EFFECTS OF NALOXONE AND POST-TETANIC STIMULATION ON ISOLATED GUINEA-PIG ILEUM FOLLOWED BY LONG EXPOSURE TO MORPHINE AND BESTATIN

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ABSTRACT — This study was performed to compare the effects of naloxone (NLX) and post-tetanic stimulation on isolated guinea-pig ileum followed by prolonged exposure to morphine and bestatin. Morphine or bestatin alone did not induce any responses. In the presence of 1 µM morphine, the challenge with 1 µM naloxone caused quick contraction and post-tetanic contraction. A longer duration of these NLX-induced contraction and post-tetanic contractions was observed at the 6th stimulation compared to those after the 2nd stimulation. By contrast, the addition of bestatin, an aminopeptidase inhibitor, did not induce any NLX-induced contraction, although the same results for post-tetanic contraction as those of morphine were observed. These different effects of morphine and bestatin on NLX-induced contraction and post-tetanic contraction in the ileum may be due to different mechanisms of action in the opioid system. That in turn may suggest the possibility that bestatin has a physical dependence liability.

KEY WORDS: Post-tetanic contraction, Naloxone-induced contraction, Bestatin, Morphine, Endogenous opioid(s), Ileum

INTRODUCTION

Studies of tolerance to and physical dependence on drugs are very important from a toxicological viewpoint. Tolerance and physical dependence as a consequence of prolonged administration of opioid alkaloids have been well documented and are associated with a characteristic abstinence syndrome that occurs after interruption of drug treatment (O’Brien, 2001) or during a challenge using naloxone (NLX) (an opioid antagonist) in vivo (Wei et al., 1973; Wei and Loh, 1976) and in vitro (Schulz and Herz, 1976; Collier et al., 1981; Chahl, 1986). These results indicate that the direct action of opioids on receptors usually results in some degree of dependence liability in vivo and in vitro.

Earlier studies demonstrated contractions that also consisted of a cholinergic component and a substance P-mediated component in the ileum that had fallen into physical dependence on opioids following exposure to an opioid antagonist in vitro (Schulz and Herz, 1976; Ehrenpreis et al., 1975; Mundey et al., 1998). This contraction is regarded as an indicator of dependence on opioids in this preparation (Chahl, 1983, 1986; Tsou et al., 1985; Wang and Tsou, 1989).

Bestatin and some other peptidase inhibitors have also been found to possess NLX-sensitive opioid properties in vivo (Roques et al., 1980; Bean and Vaught, 1984) and in vitro (Ozaki et al., 1994a, 1996), presumably by blocking degradation and by accelerated release of endogenous opioid peptides from the opioidergic neurons; i.e. indirectly activating opioidergic pathways.

However, the peptidase inhibitor, which indirectly acts on opioid receptors and increases the various opioid actions, has not been determined to have a liability to effect dependence as does morphine.

We previously reported an increase in basal tension after tetanic stimulation in the presence of a higher concentration of NLX [post-tetanic contraction] and its characteristics (Ozaki and Masuda, 2000). This post-tetanic contraction was attributed to substance P, pros-
taglandins and acetylcholine, which were the common
cotractile components in the ileum dependent on opi-
oids following exposure to an opioid antagonist in vitro
(Schulz and Herz, 1976; Ehrenpreis et al., 1975; Tsou
et al., 1985; Mundy et al., 1998).

Therefore, in the present study, I compared the
effects of morphine or bestatin, an aminopeptidase
inhibitor (Umezawa et al., 1976), on NLX-induced
contraction and post-tetanic contraction induced by
both NLX and high-frequency stimulation using
guinea-pig ileum to determine the differences in the
mechanisms of action between morphine and bestatin
in the opioid system and to detect the possibility of a
development of dependence on bestatin.

MATERIALS AND METHODS

Animals and preparation

All experiments were performed on isolated ileum obtained from randomly-bred male guinea pigs
weighing 350-450 g. The animals were stunned and
decapitated, and the ileum was quickly isolated about
10 cm distant from the ileocecal junction. The myen-
teric plexus-longitudinal muscle was prepared by the
method described previously (Ozaki et al., 1994a,
1994b; Ozaki et al., 1995, 1996; Ozaki and Masuda,
2000). A glass rod was inserted into the lumen of an
intestinal segment and the MPLM removed by rubbing
the segment with a cotton swab soaked in Krebs’
solution. The preparations (2-2.5 cm in length) were sus-
pended at a resting tension of 500 mg in a 5-ml organ
bath between platinum ring electrodes (3.5 cm apart),
placed at the top and the bottom of the bath. The bath
contained Krebs-bicarbonate solution (mM: NaCl 118;
KCl 4.7; CaCl2 2.5; KH2PO4 1.2; MgSO4 1.2;
NaHCO3 25; Glucose 10), at 37°C and was bubbled
with 95% O2 / 5% CO2.

