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ANTAGONISTIC EFFECT OF COMPOUND 48/80 ON THE INHIBITORY ACTIONS OF MORPHINE AND METHIONINE-ENKEPHALIN ON ELECTRICALLY-INDUCED CONTRACTIONS OF THE GUINEA-PIG ILEUM

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1 The effect of compound 48/80 was studied on the twitch-like contractions of the longitudinal muscle of guinea-pig ileum induced by electrical stimulation of intramural cholinergic nerves.

2 Compound 48/80 alone, at concentrations up to 30 μg/ml, had no effect on the twitch contractions. The contraction to exogenously applied acetylcholine was slightly depressed by the compound.

3 At 100 μg/ml, compound 48/80 caused a weak but long-lasting increase in tone and irregular contractile activity in the ileum, part of which was reduced but not completely abolished by pretreatment with chlorpheniramine (1 μM) or by repeated applications of compound 48/80.

4 The inhibitory effects of morphine and methionine-enkephalin on the twitches were antagonized by the presence of compound 48/80 (3 to 30 μg/ml), possibly in a competitive manner. The antagonism was not affected by pretreatment with the antihistaminics, chlorpheniramine and/or metiamide.

5 The inhibitory effects of noradrenaline and adrenaline on the twitches were slightly but significantly increased by the presence of compound 48/80 (10 or 30 μg/ml), whereas that of ATP was not modified.

6 These results indicate that compound 48/80 acts as a selective and competitive antagonist at opiate receptors located in the intramural cholinergic nerves of guinea-pig ileum.

Introduction

Compound 48/80, a condensation product of *p*-methoxyphenethylmethylamine and formaldehyde, is well known to be the most potent liberator of histamine from mast cells (Paton, 1951). However, other pharmacological properties independent of its release of endogenous histamine have also been attributed to it, e.g. blockade of ganglionic transmission in cat superior cervical ganglion (Gertner, 1955; Damjanovich, Fehér & Bokri, 1963), blockade of rabbit neuromuscular junction (Sömjén & Uyldert, 1955), inhibition of the negative chronotropic effect of vagal nerve stimulation in guinea-pig atria (Kasuya & Kamikawa, 1973), accumulation of tissue cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (Lindl, Behrendt, Heinl-Sawaja, Teufel & Cramer, 1974; Lindl, Heinl-Sawaja & Cramer, 1976), and stimulation of small granule-containing cells of the rat superior cervical ganglion (Behrendt, Lindl & Cramer, 1976). The mechanisms of these histamine-independent actions are not yet clear; however, from the early findings mentioned above (Kasuya & Kamikawa, 1973), it has been suggested that compound 48/80 may possess some actions on cholinergic neurotransmission.

The present investigation was carried out to examine the effect of compound 48/80 on the twitch-like contractions of the longitudinal muscle of guinea-pig ileum induced by electrical stimulation of intramural cholinergic nerves. In addition, the interactions between compound 48/80 and opiate agonists, catecholamines or adenine nucleotide in this preparation were also examined since it is well known that these drugs inhibit the twitch contractions by means of a decrease of acetylcholine output from intramural cholinergic nerves (Paton, 1957; Paton & Vizi, 1969; Kosterlitz, Lydon & Watt, 1970; Takagi & Takayanagi, 1972; Sawynok & Jhamandas, 1976). The results indicate that compound 48/80 acts as an antagonist on opiate receptors in this tissue.

