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Morphine and opioid peptides selectively inhibit the non-cholinergically mediated neurogenic contraction of guinea-pig isolated bronchial muscle

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Abstract—The experiments examine the actions of morphine and opioid peptides on the responses evoked by electrical field stimulation or by acetylcholine (ACh) and substance P (SP) in guinea-pig bronchial strip chain. Electrical field stimulation evoked a biphasic contraction, consisting of a cholinergically mediated fast contraction followed by a non-cholinergically mediated slow contraction. Morphine and opioid peptides caused a concentration-dependent inhibition in the height of the non-cholinergic contraction. The order of inhibitory activity was BW443C > dynorphin > morphine > β-endorphin > leucine-enkephalin > methionine-enkephalin. Cholinergically mediated contractions were less potently inhibited by these opioids. Submaximal contractions of bronchial muscle evoked by exogenous ACh (2 μM) or SP (0·2 μM) were not inhibited by morphine (100 μM) or opioid peptides (3–10 μM), rather, they were augmented. The results indicate that in guinea-pig isolated bronchial muscle, morphine and opioid peptides can selectively inhibit excitatory non-cholinergic neurotransmission via prejunctional opioid receptors.

Recently, we reported that catecholamines and adenosine inhibit excitatory cholinergic and non-cholinergic neurotransmission of the guinea-pig airways via prejunctional α_2-adrenoceptors and P_1-purinoceptors, respectively (Kamikawa & Shimo 1986, 1989, 1990). In the guinea-pig gut, morphine and related opioid peptides have been reported to inhibit transmitter release (Paton 1957; Kamikawa & Shimo 1978, 1983; Szerb 1982). Therefore we have investigated the modulating effect of morphine and opioid peptides on electrically induced neurogenic contractions of guinea-pig bronchi. A preliminary report of some of these results has been made (Kamikawa & Shimo 1988).

Materials and methods

Male guinea-pigs (300–700 g) were stunned, the tracheobronchial tree excised and the bronchial strip chain was prepared (Kamikawa & Shimo 1989). Briefly, two pieces of right and left bronchial transverse strips, 2–3 mm wide, were connected in alignment with threads and immersed in a 10 mL organ bath filled with modified Krebs bicarbonate solution of the following composition (mM); NaCl 120, KCl 4·7, $CaCl_2$ 2·5, $MgCl_2$ 1·2, $NaHCO_3$ 25, KH_2PO_4 1·2, disodium edetate 0·03, ascorbic acid 0·12 and glucose 11 (pH 7·4). The Krebs solution always contained 20 μM choline chloride and was bubbled with 5% carbon dioxide in oxygen, and maintained at 37°C.

The preparation was suspended under an initial tension of 0·5 g and 60 min was allowed to elapse before experiments were started. The bronchial response was isometrically recorded by a force-displacement transducer (Nihon Kohden SB-1T-H) and a Nihon Kohden polygraph recorder (RJG-4004). Electrical field stimulation was with rectangular pulses of 8 Hz, 0·5 ms duration and supramaximal voltage, through bipolar platinum electrodes which were 10 mm apart and connected to a Nihon Kohden stimulator (SEN-1101). The total number of stimulating pulses was kept constant at 40. For the elimination of endogenous prostaglandin biosynthesis in response to field stimulation, the Krebs solution contained 2 μM indomethacin.

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When the strip was electrically stimulated, a biphasic contraction was obtained. The response was composed of an initial fast contraction followed by a sustained contraction which was mediated by cholinergic and non-cholinergic nerve stimulation, respectively (Kamikawa & Shimo 1989). The height of these contractions was comparable to those of submaximal contractions induced by exogenous acetylcholine (ACh, 2 μM) and substance P (SP, 0·2 μM), respectively. The effects of morphine and opioid peptides on the electrically induced contractions were measured as the percentage changes of the original contraction height just before the drug was applied to the bath.

Drugs used were morphine hydrochloride (Dainippon), human β-endorphin, dynorphin A (1-17), methionine-enkephalin, leucine-enkephalin, substance P (Peptide Institute), BW443C (Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2; Wellcome Research Laboratories), naloxone hydrochloride (Endo), acetylcholine chloride (Daiichi), carbachol chloride (Sigma) and indomethacin (Sankyo). To prepare the drug solutions, indomethacin was dissolved in distilled water containing an equimolar concentration of Na_2CO_3 and diluted with 0·9% w/v NaCl solution (saline); all other drugs were dissolved in and diluted with saline. The molar concentrations of drugs in this paper refer to the final bath concentrations.