Electrical field stimulation

Rectangular electrical pulse field stimuli were
applied at 0.1 Hz, 0.5 msec pulse width and maximum
voltage using a DPS-160B stimulator (NEC-Sanei Co.,
Tokyo, Japan) with a DPS-122 isolator (NEC-Sanei
Co., Tokyo, Japan), and the responses were recorded
isometrically on an SP-H5P recorder (Riken Denshi
Co., Tokyo, Japan) by means of an SD-1T force dis-
placement transducer (Nihon Kohden Co., Tokyo,
Japan).

Tetanic stimulation

Electrical field stimulation at 0.1 Hz was contin-
uously applied throughout the experiments. Tetanic
stimulation of 10 Hz (0.5 msec pulse width, maximum
intensity for 1 min), inducing twitch contractions
brought about by electrical field stimulation at 0.1 Hz
(0.5 msec pulse width, maximum intensity) (Ozaki et
al., 1994a, 1994b, 1995, 1996; Ozaki and Masuda,
2000), was repeated every 30 min (indicated by the
stimulation number described below). A diagram of
the experimental protocol was published previously
(Ozaki et al., 1995). The first tetanic stimulation was
applied 40 to 50 min after the preparation was set up.
The preparation was washed 6 times with 5 ml of
Krebs-bicarbonate solution 13 to 15 min after tetanic
stimulation.

Effects of morphine and bestatin on post-tetanic
contraction

Morphine (1 µM), which induces withdrawal
contraction and post-tetanic contraction by NLX (1
µM) in vitro (Ehrenpreis et al., 1975), or bestatin was
added to the bath 3 min prior to the first tetanic stimu-
lation. The concentration of bestatin (100 µM), which
did not affect the twitch response but increased in post-
tetanic twitch inhibition, was adopted, as shown in our
previous study (Ozaki et al., 1994a, 1996). The prepa-
ration was washed with 30 ml of Krebs-bicarbonate
solution containing bestatin or morphine without NLX,
13-15 min after each tetanic stimulation to maintain
continuous contact with bestatin or morphine. Nalox-
one (1 µM), at a concentration adopted for induction of
the post-tetanic contraction (Ozaki and Masuda, 2000),
was added to the bath 5 min before the respective tet-
atonic stimuli (2nd and 6th).

Quantification of post-tetanic twitch inhibition

The maximum post-tetanic twitch inhibition was
quantified as previously described (Ozaki et al.,
1994a). The maximum inhibitory twitch height (mm)
after cessation of tetanic stimulation was subtracted
from the twitch height (mm) before tetanic stimulation
on the chart. This value was divided by the twitch
height (mm) before the tetanic stimulation and multi-
plied by 100 to obtain the inhibitory percentage of
post-tetanic twitch inhibition.

Quantification of NLX-induced contraction and
post-tetanic contraction

NLX-induced contraction and post-tetanic con-
traction were defined as the maximum basal tension
height (mm) after application of NLX or after cessation of tetanic stimulation, respectively, and are expressed as the contraction percentage of the twitch contraction height (mm) before NLX or tetanic stimulation (Ozaki and Masuda, 2000), indicated as the maximum contraction (Max). The duration was defined as the time (sec), measured by the number of twitches on the chart, from the application of naloxone or cessation of tetanic stimulation to the return of basal tension, and is expressed in seconds (10 sec/twitch).

**Statistical analysis**

Values are expressed as means and standard error of the mean (S.E.M.) with the number of different preparations in parentheses. The significance of differences was evaluated by one-way analysis of variance followed by Student’s t-test (Tallarida and Murray, 1987). Differences were considered significant at p<0.05.

**Drugs**

The sources of the drugs were: naloxone hydrochloride (Endo Laboratories Inc., Garden City, NY), bestatin (Peptide Institute Inc., Minoh, Osaka, Japan), and morphine hydrochloride (Takeda Pharm. Co., Osaka, Japan). All other drugs were obtained commercially and were dissolved in distilled water and added to the bath within a volume of 5 µl.