Methods

Male guinea-pigs weighing 250 to 500 g were used. The animals were stunned by a blow on the head and a 5 cm strip of the terminal ileum was excised after discarding the 10 cm nearest to the ileo-caecal junction. For most of the present experiments, the mucosa was removed in order to eliminate the stimulant effects of compound 48/80 on mast cells or enterochromaffin cells which are mostly located in the intestinal mucosa. After cutting away the mesentery, the ileum was inverted with forceps and the mucosa was gently incised and removed from the underlying muscle layers. For some experiments, intact ileum or the myenteric plexus-longitudinal muscle preparations obtained by the method of Kosterlitz *et al.* (1970) were also used. The preparation thus obtained, about 25 mm long, was immersed in a 15 ml organ bath filled with the 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 and glucose, 14 (pH, 7.4) containing 20 μM choline chloride. The Krebs solution was bubbled with 5% CO_2 and 95% O_2 and maintained at 37°C. The strip was suspended under a 0.7 g load and 40 to 60 min were allowed to elapse before experiments were started. In examining the effect of single doses of the drugs, rest intervals between doses were about 30 min. In experiments to obtain cumulative dose-response curves, the intervals were about 60 min. Responses of the longitudinal muscle of guinea-pig ileum were recorded isotonically by means of an isotonic transducer (MEC-1411) and a Nihon Kohden polygraph recorder. Electrical stimulation was carried out transmurally by means of two co-axial platinum electrodes, the anode in the lumen and the cathode in the organ bath. The stimulation parameters were 0.1 Hz, 0.5 or 0.01 ms duration and supramaximal voltage (approx. 20 V for 0.5 ms and 50 V for 0.01 ms duration). When the strip was electrically stimulated with these parameters, stable twitch-like contractions were obtained, the height of which, for a pulse duration of either 0.01 ms or 0.5 ms, was nearly equivalent to that of the contraction caused by exogenously applied acetylcholine (0.1 μM). The elicited twitch contractions seem to be mediated by the stimulation of intramural cholinergic nerves in the ileum since the responses were completely inhibited by application of atropine (0.1 μM) or tetrodotoxin (0.1 μM). The inhibition of the twitch contractions by drugs is shown as percentage inhibition compared with the original twitch height obtained just before the drug was applied to the bath. To obtain the dose for 50% inhibition of the twitch height (ID_{50} values in Tables 1 and 2), an ID_{50} was calculated from individual dose-response curves and these results were processed statistically. Statistical significance of the difference was examined by Student's *t* test.

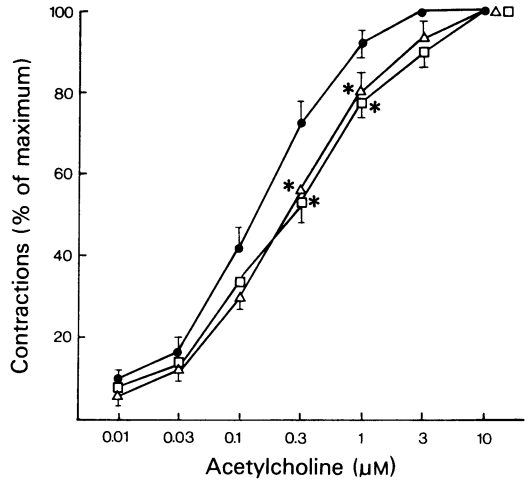

Figure 1 Cumulative dose-response curves for contractions of the inverted ileum of the guinea-pig to exogenously applied acetylcholine in the presence or absence of compound 48/80. (●) Control (n = 20); (△) in the presence of compound 48/80 10 μg/ml (n = 10); (□) in the presence of compound 48/80 30 μg/ml (n = 10). Abscissa scale: log concentrations (μM) of acetylcholine. Ordinate scale: % of the maximum control contraction. Mean values are given; vertical lines show s.e. means. Both concentrations of compound 48/80 slightly depressed the contractions to acetylcholine. In order to minimize the spontaneous activity of the ileum, experiments were carried out at 28°C. *P < 0.05.

Drugs used were: compound 48/80 (Sigma), morphine hydrochloride (Dainippon Pharmaceuticals), methionine-enkephalin (Protein Research Foundation, Osaka, Japan), (−)-noradrenaline bitartrate (Sigma), (−)-adrenaline bitartrate (Sigma), acetylcholine chloride (Daiichi Pharmaceuticals), adenosine 5′-triphosphate disodium salt (Sigma), naloxone hydrochloride (Endo Laboratories), choline chloride (Wako Pure Chem.), atropine sulphate (Wako Pure Chem.), histamine dihydrochloride (Wako Pure Chem.), chlorpheniramine maleate (Sankyo Pharmaceuticals), metiamide (Smith Kline & French Ltd.), and tetrodotoxin (Sankyo Pharmaceuticals). All drugs were dissolved in 0.9% w/v NaCl solution (saline) except adenosine triphosphate and compound 48/80 which were dissolved in the Krebs solution. The molar concentrations of drugs described in this paper refer to the final bath concentration. Only the concentration of compound 48/80 is shown as μg/ml in the organ bath since the molecular weight of compound 48/80 cannot be determined (Read & Lenney, 1972).