Results

Morphine, at concentrations higher than 0·1 μM, inhibited the electrically induced biphasic contraction of guinea-pig bronchial muscle without any influence on resting tone. The response was concentration-dependent and reversible by washing. However, its inhibitory activity was different between cholinergic and non-cholinergic components of the contraction. The highest concentration of morphine (100 μM) inhibited the electrically induced non-cholinergic contraction by approximately 90%, but the cholinergic contraction only by about 20% (Fig. 1A). The inhibitory action of morphine (100 μM) was reversed, but not completely, after 20 min treatment with naloxone (10 μM) (Table 2). Submaximal contractions of bronchial muscle to exogenously supplied ACh (2 μM) and SP (0·02 μM) were augmented in the presence of morphine (100 μM) (Table 3).

Among various opioid peptides tested, β-endorphin, dynorphin and BW443C caused a concentration-dependent inhibition of the electrically induced non-cholinergic contractions of guinea-pig bronchial muscle (Fig. 1B, C, F). BW443C was the most potent on the basis of the concentration required to inhibit

Table 1. Inhibitory activities of morphine and opioid peptides on electrically induced non-cholinergic contraction of guinea-pig isolated bronchial muscle.

Opioid	n	$-\text{Log}_{10}$IC50
Morphine	11	5·87 ± 0·08
Dynorphin	10	6·02 ± 0·12
β-Endorphin	6	5·70 ± 0·06
Met-enkephalin	4	< 4·50
Leu-enkephalin	5	< 4·50
BW443C	8	6·18 ± 0·05

All values represent the mean ± s.e.m.

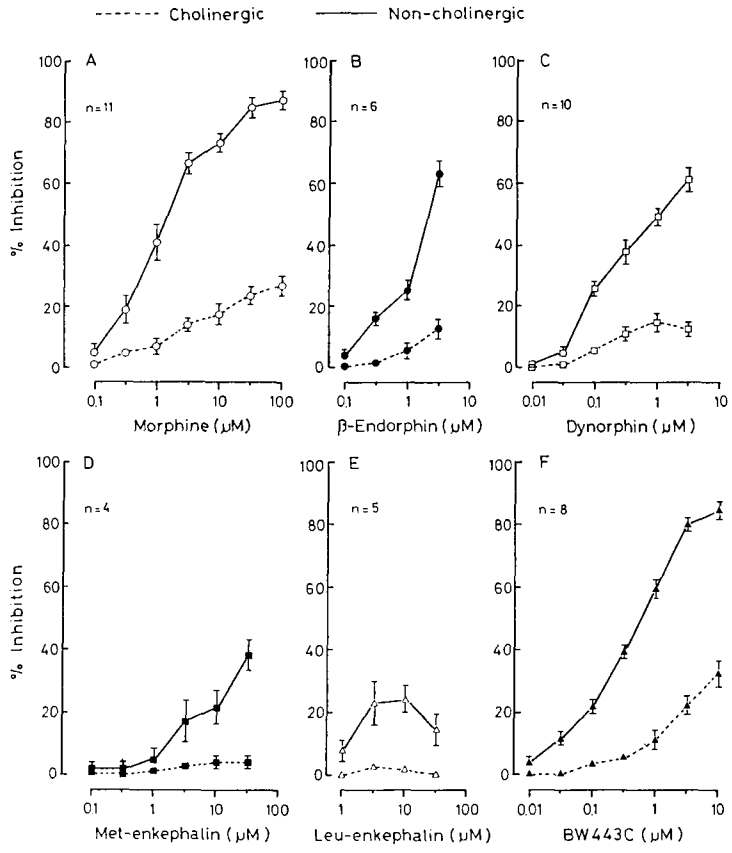

FIG. 1. Cumulative log concentration-response curves for the inhibitory actions of opioids on electrically induced cholinergic (dotted lines) and non-cholinergic (solid lines) contractions of guinea-pig isolated bronchial muscle. Each point represents the mean ± s.e.m.

Table 2. Effects of opioids on electrically induced cholinergic and non-cholinergic contractions of guinea-pig isolated bronchial muscle before and after 20 min treatment with naloxone (10 μM).

			% of control response			
			Before		After naloxone 10 μM	
Opioid	Concentration (μM)	n	Cholinergic	Non-cholinergic	Cholinergic	Non-cholinergic
Morphine	100	9	74·1 ± 4·8***	16·9 ± 2·7***	106·3 ± 2·3$^{N.S.}$	67·7 ± 6·3***
Dynorphin	1	8	94·7 ± 2·3$^{N.S.}$	41·6 ± 3·0***	112·1 ± 3·9**	82·7 ± 5·8$^{N.S.}$
BW443C	10	8	63·9 ± 3·6***	14·4 ± 2·4***	103·7 ± 2·2$^{N.S.}$	84·5 ± 4·6$^{N.S.}$

All values represent the mean ± s.e.m. as a percentage of the original contraction height evoked by 8 Hz electrical stimulation.

