Acute withdrawal after bremazocine and the interaction between μ - and κ -opioid receptors in isolated gut tissues

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1 This study was undertaken to investigate whether, after a brief exposure of guinea-pig isolated ileum and rabbit jejunum to bremazocine, a κ -opioid agonist also possessing antagonist activity at μ -opioid receptors, the addition of opioid antagonists produced withdrawal contractures. Our aim was to verify in these tissues the existence of an interaction between the μ - and κ -opioid systems.

2 In guinea-pig ileum preparations previously exposed for 5 min to bremazocine at 5.7×10^{-7} M and 5.7×10^{-8} M, naloxone (5×10^{-7} M) elicited no response whereas in tissues exposed to a lower bremazocine concentration (5.7×10^{-9} M), naloxone (5×10^{-7} M) and the selective κ -opioid antagonist, nor-binaltorphimine (3.4×10^{-8} M) both produced a strong contracture.

3 Bremazocine (5.7×10^{-7} M) administered to guinea-pig isolated ileum, previously exposed for 5 min to morphine (10^{-7} M), induced a withdrawal contracture. In contrast, lower bremazocine concentrations (1.4 and 7.1×10^{-8} M) did not elicit a withdrawal contracture.

4 Naloxone (5×10^{-7} M), added to the bath after a 5 min exposure of guinea-pig ileum to morphine (10^{-7} M), elicited the characteristic withdrawal contracture. Bremazocine (1.4 – 7.1×10^{-8} M) added 1 min before naloxone (5×10^{-7} M) inhibited the naloxone withdrawal contracture in a dose-related way whereas naloxone 5×10^{-8} M added 1 min before naloxone 5×10^{-7} M, did not affect the withdrawal response.

5 In the rabbit jejunum, bremazocine (1.4 – 7.1×10^{-8} M) caused a decrease in amplitude in the spontaneous tissue activity. In tissues exposed to these bremazocine concentrations, naloxone (5×10^{-7} M) elicited a marked contracture. A similar contracture occurred when nor-binaltorphimine (3.4×10^{-8} M) was added in place of naloxone. These effects were dose-related to the bremazocine concentration. The specific κ -agonist, U-50,488H (5×10^{-8} M), elicited the same effects as bremazocine.

6 These findings show that stimulation of κ -opioid receptors induces a state of dependence that is not prevented by blocking the μ -opioid system. The observation that low bremazocine concentrations inhibit the morphine-induced withdrawal contractures, indicates an interaction between the μ - and κ -opioid system in guinea-pig isolated ileum, similar to that observed in the whole animal.

Keywords: Bremazocine; κ -opioid agonists; μ -antagonist; κ -induced dependence; withdrawal inhibition; gut isolated tissues

Introduction

Although opiate dependence appears to be mediated primarily by μ -opioid receptors, recent studies have shown that chronic administration of κ -opioid agonists causes physical dependence to develop in monkeys and rats (Young & Khazan, 1985; Gmerek *et al.*, 1987; Jaw *et al.*, 1993). The withdrawal signs developing in animals chronically exposed to κ -agonists differ from those occurring in animals exposed to μ -agonists. μ -Agonist administration does not reverse withdrawal signs in the κ -dependent animal and *vice versa* (Gmerek *et al.*, 1987; Fukagawa *et al.*, 1989; Brent *et al.*, 1993).

Hence, the processes of adaptation leading to dependence appear to be determined by the distinct activation of either μ - or κ -opioid receptors. Accordingly, in our previous study in guinea-pig ileum and rabbit jejunum we showed that the selective κ -opioid antagonist, nor-binaltorphimine, induced a sustained contraction in isolated preparations previously exposed to the selective κ -opioid agonist, U-50,488H. The addition of nor-binaltorphimine, before opioid agonists, also prevented the naloxone-induced contracture in tissues previously exposed to U-50,488H but not in those previously exposed to morphine (Valeri *et al.*, 1990b; 1992; Morrone *et al.*, 1993).

On the other hand, some reports have indicated the existence in the development and expression of opiate dependence of interactions between the various opioid receptor subtypes. In mice, the development of morphine

dependence is prevented by the selective blockade of δ -opioid receptors (Abdelhamid *et al.*, 1991; Miyamoto *et al.*, 1993) and is potentiated by the selective blockade of the κ -opioid system (Suzuki *et al.*, 1992). In the guinea-pig ileum made dependent to μ -receptor agonists, κ -agonists suppress the withdrawal contracture to naloxone (Schulz *et al.*, 1985; Chahl, 1990).