Results

Effect of compound 48/80 on the twitch contractions

Compound 48/80 alone, at concentrations up to 30 μg/ml, did not have any significant influence on the twitch contractions caused by electrical stimulation (both 0.5 ms and 0.01 ms duration) of the longitudinal muscle of the inverted guinea-pig ileum ($n = 73$). In many cases, a transient inhibition of the twitch occurred immediately after application of the compound but the degree of inhibition was less than 10% of the original response. However, a higher concentration (100 μg/ml) of compound 48/80 caused a weak but long-lasting contracture and irregular activity of the ileum in all cases ($n = 18$); therefore, its effects on the twitch could not be quantified precisely. The responses of the ileum to high concentrations of compound 48/80 were reduced but not abolished completely by preincubation with chlorpheniramine (1 μM) or by repeated applications of compound 48/80. This may indicate the involvement of histamine release from mast cells remaining in the muscle layers. In the intact ileum ($n = 10$) or in the myenteric plexus-longitudinal muscle preparation ($n = 6$), compound 48/80 caused a marked contracture and irregular activity of the ileum even at a concentration of 30 μg/ml; therefore, most of the present experiments were carried out with the inverted and mucosa-free segment of ileum.

At a concentration of 30 μg/ml, compound 48/80 did not significantly modify contractions caused by exogenously applied acetylcholine (93.4 ± 7.8% of control, $n = 4$), given in a concentration (0.1 μM) that produced responses equivalent to those of the twitch contractions of electrical stimulation of the ileum. However, the dose-response curve to acetylcholine obtained at a bath temperature of 28°C was slightly but significantly shifted to the right in the presence of compound 48/80 (10 or 30 μg/ml, Figure 1).

Effects of compound 48/80 on the inhibitory responses to opiate agonists

The influence of compound 48/80 was examined on the inhibitory effects of morphine and methionine-enkephalin (one of the endogenous morphine-like pentapeptides found by Hughes, Smith, Kosterlitz, Fostergill, Morgan & Morris, 1975), on twitch con-

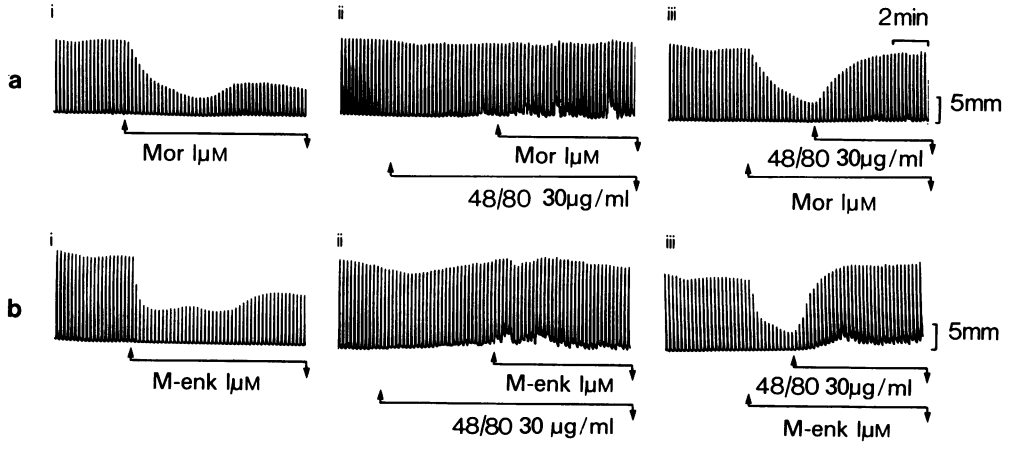

Figure 2 Effect of compound 48/80 on the inhibitory effects of morphine (Mor, a, 1 μM) and methionine-enkephalin (M-Enk, b, 1 μM) on the twitch contractions of the inverted ileum induced by electrical stimulation (0.1 Hz, 0.5 ms and supramaximal voltage). (i) Control; (ii) pretreatment with compound 48/80 30 μg/ml) 5 min before the addition of drugs; (iii) inhibitory responses to these drugs 30 min after washing out compound 48/80, and reversal of their effects by further application of compound 48/80. The preparations were washed 3 times with fresh Krebs solution and rested at least 30 min between (i) and (ii) or (ii) and (iii). Compound 48/80 completely reversed inhibitory responses to both morphine and methionine-enkephalin when given either before or after these drugs. Vertical calibration, 5 mm twitch height of the ileum; horizontal calibration, 2 min.

