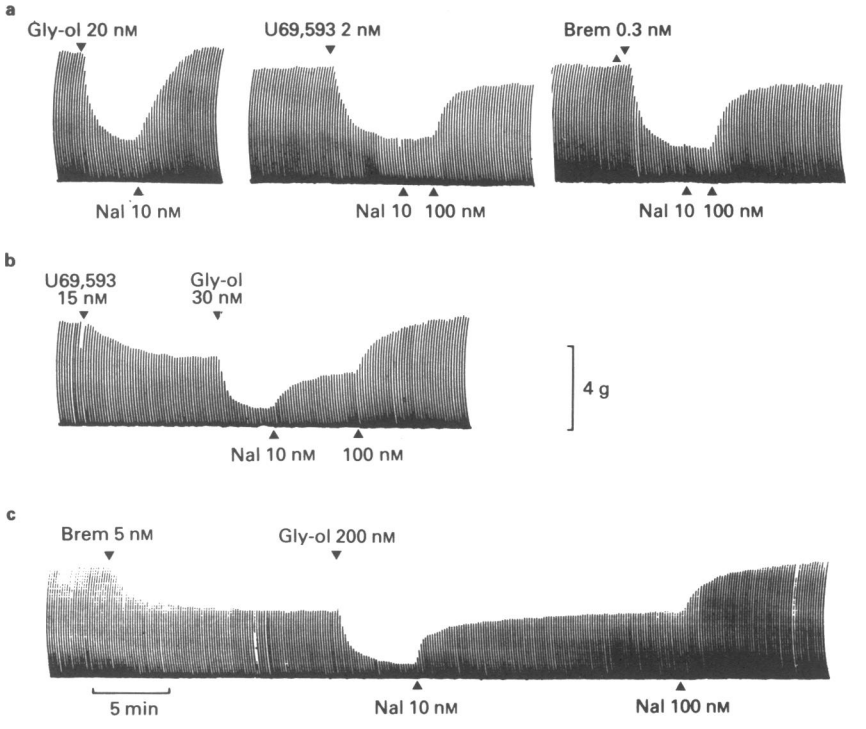

Figure 1 The effects of some opioid agonists and antagonists on the electrically-evoked contractions of the guinea-pig myenteric plexus-longitudinal muscle preparation. Drugs were added at the arrows. Brem is bremazocine; U-69,593 is a selective κ-agonist; Gly-ol is the selective μ-agonist [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin; Nal is naloxone. (a) Control preparation; (b and c) after pre-incubation of guinea-pig myenteric plexus with β-chlornaltrexamine and protection of μ-receptors with [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin. Note that in (a) and (b) 20 nM and 30 nM [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin were used whereas in (c), in the presence of 5 nM bremazocine, 200 nM [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was required to produce a similar depression.

Table 1 The effects of pre-incubation with β-chlornaltrexamine in the presence of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin on the agonist potencies of opioids in the myenteric plexus-longitudinal muscle preparation of the guinea-pig

	Agonist potency (IC_{50}, nM)		
Opioid	*Before* β-CNA	After β-CNA	Dose-ratio
[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin	16.6 ± 1.35	35.9 ± 6.6	2 (9)
U-69,593	1.28 ± 0.05	41.3 ± 3.33	32 (3)
(−)-Bremazocine	0.16 ± 0.05*	10.0 ± 4.9*,†	62 (4)

The values are the means ± s.e.mean; the number of observations is given in parentheses. Individual preparations of myenteric plexus were pre-incubated with 30 nM β-chlornaltrexamine (β-CNA) for 30 min, then washed for 90 min with drug-free Krebs solution. [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (10 μM) was added to the organ bath 5 min before β-CNA. Dose-ratio is the ratio, IC_{50} after pre-incubation with β-CNA to IC_{50} before treatment. *Agonist potencies before and after β-CNA were determined in different tissues. †The IC_{50} value of bremazocine was determined by the 'single-dose' method (Kosterlitz & Watt, 1968).

Table 2 The agonist and antagonist activity of some opioids in the guinea-pig myenteric plexus preparation

Drug	*Agonist activity* (IC_{50}, nM)	*Antagonist activity* (K_e, nM)	*Effective antagonist potency* (IC_{50}/K_e)
[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin	16.6	None	
U-69,593	1.3	None	
Bremazocine	0.16	None	
Naloxone*	>68,000	1.2	>56,000
Nalorphine*	24.3	4.5	5.4
After pre-incubation with β-FNA†			
[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin	820	None	
U-69,593	1.6	None	
Bremazocine	0.10	None	
After pre-incubation with β-CNA *and μ-protection‡*			
[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin	36	None	
U-69,593	41	None	
Bremazocine	10	1.6	6.1

The values are the means of at least 3 observations. *From Kosterlitz & Watt (1968). †Tissues were pre-incubated with 100 nM β-funaltrexamine (β-FNA) for 90 min, then washed for 90 min with drug-free Krebs solution. ‡Tissues were pre-incubated with 30 nM β-chlornaltrexamine (β-CNA) for 30 min, then washed for 90 min with drug-free Krebs solution. To protect the μ-receptors, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was added to the organ bath 5 min before β-CNA.