When challenged with naloxone or with selective antagonists, gut isolated preparations briefly exposed to opiates display a withdrawal contracture, the expression of acute dependence (Chahl 1986; Valeri *et al.*, 1990a,b; 1992; Morrone *et al.*, 1993).

We undertook this work to investigate whether the brief activation of κ -opioid receptors in isolated gut preparations, by a drug also having antagonistic activity at μ -opioid receptors, would induce dependence, as does the selective κ -agonist, U-50,488H (Morrone *et al.*, 1993). We also studied the effects of κ -receptor activation on the naloxone-induced contracture after exposure of the gut isolated tissues to morphine, to observe the possible interaction between κ - and μ -receptors under experimental conditions unlike those of previous studies (Schulz *et al.*, 1985; Chahl, 1990). For these purposes, we used bremazocine, a drug known to be a potent κ -agonist and a μ -antagonist (Romer *et al.*, 1980; Petrillo *et al.*, 1984; Corbett & Kosterlitz, 1986). The study was carried out in guinea-pig isolated ileum, which contains μ - and κ -opioid receptors and rabbit jejunum, which possesses κ - and δ -opioid receptors (Hutchinson *et al.*, 1975; Valeri *et al.*, 1992). These tissues are currently used to study the homeostatic mechanism involved in the dependence phenomena.

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Methods

The experimental procedures were essentially those used previously (Valeri *et al.*, 1990a; 1992). Adult male guinea-pigs weighing 300–350 g and New Zealand White rabbits weighing 2–2.5 kg were killed by a blow on the neck and bled. Three to five segments of guinea-pig ileum or rabbit jejunum, 2–3 cm long, were removed from the same animal, suspended under 1 g tension in a 10 ml tissue organ bath containing Tyrode solution at 37°C and gassed with 95% O₂ and 5% CO₂. The tissues were connected to an isotonic force transducer connected to a pen recorder (Ugo Basile, 7006 and 7050 Italy) and allowed to equilibrate for 30–45 min.

At the start of each experiment a maximum response to acetylcholine (ACh, 10⁻⁶ M) was obtained in each tissue in order to check its suitability and to express the responses to opioid antagonists as percentages of the ACh maximum. Each experiment was repeated on at least four separate preparations obtained from different animals.

Naloxone- and nor-binaltorphimine-induced withdrawal contractures after bremazocine exposure of guinea-pig ileum and rabbit jejunum

Because previous studies with the κ -agonist, U-50,488H, had shown that the state of dependence of the tissue could not be revealed if the agonist concentration was too high (Morrone *et al.*, 1993), we tested decreasing doses of bremazocine, starting from 6.3×10^{-7} M. The effects of naloxone (5×10^{-7} M) and nor-binaltorphimine (5×10^{-8} M), a selective κ -opioid antagonist, were tested after leaving the tissues for 5 min in contact with bremazocine or U-50,488H. Regardless of whether a contractile response occurred, after the addition to the bath of the opioid antagonists the preparations were washed out. The antagonists were therefore in contact with the tissues for no more than 2–3 min. In experiments with guinea-pig ileum, after washout and a resting period of 20–30 min the same tissue sample was used for further tests. Because the antagonist activity of nor-binaltorphimine faded out very slowly, after contact with this antagonist the preparations were washed out again three or four times during the resting interval. Rabbit jejunum tissues were always discarded after the first exposure to nor-binaltorphimine, because preliminary observations had shown that they no longer yielded reproducible responses. In experiments using naloxone as the antagonist, after washout and a 25–30 min resting period the same tissue preparation continued to provide reproducible responses.

Bremazocine-induced withdrawal contractures after exposure of guinea-pig ileum to morphine

The tissues were exposed to morphine (10⁻⁷ M) for 5 min before the addition of naloxone (5×10^{-7} M) or bremazocine (1.4 – 63×10^{-8} M). The same tissue sample was used for several tests, after washout and a resting period of 20–30 min. Between tests with bremazocine control tests were performed with morphine and naloxone.

Bremazocine inhibition of naloxone-induced withdrawal contractures in guinea-pig ileum after morphine exposure

The tissues were exposed to morphine (10⁻⁷ M) for 5 min before the injection of naloxone (5×10^{-7} M). Bremazocine (1.4 – 7.1×10^{-8} M) and naloxone 5×10^{-8} M were injected into the bath 1 min before naloxone 5×10^{-7} M.