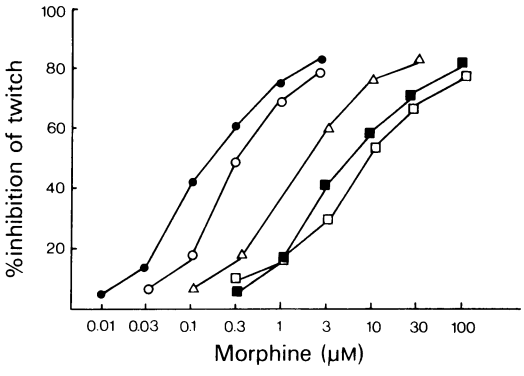

Figure 3 Cumulative dose-response curves for the inhibitory effects of morphine on the twitch contractions of the inverted ileum induced by electrical stimulation (0.1 Hz, 0.01 ms and supramaximal voltage) in the presence or absence of compound 48/80 and naloxone. Control (●); in the presence of compound 48/80, 3 μg/ml (○), 10 μg/ml (△) or 30 μg/ml (□); in the presence of naloxone 0.1 μM (■). Abscissa scale: log concentrations (μM) of morphine. Ordinate scale: % inhibition of the twitch produced by morphine. Each point is the mean value from 9–30 experiments shown in parentheses in Table 1. For any value, s.e. mean was never greater than ±5%. Note parallel shift of the curve for morphine to the right by pretreatment of the ileum with compound 48/80, depending on the dose, or with naloxone.

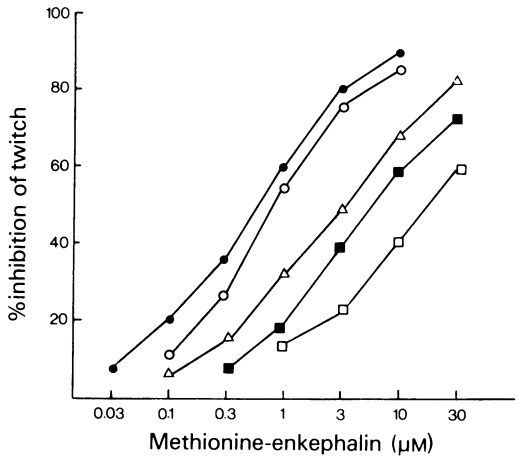

Figure 4 Cumulative dose-response curves for the inhibitory effects of methionine-enkephalin on the twitch contractions of the inverted ileum induced by electrical stimulation (0.1 Hz, 0.01 ms and supramaximal voltage) in the presence or absence of compound 48/80 and naloxone. Control (●); in the presence of compound 48/80, 3 μg/ml (○), 10 μg/ml (△) or 30 μg/ml (□); in the presence of naloxone 0.1 μM (■). Abscissa scale: log concentration (μM) of methionine-enkephalin. Ordinate scale: % inhibition of the twitch produced by methionine-enkephalin. Each point is the mean value from 9 or 27 experiments shown in parentheses in Table 1. For any value, s.e. mean was never greater than 5%. Note parallel shift to the right of the curve for methionine-enkephalin by pretreatment of the ileum with compound 48/80, depending on the dose, or with naloxone. A higher concentration of enkephalin than 30 μM could not be prepared from the commercial enkephalin used here.

tractions induced by electrical stimulation (0.5 or 0.01 ms duration) of the inverted ileum. The inhibitory effects of morphine 1 μM (Figure 2a) and methionine-enkephalin 1 μM (Figure 2b) were completely prevented by preincubation of the ileum with compound 48/80 (30 μg/ml) 5 min before the addition of the opiates (Figure 2 aii, bii). This antagonism was fully reversible; the inhibitory effects of the opiate agonists were restored after washing out compound 48/80 from the bath (Figure 2 aiii, biii) and the inhibition by opiate agonists was completely reversed again by further application of compound 48/80 (Figure 2 aiii, b iii). It should be noted that the antagonistic effect of compound 48/80 could be reproduced even after repeated application of the compound and was not affected by pretreatment of the ileum with the H_1- and H_2-receptor antagonists, chlorpheniramine (1 μM) and/or metiamide (100 μM). The partial antagonism between compound 48/80 (10 μg/ml) and morphine (0.5 μM) or methionine-enkephalin (1 μM) was also observed both in the myenteric plexus-longitudinal muscle preparation ($n = 6$) and in the intact segment of the ileum ($n = 10$).