**RESULTS**

**Post-tetanic twitch inhibition, naloxone-induced contraction and post-tetanic contraction in the absence of morphine or bestatin**

Post-tetanic twitch inhibition was seen, as we previously reported, in the absence of morphine or bestatin, and NLX inhibited these post-tetanic twitch inhibitions to the same level as non-treatment, as we also previously reported (Ozaki et al., 1994a, 1994b, 1995, 1996). In addition, naloxone-induced contraction in the absence of morphine or bestatin could not be observed (indicated by N in Table 1 and 2). However, post-tetanic contraction was observed as we previously reported (Ozaki and Masuda, 2000). The maximum or duration of post-tetanic contraction at 2nd stimulation was not significantly different from those at 6th stimulation in the absence of morphine or bestatin, respectively (Table 1 and 2; Non-treatment).

**Effects of morphine on post-tetanic twitch inhibition, naloxone-induced contraction and post-tetanic contraction**

Typical traces of the effects of morphine on post-tetanic twitch inhibition, NLX-induced contraction and post-tetanic contraction are shown in Fig. 1. Morphine inhibited the twitch response to 77.9% (Fig. 1, 1st upper trace stimulation), and did not induce contraction after tetanic stimulation without NLX (Fig. 1, 2nd and 6th upper trace stimulation). However, in the continuous presence of morphine, twitch inhibition was still seen and was completely antagonized by NLX (1 µM). Contraction in basal tension and subsequent recovery of this contraction to the original levels were observed in both NLX-induced and post-tetanic contractions (Fig. 1, lower trace, 2nd stimulation). In the continuous presence of morphine, the complete recovery of twitch inhibition, NLX-induced and post-tetanic contraction and the subsequent recovery of these contractions to the original levels were also observed, as in the 2nd stimulation (Fig. 1, lower trace, 6th stimulation). Values of NLX-induced and post-tetanic contraction in the presence of morphine are shown in Table 1. In the presence of morphine alone, post-tetanic contraction could not be detected at 1st, 2nd and 6th stimulation (Fig. 1, upper trace) (indicated by N in Table 1). However, in the presence of morphine (Fig. 1, lower trace), the maximum NLX-induced contraction at 6th stimulation (21.5±4.0%, n=4) was not different from that of 2nd stimulation (23.3±3.4%, n=4), but the duration of this contraction was significantly increased (77.5±8.5 sec, n=4 at 2nd stimulation and 117.5±13.1 sec, n=4 at 6th stimulation, p<0.05). Max of post-tetanic contraction at 2nd stimulation (39.9±4.4%, n=4) was not different from that of 6th stimulation (31.8±7.6%, n=4). The duration of this contraction was slightly increased, but this effect was not significant (65.0±6.5 sec, n=4 at 2nd stimulation and 87.5±8.5 sec, n=4 at 6th stimulation). However, the duration of this contraction was significantly different when compared with non-treatment (62.5±4.8 sec, n=4 at 6th stimulation, p<0.05). Post-tetanic twitch inhibition in the presence of morphine was observed at 1st stimulation (100±0.0%, n=8) and was antagonized by NLX at 2nd stimulation (37.1±3.5%, n=4, p<0.01) and 6th (26.2±4.9%, n=4, p<0.01) (Table 1), as shown in our previous studies (Ozaki et al., 1994a, 1994b, 1995).
Table 1. Summary of the effects of morphine on naloxone-induced or post-tetanic contraction and post-tetanic inhibition.

<table>
<thead>
<tr>
<th>Tetanic stimulation Number</th>
<th>1st</th>
<th>2nd</th>
<th>6th</th>
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<tr>
<td></td>
<td>N</td>
<td>64.1±5.2</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td>(4)</td>
</tr>
<tr>
<td>Non-treatment</td>
<td>N</td>
<td>100 ±0.0</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td>(4)</td>
</tr>
<tr>
<td>Morphine (1 μM) alone</td>
<td>72.5±4.4</td>
<td>N</td>
<td>23.3±3.4</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(NLX not applied)</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>Morphine (1 μM)</td>
<td>77.9±6.1</td>
<td>77.5</td>
<td>39.9±4.4</td>
</tr>
<tr>
<td></td>
<td>±6.1</td>
<td>(8)</td>
<td>±4.4</td>
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<tr>
<td></td>
<td>35.8±3.2**</td>
<td>N</td>
<td>35.4±5.6</td>
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The values were quantified as described in the "MATERIALS AND METHODS" section. Ns indicate that no contraction or inhibition occurred with these drugs or tetanic stimulation. The values represent the means±S.E.M. obtained from the number of different preparations in parentheses. The differences from 2nd stimulation correspond to the column labeled Max or duration of naloxone-induced or post-tetanic contraction at 6th stimulation. * p<0.05 compared with the duration of naloxone-induced contraction in the presence of morphine at 2nd stimulation. ** p<0.01 compared with morphine alone at the 2nd and 6th stimulation, respectively. † p<0.05 compared with non-treatment corresponds to the column labeled Max or duration of naloxone-induced or post-tetanic contraction at each numbered stimulation.
Table 2. Summary of the effects of bestatin on naloxone-induced or post-tetanic contraction and post-tetanic inhibition.