Drugs

Morphine hydrochloride and naloxone hydrochloride were purchased from SIFAC (Milan, Italy), nor-binaltorphimine dihydrochloride from Research Biochemical Inc. (Natick,

MA, U.S.A.), U-50,488H (trans-($\pm$)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzenacetamide methane sulphonate salt) from Upjohn Co. (Kalamazoo, MI, U.S.A.) and bremazocine from Sandoz Ltd (Basel, Switzerland).

Results

Withdrawal responses after exposure of guinea-pig ileum to decreasing concentrations of bremazocine

In tissues exposed for 5 min to U-50,488H (5.4×10^{-8} M), naloxone (5×10^{-7} M) elicited a strong contractile response (Figure 1a). When the same preparation was washed out, left to rest for 25 min and exposed to bremazocine at 5.7×10^{-7} M or 5.7×10^{-8} M, naloxone did not elicit a contracture (Figure 1b and c). In the control tests performed after every dose of bremazocine, naloxone injected after exposure to U-50,488H caused in most tissues a contractile response similar in intensity to the first response. In some tissues, however, particularly those exposed to the highest concentrations of bremazocine, naloxone elicited only a very low-intensity contracture or even no response at all (not shown). These preparations were discarded.

When the bremazocine concentration was reduced to 5.7×10^{-9} M, naloxone caused a sustained contractile response (Figure 1d). In tissues treated with this bremazocine concentration, washed out and left to rest for 20–30 min, naloxone elicited reproducible responses.

The ileum preparations exposed for 5 min to the lowest dose of bremazocine contracted also when challenged with the specific κ -opioid antagonist, nor-binaltorphimine (3.4×10^{-8} M); after washout and a 20–30 min interval between tests, a further exposure to the same concentration of bremazocine followed by nor-binaltorphimine continued to elicit responses of the same intensity as before (Figure 2a). These responses appeared to be maximal, because after a 20 min contact with bremazocine the antagonist still determined a contractile response of the same height ($81 \pm 12.5\%$ of the maximum to ACh in 10 preparations from 5 individual animals) as that obtained after 5 min of contact (Figure 2b).

Withdrawal responses after exposure of rabbit jejunum to bremazocine

Addition of bremazocine (5.5×10^{-8} M) to rabbit jejunum caused a decrease in the amplitude of the spontaneous tissue

Figure 1 Guinea-pig ileum contractile responses to naloxone (N) after a 5 min exposure of the tissue to U-50,488H (U-50) or bremazocine (BM). A contractile response is elicited by N (5×10^{-7} M) after exposure to U-50 (5.4×10^{-8} M) (a). After exposure to BM concentrations of 5.7×10^{-7} (b) and 5.7×10^{-8} M (c), N elicited no response. An N-induced contracture was obtained after exposure to BM 5.7×10^{-9} M (d). Tracings (a) and (b) were recorded from the same preparation, tracings (c) and (d) from another. Interval between tests: 25–30 min. ACh max: acetylcholine (10^{-6} M); (■) washout.

activity, sometimes accompanied by a relaxation of the muscle, as did U-50,488H (5×10^{-8} M). After a 5 min contact with bremazocine or with U-50,488H (same concentrations) the addition of naloxone (5×10^{-7} M) caused a marked contracture of the tissue (Figure 3a and b). After washout and a 25–30 min interval, and a further 5 min exposure to bremazocine, naloxone (5×10^{-7} M) caused responses of almost identical intensity in many preparations, so that the same tissue could be used for several tests. The decreases in spontaneous activity and in the height of the contractile responses to naloxone were dose-related to the bremazocine concentrations studied (1.4 – 7.1×10^{-8} M).

After exposure of the tissue to bremazocine or U-50,488H (same concentrations as above), nor-binaltorphimine (3.4×10^{-8} M) added in place of naloxone caused a similar contractile response (Figure 3c and d). The tissues completely lost their responsiveness to the κ -opioid agonist and antagonist after contact with nor-binaltorphimine, but retained their responsiveness after contact with naloxone. Only few preparations recovered their responsiveness after contact with norbinaltorphimine and did only after a prolonged interval, with several washouts (not shown).