Cumulative dose-response curves for inhibitory effects of morphine and methionine-enkephalin in the presence or absence of compound 48/80 are shown in Figures 3 and 4. The curves for morphine (Figure 3) and methionine-enkephalin (Figure 4) were shifted to the right in a parallel and dose-dependent manner by pretreatment with compound 48/80 (3 to 30 μg/ml) 5 min before the addition of the first dose of the agonists. The concentration of morphine producing a 50% inhibition of the twitch (ID_{50}) was increased 2.1, 12.4 and 49.5 times in the presence of compound 48/80, 3, 10 and 30 μg/ml respectively (Table 1); the corresponding ID_{50} for methionine-enkephalin was increased to a smaller extent (Table 1). In this preparation, pretreatment with naloxone (0.1 μM), a pure narcotic antagonist, caused a parallel shift to the right of dose-response curves for both morphine and methionine-enkephalin (Figures 3 and 4), and increased

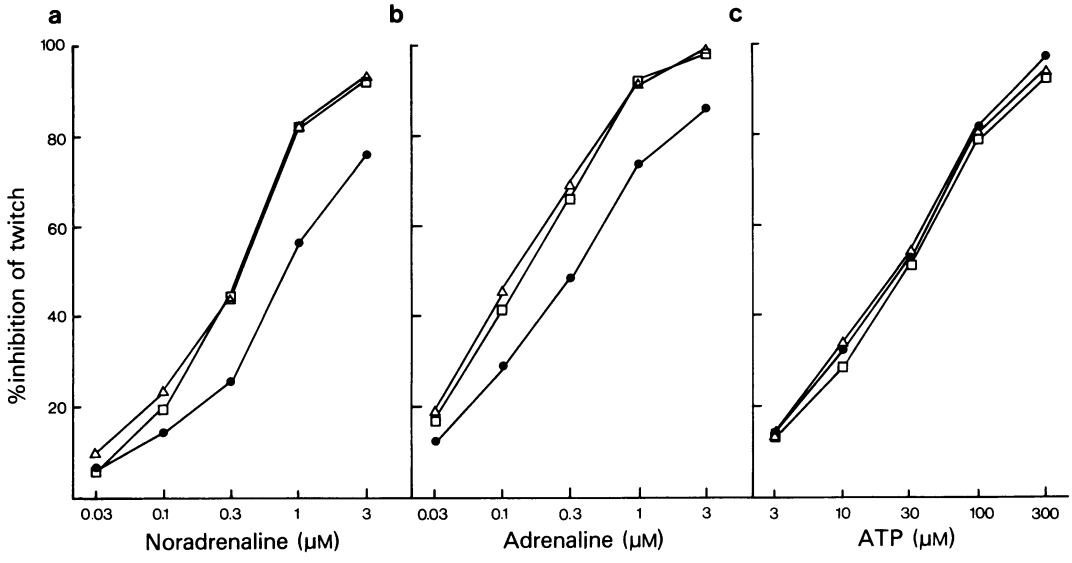

Figure 5 Cumulative dose-response curves for the inhibitory effects of noradrenaline (a), adrenaline (b) and ATP (c) on the twitch contractions of the inverted ileum induced by electrical stimulation (0.1 Hz, 0.01 ms and supramaximal voltage) in the presence or absence of compound 48/80. Control (●); in the presence of compound 48/80, 10 μg/ml (△) and 30 μg/ml (□). Abscissa scale: log concentrations (μM) of catecholamines or ATP. Ordinate scale: % inhibition of the twitch contractions produced by catecholamines or ATP. Each point is the mean value from 6–17 experiments shown in parentheses in Table 2. For any value, s.e. mean was never greater than ±6%. Curves for both noradrenaline and adrenaline shifted slightly upward and to the left in the presence of compound 48/80 but the curve for ATP was not affected.

ID_{50} values for both agonists by 26.6 and 12.3 times, respectively (Table 1).

Effects of compound 48/80 on the inhibitory responses to catecholamines and adenine nucleotide

The effects of compound 48/80 on the inhibitory actions of noradrenaline, adrenaline and adenosine triphosphate (ATP) on the twitch contractions were also examined. Figure 5 shows cumulative dose-response curves for noradrenaline (a), adrenaline (b) and ATP (c) in the presence or absence of compound 48/80 (10 or 30 μg/ml). The curves for noradrenaline and adrenaline were shifted upward and to the left by compound 48/80 pretreatment but not in a dose-dependent manner. As shown in Table 2, both ID_{50}

Table 1 Comparisons of the dose causing 50% inhibition of the twitch contractions (ID_{50}) for morphine and methionine-enkephalin in the presence or absence of pretreatment with compound 48/80 or naloxone