<table>
<thead>
<tr>
<th>Tetanic stimulation Number</th>
<th>1st</th>
<th>2nd</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naloxone (1 μM) -induced contraction</td>
<td>Post-tetanic twitch inhibition (%)</td>
<td>Max (%)</td>
<td>Duration (sec)</td>
</tr>
<tr>
<td>Non-treatment</td>
<td>N (8)</td>
<td>69.3 ± 5.2 (8)</td>
<td>N (4)</td>
</tr>
<tr>
<td>N (NLX not applied) (4)</td>
<td>47.5 ± 7.5 (4)</td>
<td>N (NLX not applied) (4)</td>
<td>N (NLX not applied) (4)</td>
</tr>
<tr>
<td>Bestatin (100 μM) alone</td>
<td>N (8)</td>
<td>100 ± 0.0 (8)</td>
<td>N (NLX not applied) (4)</td>
</tr>
<tr>
<td>Bestatin (100 μM)</td>
<td>N (8)</td>
<td>100 ± 0.0 (8)</td>
<td>N (NLX not applied) (4)</td>
</tr>
</tbody>
</table>

The values were quantified as described in the “MATERIALS AND METHODS” section. Ns indicate that no contraction or inhibition occurred with these drugs or tetanic stimulation. The values represent the means±S.E.M. obtained from the number of different preparations in parentheses. The differences from 2nd stimulation correspond to the column labeled Max or duration of naloxone-induced or post-tetanic contraction at 6th stimulation. *p<0.05 compared with the duration of post-tetanic contraction in the presence of bestatin at 2nd stimulation. **p<0.01 compared with bestatin alone at 2nd and 6th stimulation, respectively. +p<0.05 compared with non-treatment corresponds to the column labeled Max or duration of naloxone-induced or post-tetanic contraction at each numbered stimulation.
Effects of bestatin on post-tetanic twitch inhibition, NLX-induced contraction and post-tetanic contraction

Typical traces of the effects of bestatin on post-tetanic twitch inhibition, NLX-induced contraction and post-tetanic contraction are shown in Fig. 2. Bestatin did not affect the twitch response and did induce post-tetanic twitch inhibition, as we reported previously (Ozaki et al., 1994a) (Fig. 2, upper trace). Contraction after tetanic stimulation was not induced in the presence of bestatin alone (Fig. 2, upper, lower traces at 1st stimulation). NLX (1 µM) had no significant effect on the twitch response in the presence of bestatin and did not affect the basal tension (Fig. 2, lower trace, 2nd and 6th stimulation). However, post-tetanic contraction was observed in the presence of bestatin. The subsequent recovery of these contractions to the original levels was also observed, similarly to observations with morphine (Fig. 1 and 2, lower trace, 2nd and 6th stimulation). Values of NLX-induced and post-tetanic contraction in the presence of bestatin are also shown in Table 2. In the presence of bestatin alone, twitch inhibition and post-tetanic contraction at 1st, 2nd and 6th stimulation could not be detected (Fig. 2, upper trace) (indicated by N in Table 2). However, post-tetanic twitch inhibition was observed by bestatin alone at 1st stimulation (100±0.0%, n=8), 2nd (89.6±4.4%, n=4) and 6th (77.3±2.8%, n=4), and was significantly antagonized by NLX added at 2nd stimulation (43.3±5.0%, n=4, p<0.01) and 6th (28.8±3.3%, n=4, p<0.01) (Table 2) (Fig. 2, lower trace), as shown in our previous study (Ozaki et al., 1994a). On the other hand, Max of post-tetanic contraction at 6th stimulation (32.1±7.0%, n=4) was not different from that of 2nd stimulation (34.1±4.5%, n=4), but the duration of this contraction at 6th stimulation (77.5±4.8 sec, n=4) was significantly increased compared to that of 2nd stimulation (55.0±6.5 sec, n=4, p<0.05), even when