Bremazocine-induced withdrawal responses in guinea-pig ileum after exposure to morphine

In guinea-pig ileum exposed to morphine (10^{-7} M) for 5 min, naloxone (5×10^{-7} M) induced a contractile response (Figure 4a). In experiments designed to study the μ -antagonist activity of bremazocine in the guinea-pig isolated ileum, after a 5 min exposure of the tissue to morphine (10^{-7} M) bremazocine was added to the bath in place of naloxone. Whereas bremazocine concentrations of 1.4×10^{-8} M and 7.1×10^{-8} M failed to contract the morphine-exposed tissues (Figure 4a and b) a higher bremazocine concentration, 5.7×10^{-7} M, close to that of naloxone, yielded a strong contracture as intense as that elicited by naloxone (Figure 4c).

After every dose of bremazocine and before a further bremazocine test, the suitability of the preparation was checked by challenging the tissue with morphine and naloxone. These control tests showed that about 50% of the isolated preparations lost their ability to contract to naloxone, sometimes even after the first exposure to bremazocine.

Figure 2 Reproducible contractile responses of guinea-pig ileum to nor-binaltorphimine (nor-BNI) after 5 and 20 min exposure of the tissue to bremazocine (BM). After a 5 min exposure to BM (5.7×10^{-9} M), nor-BNI (3.4×10^{-8} M) elicited a contractile response; after a 25–30 min interval, including 3–4 washouts, and a further 5 min exposure to BM (5.7×10^{-9} M), nor-BNI (3.4×10^{-8} M) induced a contracture of the same amplitude as the first (a). After a 20 min exposure to BM (5.7×10^{-9} M) nor-BNI (3.4×10^{-8} M) still elicited a contracture of the same amplitude (b). All tracings shown in figure were obtained from the same tissue preparation, with a 25–30 min interval and 3–4 washouts between tests. ACh max: acetylcholine (10^{-6} M); (■) washout.

Antagonist activity of bremazocine on naloxone-induced contractures after morphine

As described above, naloxone (5×10^{-7} M) induced a contracture in guinea-pig ileum that had been exposed to morphine (10^{-7} M) for 5 min (Figure 5a). The addition, 1 min before the antagonist, of bremazocine 1.4×10^{-8} M reduced the naloxone-induced contracture (Figure 5b); this response was completely inhibited by bremazocine at 7.1×10^{-8} M (Figure 5c), i.e., a concentration 10 fold lower than that causing a contractile response in the morphine-exposed tissue.

To verify that this inhibitory effect was not due to antagonist activity at μ -receptors, a low dose of naloxone (5×10^{-8} M) was administered 1 min before the naloxone concentration known to elicit a withdrawal response (5×10^{-7} M). In most preparations, when this low concentration of naloxone was administered 4 or 5 min after morphine, it did not elicit contractures, as previously observed (Valeri *et al.*, 1990). Administration of naloxone (5×10^{-8} M) 1 min before naloxone (5×10^{-7} M) left the withdrawal response to the higher dose unchanged (Figure 5d).

Figure 3 Typical tracings showing the effect of U-50,488H (U-50) and bremazocine (BM) on the rabbit jejunum spontaneous activity and the contractile response of the tissue to naloxone (N) and nor-binaltorphimine (nor-BNI) after a 5 min exposure of the tissue to U-50 or BM. Both U-50 (5.4×10^{-8} M; a and c) and BM (5.5×10^{-8} M; b and d) caused a decrease in the spontaneous tissue activity. N (5×10^{-7} M) elicited a strong contracture after a 5 min exposure of the tissue to U-50 (a) or BM (b). Nor-BNI (3.4×10^{-8} M) also elicited a contractile response, after a 5 min exposure to U-50 (c) or BM (d). Tracings (a), (b) and (c) were recorded from the same tissue preparation with a 25–30 min interval between tests; tracing (d) was from another preparation. ACh max: acetylcholine (10^{-6} M); (■) washout.

Figure 4 Guinea-pig ileum contractile responses to naloxone (N) and bremazocine (BM) after exposure of the tissue to morphine (M). N (5×10^{-7} M) elicited a contractile response after a 5 min exposure to M (10^{-7} M) (a). Neither BM 1.4×10^{-8} M (a) nor 7.1×10^{-8} M (b) elicited a contracture after exposure to M 10^{-7} M. In contrast, BM 5.7×10^{-7} M elicited a contractile response, after the usual 5 min exposure to M 10^{-7} M. ACh max: acetylcholine (10^{-6} M); (■) washout.