	Morphine ID_{50} *(μM)*	*Ratio*	*Methionine-enkephalin* ID_{50}*(μM)*	*Ratio*
Control	0.21 ± 0.03 (30)		0.66 ± 0.07 (27)	
Compound 48/80				
3 μg/ml	0.44 ± 0.06***(10)	2.1	0.98 ± 0.16* (9)	1.5
10 μg/ml	2.6 ± 0.5***(10)	12.4	5.4 ± 2.1***(9)	8.2
30 μg/ml	10.4 ± 1.8***(10)	49.5	19.1 ±4.1***(9)	28.9
Naloxone 0.1 μM	7.46 ± 1.4***(9)	26.6	8.1 ± 2.2***(9)	12.3

ID_{50} values (mean ± s.e. mean) were calculated from experimental results of the cumulative dose-response relations. Ratio = ID_{50} after pretreatment/ID_{50} of control.
*$P < 0.05$; ***$P < 0.001$. Numbers in parentheses show numbers of observations.

values for noradrenaline and adrenaline significantly decreased by about 40% of the control value in the presence of either concentration of compound 48/80. The inhibitory effect of ATP was not significantly modified by compound 48/80 pretreatment.

Discussion

Compound 48/80 itself, at concentrations less than 30 μg/ml, did not have any significant effect on the twitch contractions of the longitudinal muscle of the inverted and mucosa-free segment of guinea-pig ileum induced by stimulation of intramural nerves. In the intact ileum or in the myenteric plexus-longitudinal muscle preparation, compound 48/80 caused a marked contracture and irregular activity of the ileum even at a concentration of 30 μg/ml and, therefore, its effects on the twitch could not be quantified accurately. Most of the present experiments were carried out on the inverted and mucosa-free segment of ileum. Compound 48/80 slightly depressed contractions of this preparation to exogenously applied acetylcholine. However, inhibitory effects of both morphine and methionine-enkephalin on the twitch contractions were selectively antagonized by compound 48/80. The ability of compound 48/80 to release endogenous histamine from mast cells does not seem to be involved in this antagonism, in view of the following findings: the antagonism was fully reversible and could be reproduced even after repeated applications of compound 48/80; it was not affected by pretreatment with the antihistaminics, chlorpheniramine and/or metiamide; histamine itself did not have any antagonistic effect on the inhibitory effects of morphine. It is well known that the effects of morphine on the electrically induced contractions of guinea-pig ileum decreases with repeated applications of the drug at short intervals (Paton, 1957; Fennessy, Heimans & Rand, 1969). However, the antagonism observed in the present experiments is not due to the development of an acute tolerance since the inhibitory actions of morphine (1 μM) and methionine-enkephalin (1 μM) remained constant for 6 h when the drugs were repeatedly applied every 30 min in the absence of compound 48/80. Furthermore, the cumulative dose-response curves for these drugs were obtained every 60 min and were always accompanied by the paired control curve without compound 48/80. In order to obtain reproducible responses and sensitivity to morphine in the guinea-pig ileum, the time intervals for drug application, the doses of the drug and the parameters for electrical stimulation inducing the twitch contractions are important factors (Paton, 1957; Gyang & Kosterlitz, 1966; Cox & Weinstock, 1966). The present experiments adequately fulfilled these conditions. The sensitivity of the present preparation to opiates, catecholamines and ATP was lower than that described in earlier papers in which the intact ileum or the myenteric plexus-longitudinal muscle preparation were used (Kosterlitz & Watt, 1968; Kosterlitz *et al.*, 1970; Hughes *et al.*, 1975; Sawynok & Jhamandas, 1976). The difference may be due to the cumulative dose methods, stimulus parameters or the changed anatomical arrangement of the ileum used in the present experiments. However, both twitch contractions and the relationship between drug concentration and response of the inverted and mucosa-free segments of ileum were exceedingly stable during experiments.

The inhibitory effects of opiate agonists on the twitch contractions of the guinea-pig ileum have been antagonized by narcotic antagonists, especially by naloxone; which is a pure antagonist without any agonist effects (Paton, 1957; Gyang & Kosterlitz, 1966; Cowie, Kosterlitz & Watt, 1968; Kosterlitz & Watt, 1968; Hughes *et al.*, 1975). The antagonism between opiate agonists and naloxone occurs by competition for the opiate receptors, the selective distribution of which has been demonstrated in the guinea-pig myenteric plexus and brain (Kosterlitz, Lord & Watt, 1972; Terenius, 1973; Pert & Snyder, 1973).