**, $P < 0.01$; ***, $P < 0.001$; $^{N.S.}$, not significant. These were statistically compared with control response using the paired t-test.

the contraction height by 50% (IC50 in Table 1). As did morphine, however, these opioid peptides showed only a weak inhibition of the cholinergically mediated contraction. Both methionine- and leucine-enkephalins, up to 30 μM, produced a less potent inhibition of the electrically induced non-cholinergic contraction (Fig. 1D, E). The inhibitory actions of dynorphin (1 μM) and BW443C (10 μM) were completely reversed after 20 min treatment with naloxone (10 μM) (Table 2). Submaximal contractions to exogenous ACh (2 μM) and SP (0·2 μM) were unaffected by the highest concentration (3 μM) of dynorphin or β-endorphin, but rather augmented by BW443C (10 μM) (Table 3). Furthermore, we examined the concentration-response curves to morphine and BW443C for their inhibition of electrically induced non-cholinergic contractions with or without naloxone (0·2–2 μM) pretreatment for 20 min, and determined the dissociation constant (K_e) of naloxone by estimating ($-\log_{10}$IC50 ratio $-$ 1) versus molar concentration of naloxone (Kosterlitz & Watt 1968). The mean K_e value of naloxone was 44 nM against morphine (n = 6) and 10 nM against BW443C (n = 6).

Discussion

In this study we have demonstrated that morphine and opioid peptides selectively inhibit the electrically induced non-cholinergic contractions of guinea-pig bronchial muscle. The order of inhibitory activity was BW443C > dynorphin > morphine > β-endorphin and, much weaker, methionine-and leucine-enkephalins. Because neither submaximal contraction to exogenous ACh nor that to SP was inhibited by these opioid agonists, the inhibitory effects are thought to be mediated by a prejunctional

Table 3. Effects of opioids on submaximal contractions to exogenously supplied acetylcholine (ACh 2 μM) and substance P (SP 0·2 μM) of guinea-pig isolated bronchial muscle.

Opioid	Concentration (μM)	n	% of control response ACh 2 μM	% of control response SP 0·2 μM
Morphine	100	10	123·0±4·5***	113·5±6·0*
Dynorphin	3	10	103·9±6·5$^{N.S.}$	96·8±7·2$^{N.S.}$
β-Endorphin	3	4	123·5±11·2$^{N.S.}$	111·0±11·8$^{N.S.}$
BW443C	10	8	122·4±8·2*	149·3±9·1***

All values represent the mean±s.e.m.
*, $P<0·05$; ***, $P<0·001$; $^{N.S.}$, not significant. These were statistically compared with control response using the paired t-test.

reduction of the transmitter release from non-cholinergic nerves. At present, opioid receptors in peripheral tissues are subclassified into three subtypes and morphine, enkephalins and dynorphins preferentially act through μ, δ- and κ-receptors, respectively (Wüstar et al 1981). BW443C, a newly synthesized enkephalin analogue with peripherally acting anti-nociceptive and antitussive actions (Follenfant et al 1988; Adcock et al 1988), appears similar to the κ-receptor agonists. The order of potency observed in the present study suggests the involvement of κ-receptors in inhibiting the non-cholinergic neurotransmission of guinea-pig bronchi. This is also supported by the antagonist potency of naloxone. The K_e value of naloxone against μ-receptor agonists was between 1 and 3 nM (Lord et al 1977), while that against κ-receptor agonists was about 10 times higher, 10–40 nM (Yoshimura et al 1982). The K_e values of naloxone against morphine and BW443C obtained in the present experiments were close to the latter value. Previously, Frossard & Barnes (1987) had reported that morphine, but not dynorphin-(1-13), inhibited non-cholinergic bronchoconstriction in guinea-pig airways via μ-opioid receptors. The discrepancy may be due to the difference of stimulus parameters. The non-cholinergic response in the present study was evoked by low frequency (8 Hz) electrical stimulation, whereas they used a high frequency (32 Hz). It is well known that presynaptic modulation of the transmitter release by opioids is inversely dependent on the stimulus frequency (Szerb 1982; Kamikawa & Shimo 1983).

Much evidence suggests that SP or related tachykinins might function as the transmitter of non-cholinergic nerves in guinea-pig bronchi (Andersson & Grundström 1987; Kamikawa & Shimo 1989). The tachykinin-containing nerves in airways are thought to be sensory nerves in origin. The present finding is in agreement with the demonstration of opioid inhibition of SP release from peripheral sensory nerves (Brodin et al 1983). Since the pulmonary sensory nerves are considered to contribute to the pathophysiology of bronchial asthma via antidromic axon reflexes (Barnes 1987), opioid agonists such as BW443C may represent a novel prophylactic agent in bronchial asthma.

In conclusion, morphine and opioid peptides can selectively inhibit electrically induced non-cholinergic contractions of guinea-pig isolated bronchial muscle via prejunctional opioid receptors, probably κ-receptors.

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