Figure 5 Bremazocine (BM) inhibition of guinea-pig ileum contractile response to naloxone (N) after exposure of the tissue to morphine (M). N (5×10^{-7} M) elicited the characteristic contractile response after a 5 min exposure to M (10^{-7} M) (a). At a concentration of 1.4×10^{-8} M BM added 1 min before N (5×10^{-7} M) reduced (b) and at a concentration of 7.1×10^{-8} M completely inhibited (c) the contractile response to N. N 5×10^{-8} M, added 1 min before N 5×10^{-7} M, failed to affect the contractile response to the higher dose of N. Tracings (a) and (b) were recorded from the same tissue preparation, tracings (c) and (d) from two other preparations. ACh max: acetylcholine (10^{-6} M); (■) washout.

Discussion

This study shows that after a brief exposure to bremazocine, isolated preparations of guinea-pig ileum and rabbit jejunum respond to the addition of both the non-selective opioid antagonist, naloxone and the selective κ -opioid antagonist, nor-binaltorphimine, with a withdrawal contracture resembling that induced by exposure to U-50,488H. These observations confirm that acute activation of κ -opioid receptors induces homeostatic changes that lead to the development of dependence (Valeri et al., 1992; Morrone et al., 1993). Our *in vitro* results agree with those obtained in the whole animal. *In vivo* experiments, however, reveal a difference between withdrawal behaviours induced by κ -opioid agonists and those induced by morphine (Gmerek et al., 1987; Fukagawa et al., 1989; Brent et al., 1993). This distinction cannot be revealed in guinea-pig isolated ileum and rabbit jejunum, because in these tissues the acute activation of μ -, κ - or δ -opioid receptors induces the same withdrawal symptom, i.e., the contractile response to opioid antagonists (Valeri et al., 1992; Morrone et al., 1993; Brent et al., 1993). In the myenteric plexus, the development of dependence to μ -, κ - and δ -opioid receptors is due to multistep homeostatic changes that converge to depress the cholinergic neurone and the expression of dependence is revealed mainly by excitation of this neurone. In the central nervous system, opioid-induced homeostatic changes of action and reaction involve many integrated circuits working with characteristic neurotransmitters and neuromodulators, thus leading to a variety of withdrawal signs. The differences between withdrawal responses in gut isolated preparations and in the whole animal therefore reflect the different complexity of the neuronal circuits involved, but the two models probably share the same basic mechanisms.

Our results also suggest that μ -opioid receptor blockade does not prevent the development of dependence to κ -antagonists, since κ -opioid antagonists induce a withdrawal contracture in tissues exposed to bremazocine despite the intrinsic μ -antagonist activity of the drug. The failure of naloxone to evoke contractures in tissues exposed to bremazocine concentrations higher than 5.7×10^{-9} M depends not on the concentration-related increase of μ -antagonist activity but on the inability of naloxone to displace these high doses of bremazocine, as previously observed with the κ -antagonist U-50,488H (Morrone et al., 1993). This conclusion is confirmed by the observation that the addition of cyprodime

(10^{-6} M), a recently available selective μ -opioid antagonist, 1 min before exposure to bremazocine (5.7×10^{-9} M) does not reduce the height of the contracture to nor-binaltorphimine (unpublished observation by the authors). The findings of this and previous (Morrone et al., 1993) studies thus indicate that in the guinea-pig ileum, blocking μ -opioid receptors does not prevent the development of dependence to κ -opioid agonists and *vice versa*. In rabbit jejunum, which appears to have few μ -opioid receptors (Valeri et al., 1992), this conclusion cannot be drawn. In addition, these two isolated preparations differ in their interaction with opioid agonists and antagonists. In particular, we observed that nor-binaltorphimine is harder to wash out from rabbit jejunum than from guinea-pig ileum and naloxone displaces bremazocine more effectively in the jejunum than in the ileum.