Table 2 ID_{50} values for noradrenaline, adrenaline and ATP with or without compound 48/80 pretreatment

	ID_{50} (μM, mean ± s.e. mean)		
	Noradrenaline	*Adrenaline*	*ATP*
Control	0.96 ± 0.15 (17)	0.41 ± 0.08 (17)	28.6 ± 5.5 (12)
Compound 48/80			
10 μg/ml	0.37 ± 0.06***(9)	0.15 ± 0.03***(9)	33.2 ± 7.1 (6) NS
30 μg/ml	0.38 ± 0.05***(8)	0.18 ± 0.05***(8)	33.5 ± 5.3 (6) NS

ID_{50} values were calculated from experimental results of the cumulative dose-response relations. Numbers in parentheses show numbers of observations.
***$P < 0.001$; NS, not significant.

Also in the present experiments, pretreatment of the ileum with naloxone (0.1 μM) caused a parallel shift to the right of both dose-response curves to morphine and methionine-enkephalin (see Figures 5 and 6). Therefore, the antagonism between compound 48/80 and opiate receptor agonists observed in the present experiment is probably also due to competition for opiate receptors, located in the intramural cholinergic nerves of guinea-pig ileum since it was fully reversible and both dose-response curves for morphine and methionine-enkephalin were displaced to the right in a parallel and dose-dependent manner by compound 48/80 pretreatment. This view is also supported by the findings that compound 48/80, itself, had no effect on the twitch contractions to electrical stimulation of ileum at the concentration which antagonized the responses to opiate agonists, and in addition, that the contractions to exogenously applied acetylcholine were rather depressed by this compound. However, these findings do not constitute conclusive evidence of competitive antagonism, since it is essential to obtain plots of log (dose-ratio − 1) versus log molar concentrations of antagonist and a value of K_e as parameters of antagonist activity (Arunlakshana & Schild, 1959; Kosterlitz & Watt, 1968). Competitive antagonism between compound 48/80 and opiate receptor agonists, however, cannot be convincingly demonstrated at the present time since compound 48/80 is composed of various polymers (dimer, trimer, tetramer and higher oligomers obtained by reacting equimolar concentrations of *p*-methoxyphenethylmethylamine and formaldehyde; Baltzly, Buck, de Beer & Webb, 1949; Read & Lenny, 1972); thus, a constituent acting as the opiate receptor antagonist cannot be identified.

It is well known that binding between opiate agonists or antagonists and opiate receptors is highly stereospecific (Cox & Weinstock, 1966; Goldstein, Lowney & Pal, 1971; Terenius, 1973). The chemical structure of compound 48/80 is apparently different from that of opiate agonists or antagonists. Recently, Horn & Rodgers (1977) showed that the presence of a tyrosine residue (and hence a 'tyramine' moiety) in the terminal amino position of enkephalins is the key factor in explaining why they possess morphine-like activities. From the X-ray crystallographic studies, they also found that in several classes of opiate drugs and analogues the 'tyramine' fragment or a conformationally equivalent moiety seems to be a crucial element. This may also explain why compound 48/80 antagonizes the inhibitory response to opiate agonists, since this compound also possesses the substituted *p*-hydroxyphenethylamine group ('tyramine' moiety) in its structure and thereby may have an affinity for opiate receptors.

The inhibitory actions of noradrenaline and adrenaline on the twitch contractions were slightly but significantly potentiated by compound 48/80. The cause of this potentiation is not yet clear. It may involve noradrenaline release from tissue or accumulation of tissue cyclic AMP by this compound, as reported by Lindl *et al.* (1974; 1976), or a weak depressant effect of this compound on the acetylcholine-induced contractions (Figure 1).

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References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BALTZLY, R., BUCK, J.S., de BEER, E.J. & WEBB, F.J. (1949). A family of long acting depressors. *J. Am. Chem. Soc.*, **71**, 1301–1305.

BEHRENDT, H., LINDL, T. & CRAMER, H. (1976). *In vitro* effects of histamine liberator compound 48/80 on rat superior cervical ganglia with special regard to the small granule-containing cells. *Cell Tiss. Res.*, **166**, 71–81.

COWIE, A.L., KOSTERLITZ, H.W. & WATT, A.J. (1968). Mode of action of morphine-like drugs on autonomic neuro-effectors. *Nature, Lond.*, **220**, 1041–1042.

COX, B.M. & WEINSTOCK, M. (1966). The effect of analgesic drugs on the release of acetylcholine from electrically stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **27**, 81–92.

DAMJANOVICH, S.O., FEHÉR, O. & BOKRI, E. (1963). The effect of 48/80 on the transmission of impulses in a sympathetic ganglion. *Archs int. Pharmacodyn.*, **141**, 490–499.