Fig. 1. Representative dynograph tracings of the effects of naloxone and tetanic stimulation in morphine-exposed ileal preparations. Morphine (1 µM), naloxone (1 µM) and tetanic stimulation (10 Hz, 0.5 msec duration, maximum voltage, for 1 min) were applied according to the protocol described in the “MATERIALS AND METHODS” section. Each drug was added to the bath at the dot and was present throughout the experiments except naloxone at 2nd and 6th stimulation, as indicated by the solid lines. Arrows indicate the naloxone-induced contraction and post-tetanic contraction, respectively.
Morphine and bestatin on opioid system in ileum.

**DISCUSSION**

Post-tetanic contraction was attributed to substance P, prostaglandins and acetylcholine (Ozaki and Masuda, 2000), which were the common cotractile components in the ileum dependent on opioids following exposure to an opioid antagonist in vitro (Schulz and Herz, 1976; Ehrenpreis et al., 1975; Tsou et al., 1985; Mundy et al., 1998). The present study was intended to compare the effects of morphine or bestatin, an aminopeptidase inhibitor (Umezawa et al., 1976), on NLX-induced contraction and post-tetanic contraction induced by both NLX and high-frequency stimulation to determine the differences in the mechanisms of action between morphine and bestatin in the opioid system and to detect the possibility of a development of dependence on bestatin.

Naloxone-induced contraction in the absence of morphine or bestatin could not be observed in the absence of morphine or bestatin (Table 1 and 2). However, post-tetanic contraction was observed as we previously reported (Ozaki and Masuda, 2000). The maximum or duration of post-tetanic contraction at 2nd stimulation was no different from those at 6th stimulation in the absence of morphine or bestatin, respectively (Table 1 and 2). These results indicate that NLX alone does not change the post-tetanic contraction.

However, a high concentration of morphine (1 µM) maximally inhibited the twitch response, which coincides with our previous study (Ozaki et al., 1995), and contraction after tetanic stimulation was not observed (Fig. 1, upper trace, 1st, 2nd and 6th stimulation). Post-tetanic twitch inhibition was also observed, which means the additive effect of morphine and endogenous opioids, since both twitch inhibition by morphine and various endogenous opioids released by
tetanic stimulation showed inhibition of twitch response (Ozaki et al., 1994a, 1994b, 1995, 1996). However, the application of NLX induced the reverse in twitch inhibition and the increase in basal tension (NLX-induced contraction) in the presence of morphine (Fig. 1, lower trace, 2nd and 6th stimulation), although NLX alone did not induce basal contraction as shown in our previous study (Ozaki and Masuda, 2000). This contraction induced by NLX was also seen with longer contact with morphine, and the duration was significantly prolonged (Fig. 1, lower trace, 2nd vs. 6th stimulation). These results indicated that NLX-induced contraction, which is considered to be an indicator of withdrawal in the ileum pre-exposed to opioids in vitro (Schulz and Herz, 1976; Collier et al., 1981; Chahl, 1986; Mundey et al., 1998) and in vivo (Wei et al., 1973; Wei and Loh, 1976), developed under these conditions with 1 µM morphine. In addition, NLX alone did not induce longer duration as shown in our previous study (Ozaki and Masuda, 2000), and the maximum height of the post-tetanic contraction at 2nd stimulation was not different from that of 6th stimulation, which had longer contact with morphine. The durations in both contractions were prolonged, although the duration of post-tetanic contraction was slightly prolonged compared with that of 2nd stimulation (Fig. 1, lower trace, 2nd and 6th stimulation). The maximum heights of NLX-induced contraction and the post-tetanic contraction were not different, but the durations in both contractions were prolonged by longer contact with each drug. These results may suggest that the longer duration is due to the post-synaptic event, since the concentration of NLX or the condition of tetanic stimulation which induce the release of the contractile substances, such as substance P etc., are constant. These results also indicated that longer exposure to morphine induced more intense naloxone-induced and post-tetanic contractions. This may suggest the possibility that both naloxone-induced and post-tetanic contraction may be dependent on the intensity of dependence, and may also suggest the possibility that post-tetanic contraction may be one of the indicators of morphine dependence, similarly to the naloxone-induced contraction which was previously shown (Schulz and Herz, 1976; Collier et al., 1981; Chahl, 1986; Mundey et al., 1998). However, further confirmation is necessary for a decisive conclusion. In particular, it is essential to know whether the post-tetanic contraction may occur similarly to naloxone-induced contraction in a preparation isolated from morphine-dependent animals in vivo.