Even though the blockade of μ -receptors does not suppress the development of dependence to κ -opioid receptors, this finding does not exclude an interaction between μ - and κ -receptors, as shown by the dose-dependent inhibition of bremazocine on naloxone-induced contracture in the guinea-pig ileum, previously exposed to morphine. This inhibition presumably arises from the activation of κ -opioid receptors rather than from the antagonist activity of bremazocine at μ -receptors, since the naloxone-induced contracture is not affected by the addition, 1 min before the higher dose, of a naloxone concentration ten fold lower than that producing a contracture (see Figure 5). This lower naloxone concentration (5×10^{-8} M) is in the range of the bremazocine concentrations inhibiting the naloxone-induced contracture (1.4 – 7.1×10^{-8} M) and naloxone has an antagonist potency at μ -receptors equal to that of bremazocine (see Figure 4). The observed inhibition of the naloxone-induced withdrawal response after morphine exposure, elicited by the activation of κ -opioid receptors agrees with previous results obtained *in vitro*, although under different experimental conditions (Schultz et al., 1985; Chahl, 1990), and in the whole animal (Green & Lee, 1988). The finding that a κ -opioid system blockade may aggravate naloxone-precipitated symptoms in morphine-dependent mice and rats (Suzuki et al., 1992) provides further evidence of an interaction between μ - and κ -receptors. Under our experimental conditions it was not possible to verify whether μ -receptors blockade could increase the κ -induced withdrawal response, because we used agonist and antagonist concentrations that yielded a withdrawal contractile response that was already maximal. The biochemical mechanisms that underlie and the physiological meaning of this interaction between μ - and κ -receptors need more detailed studies.

The observation that low concentrations of bremazocine determine a state of dependence in isolated preparations supports studies reporting the high potency and binding affinity of this drug to κ -opioid receptors (Romer et al., 1980; Corbett & Kosterlitz, 1986; Dissanayake et al., 1990). As with morphine and U-50,488H (Valeri et al., 1990a; Morrone et al., 1993) the use of low bremazocine concentrations makes it possible to obtain reproducible responses. The reported inability to reveal the development of dependence or to obtain reproducible responses from isolated preparations after exposure to opioid agonists with high receptor affinity (Chahl, 1986; 1990; Brent et al., 1993) probably depends on the fact that the antagonists tested cannot adequately displace the high agonist concentrations used. Reproducible responses are very useful not only for practical but also for theoretical reasons. The loss of reproducibility implies the occurrence of biochemical events that have changed the responsiveness of the tissue. Detailed examination of these changes may help to explain some of the mechanisms involved in development or expression of dependence. Thus, the observation that several guinea-pig ileum preparations previously tested with morphine and bremazocine did not give reproducible responses to naloxone when further tested with morphine and naloxone, suggested that bremazocine

inhibited the expression of morphine dependence, as we subsequently showed.

Bremazocine precipitates an abstinence syndrome in morphine-dependent rats and mice (Vonvoigtlander *et al.*, 1983; Petrillo *et al.*, 1984). As our results show, this antagonist activity at μ -opioid receptors can be easily revealed even *in vitro*, by the contracture that bremazocine elicits in tissues briefly exposed to morphine, without recourse to the complex experimental procedures previously used (Corbett & Kosterlitz, 1985).

Bremazocine has been shown to act as a partial agonist in some electrically-stimulated isolated tissues (Miller *et al.*, 1986; Hunter *et al.*, 1990). Yet, guinea-pig ileum has been shown to have a high κ -receptor reserve (Miller *et al.*, 1986) and the features of rabbit jejunum responses to κ -agonists and antagonists suggest that also this tissue has a high number of spare κ -receptors. It is known that in tissues with a high receptor reserve, partial agonists behave like full agonists. In addition, it must be remembered that in field-stimulated tissues the activity of opioids may depend also on the stimulus intensity (Smith, 1984). Hence, some observations made using electric stimulation may not apply to unstimulated models. Thus, under our experimental procedure,

i.e., in unstimulated tissues which possess a high κ -receptor reserve, it was not possible to reveal the κ -antagonist activity of the drug.

In conclusion, in this study we have shown that, as we found for the selective κ -agonist U-50,488H (Morrone *et al.*, 1993), a withdrawal contractile response can be obtained in guinea-pig ileum and rabbit jejunum briefly exposed to bremazocine, despite the intrinsic μ -antagonist activity of bremazocine. These findings confirm that the acute activation of κ -opioid receptors induces biochemical changes leading to dependence. In addition, they indicate that a μ -opioid system blockade does not prevent the development of the dependence induced by κ -opioid agonists. Yet, our observation that the bremazocine-induced activation of κ -opioid receptors prevents the naloxone-precipitated contracture in tissues briefly exposed to morphine shows that interactions between μ - and κ -opioid systems operate in the guinea-pig ileum, as reported in whole animal. The possibility of studying the interactions between the opioid systems, in isolated preparations, gives us a simple tool for characterizing the complex homeostatic biochemical events leading to the development and expression of dependence.

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