FENNESSY, M.R., HEIMANS, R.L.H. & RAND, M.J. (1969). Comparison of effect of morphine-like analgesics on transmurally stimulated guinea-pig ileum. *Br. J. Pharmac.*, **37**, 436–449.

GERTNER, S.B. (1955). The effect of compound 48/80 on ganglionic transmission. *Br. J. Pharmac. Chemother.*, **10**, 103–109.

GOLDSTEIN, A., LOWNEY, L.I. & PAL, B.K. (1971). Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. *Proc. natn. Acad. Sci., USA.*, **68**, 1742–1747.

GYANG, E.A. & KOSTERLITZ, H.W. (1966). Agonist and antagonist actions of morphine-like drugs on the

guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.*, **27,** 514–527.

HORN, A.S. & RODGERS, J.R. (1977). The enkephalins on opiates: Structure-activity relations. *J. Pharm. Pharmac.*, **29,** 257–265.

HUGHES, J., SMITH, T.W., KOSTERLITZ, H.W., FOTHERGILL, L.A., MORGAN, B.A. & MORRIS, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature, Lond.*, **258,** 577–579.

KASUYA, Y. & KAMIKAWA, Y. (1973). Studies on the cardiac anaphylaxis in the guinea-pig. 1. Changes in the sensitivity to some drugs induced by anaphylactic desensitization. *Jap. J. Pharmac.*, **23,** Suppl. 114.

KOSTERLITZ, H.W., LORD, J.A.H. & WATT, A.J. (1972). Morphine receptors in the myenteric plexus of the guinea-pig ileum. In *Agonist and Antagonist Actions of Narcotic Analgesic Drugs.* ed. Kosterlitz, H.W., Collier, H.O.J. & Villarreal, J.E. pp 45–61. London: Macmillan.

KOSTERLITZ, H.W., LYDON, R.J. & WATT, A.J. (1970). The effects of adrenaline, noradrenaline and isoprenaline on inhibitory α- and β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum. *Br. J. Pharmac.*, **39,** 398–413.

KOSTERLITZ, H.W. & WATT, A.J. (1968). Kinetic parameters of narcotic agonists and antagonists, with particular reference to N-allylnoroxomorphone (naloxone). *Br. J. Pharmac. Chemother.*, **33,** 266–276.

LINDL, T., BEHRENDT, H., HEINL-SAWAJA, M.C.B., TEUFEL, E. & CRAMER, H. (1974). Effects of compound 48/80 on mast cells, histamine, and cyclic AMP in isolated superior cervical ganglia. *Naunyn-Schmiedebergs Arch. Pharmac.*, **286,** 283–296.

LINDL, T., HEINL-SAWAJA, M.C.B. & CRAMER, H. (1976). Effects of compound 48/80, a histamine-releasing agent, on accumulation and release of cyclic AMP in various regions of rat brain *in vitro*. *Res. Comm. Chem. Path. Pharmac.*, **13,** 65–74.

PATON, W.D.M. (1951). Compound 48/80: A potent histamine liberator. *Br. J. Pharmac., Chemother.*, **6,** 499–508.

PATON, W.D.M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **12,** 119–127.

PATON, W.D.M. & VIZI, E.S. (1969). The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmac.*, **35,** 10–28.

PERT, C.B. & SNYDER, S.H. (1973). Opiate receptors: Demonstration in nervous tissue. *Science, N.Y.*, **179,** 1011–1014.

READ, G.W. & LENNEY, J.F. (1972). Molecular weight studies on the active constituents of compound 48/80. *J. med. Chem.*, **15,** 320–323.

SAWYNOK, J. & JHAMANDAS, K.H. (1976). Inhibition of acetylcholine release from cholinergic nerves by adenosine, adenine nucleotides and morphine: antagonism by theophylline. *J. Pharmac. exp. Ther.*, **197,** 379–390.

SÖMJÉN, G. & UYLDERT, E. (1955). The effect of compound 48/80 on mammalian skeletal muscle. *Br. J. Pharmac. Chemother.*, **10,** 413–417.

TAKAGI, K. & TAKAYANAGI, I. (1972). Effect of N^6,2′-o-dibutyryl 3′,5′-cyclic adenosine monophospate, 3′,5′-cyclic adenosine monophosphate and adenosine triphosphate on acetylcholine output from cholinergic nerves in guinea-pig ileum. *Jap. J. Pharmac.*, **22,** 33–36.

TERENIUS, L. (1973). Stereospecific uptake of narcotic analgesics by a subcellular fraction of the guinea-pig ileum. *Uppsala J. med. Sci.*, **78,** 150–152.

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