(18 nM) but higher than the K_e value against the selective μ-agonist [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (1.9 nM). However, it is important to note that bremazocine is a μ-opioid antagonist in the rat vas deferens (Gillan *et al.*, 1981) and also a δ-opioid antagonist in the hamster vas deferens (McKnight *et al.*, 1985). Furthermore, bremazocine antagonizes the antinociceptive effect of morphine in the mouse and precipitates abstinence jumping in morphine-dependent mice and rats (Römer *et al.*, 1980; Von Voigtlander *et al.*, 1982; 1983; Petrillo *et al.*, 1984). These *in vivo* findings also indicate that bremazocine has an antagonist action at μ-receptors.

In order to substantiate such a μ-antagonist action, it would be necessary to have an *in vitro* preparation which has a homogeneous population of μ-receptors. The guinea-pig myenteric plexus-longitudinal muscle preparation contains μ- and κ-opioid receptors (Hutchinson *et al.*, 1975; Chavkin & Goldstein, 1981). To show the antagonist activity of bremazocine at the μ-receptors in this preparation, it was necessary to block selectively the κ-receptors at which bremazocine is a potent agonist. This aim was achieved in myenteric plexus preparations pre-incubated with the opioid receptor alkylating agent β-chlornaltrexamine in the presence of the μ-ligand [D-Ala^2, MePhe^4,Gly-ol^5]enkephalin at a concentration sufficiently high (10 μM) to protect μ-receptors. It was found that after this treatment, the IC_{50} value of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was increased only 2 fold whereas the IC_{50} value of the κ-ligand U-69,593 was increased by more than 30 fold. Thus, although full protection of the μ-receptors was not achieved in our experiments a large proportion of the κ-receptors was blocked. With an identical experimental design the IC_{50} value of bremazocine was increased 62 fold, which is further evidence for the agonist action of bremazocine at κ-receptors. This view is supported by the fact that a high concentration of naloxone was required to antagonize the action of bremazocine after β-chlornaltrexamine pretreatment.

Blockade of κ-receptors by pre-incubation with β-chlornaltrexamine made it possible to observe the antagonist action of bremazocine at the protected μ-receptors when the K_e value against [D-Ala^2,MePhe^4,Gly-ol^5] enkephalin was 1.6 nM. A measure of the antagonist activity of opioids with both agonist and antagonist actions is obtained by calculation of the effective antagonist potency, IC_{50}/K_e (Kosterlitz & Watt, 1968). The effective antagonist potency of a pure agonist such as [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin or U-69,593 is zero whereas that of a pure antagonist is very high, e.g. >56,000 for naloxone (Table 2). An agonist-antagonist compound has an effective antagonist potency lying somewhere between these extremes, such as 5.4 for nalorphine. In myenteric plexus preparations not treated with β-chlornaltrexamine the effective antagonist potency of

bremazocine is zero, as it is in preparations pre-incubated for 90 min with the irreversible, μ-receptor blocking agent, β-funaltrexamine (Portoghese *et al.*, 1980). We have previously shown that in guinea-pig myenteric plexus this pretreatment selectively blocks the μ-opioid receptors without affecting the κ-receptors (Corbett *et al.*, 1985b). However, when agonist activity at the κ-receptors is almost blocked by pre-incubation with β-chlornaltrexamine and the μ-receptors protected, the effective antagonist potency of bremazocine is 6.1. Although bremazocine has retained some agonist action at κ-receptors, it is now an antagonist at the μ-receptors protected with [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin. Therefore, after partial blockade of κ-receptors in the myenteric plexus, bremazocine is a compound with the dual actions of a κ-agonist and a μ-antagonist but without β-chlornaltrexamine pretreatment only the agonist activity at κ-receptors can be shown. A corollary is the lack of agonist activity of bremazocine in the rat vas deferens which has μ-receptors but no κ- or δ-receptors since in this tissue it is an antagonist (Gillan *et al.*, 1981).

Thus, the pharmacological activity of bremazocine is determined by the relative proportion of μ, δ- and κ-opioid receptors at an effector tissue; furthermore, it is important, if possible, to use agonist and antagonist ligands that are selective for only one opioid receptor.

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