Bestatin, which is an aminopeptidase inhibitor in this system (Umezawa et al., 1976; Ozaki et al., 1994a, 1996), did not show any effect on twitch response, basal tension or that after tetanic stimulation (Fig. 2, upper, and lower, 1st stimulation and our previous report; Ozaki et al., 1994a), and these contractions were not observed even after longer exposure to bestatin alone (Fig. 2, upper, 6th stimulation). In addition, unlike morphine, the NLX-induced contraction was not observed in the presence of bestatin. However, post-tetanic twitch inhibition was reversed by NLX (Fig. 2, lower, 2nd and 6th stimulation). These results indicated the different mechanisms between morphine- and bestatin-action. Post-tetanic contraction was also observed with bestatin, and the duration of this contraction was prolonged by long exposure to bestatin as well as morphine (Fig. 2, lower trace, 2nd and 6th stimulation). However, it is not yet clear which drug is easier or more intensive for the development of these contractions, since both drugs have different sites of action and were used in only one concentration in this experiment. Therefore, various differences between the two drugs may be made much clearer by results for different concentrations of these drugs, stimulation conditions and duration of contact of these drugs with the test preparation.

These results may indicate the participation of opioid receptors in these actions of a peptidase inhibitor, since the twitch inhibition of met-enkephalin and other endogenous opioid subtype ligands, β-endorphin and dynorphin 1-13, and their antagonism by NLX were observed (Ozaki et al., 1994a, 1996). Moreover, participation of opioid receptor types on post-tetanic twitch inhibition were also observed (Ozaki et al., 1994b). From these results, the increased endogenous ligand effect by the peptidase inhibitor may act on the opioid receptors. However, experiments on participation of opioid receptor types on post-tetanic contraction have not been done. Therefore, further investigations of the effects of the opioid receptor subtype antagonist on post-tetanic contraction containing contractile substances, substance P etc., which may be released by tetanic stimulation, must be done. Additionally, the relationships between tolerance and these contractions should be clear, if these contractions are indeed the indicator of physical dependence induced by continuous contact with morphine or bestatin to the preparation, since it is well known that tolerance and dependence are induced by chronic administration of morphine in vivo. Also, the specificity of bestatin as a peptidase inhibitor in this experimental system should be considered.
These results also indicated that high-frequency stimulation, i.e., the firing of opioidergic neurons, is essential for the NLX-induced contraction in the presence of bestatin, which may act indirectly on opioid receptors.

From these observations, the author proposes that bestatin and some peptidase inhibitors can produce NLX-induced contraction in the endogenous ileal opioid system with qualitatively different mechanisms from that reported previously with morphine treatment in vivo (Wei et al., 1973; Wei and Loh, 1976) and in vitro (Schulz and Herz, 1976; Collier et al., 1981; Chahl, 1986; Mundey et al., 1998). Thus, NLX-induced contraction, a physical dependence sign in ileum, can be constructed through opioidergic neuron(s) by endogenous opioid peptides in addition to exogenous opioids such as morphine. In addition, the firing of opioidergic neurons may be one of the mechanisms participating in the development of NLX-induced contraction besides antagonism by NLX on opioid receptors. From a clinical viewpoint, in order to prevent dependence on peptidase inhibitors used to assist opioid analgesics, it may be critical that opioidergic neurons should not fire, even in the case of long-term treatment with peptidase inhibitors, although specificity only for the endogenous opioid system is questionable.

Further toxicological, pharmacological and clinical implications of these observations remain to be determined. The relationships between twitch inhibition by exogenous opioids, post-tetanic twitch inhibition by endogenous opioids, which we reported previously (Ozaki et al., 1994a, 1994b, 1995, 1996), and these NLX-induced contractions are still unclear. However, because of the different mechanisms in the development of withdrawal signs with respect to exogenous and endogenous opioids, these results should be considered further in the study of the mechanism of development of physical dependence and withdrawal signs.

In conclusion, bestatin and some peptidase inhibitors can produce NLX-induced contraction in the endogenous ileal opioid system with qualitatively different mechanisms from that reported previously with morphine treatment in vivo and in vitro. A physical dependence sign in ileum can be constructed through the opioidergic neuron(s) by endogenous opioid peptides in addition to exogenous opioids such as morphine.

REFERENCES


