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Effect of pertussis toxin on normorphine-dependence and on acute inhibitory effects of normorphine and clonidine in guinea-pig isolated ileum

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In guinea-pig isolated ileum from animals pretreated with Pertussis toxin, the acute inhibitory effects of normorphine and clonidine on electrically induced contractions were markedly attenuated whilst responses to acetylcholine and electrical stimulation were unaltered. Pertussis toxin treatment also reduced naloxone-precipitated withdrawal contractures in normorphine-dependent tissues. These results suggest that the acute and chronic effects of normorphine are mediated by the same mechanism, namely that of adenylate cyclase inhibition.

Introduction It has been proposed that adenylate cyclase is involved in the presynaptic inhibitory effects of both opiates and adrenoceptor agonists (Collier & Roy, 1974; Pelayo *et al.*, 1978) and the dependence resulting from chronic opiate exposure (Sharma *et al.*, 1975). The demonstration that islet-activating protein, a toxin isolated from cultures of *Bordetella pertussis*, can abolish receptor-mediated inhibition of adenylate cyclase (Katada & Ui, 1982) enables these proposals to be explored.

In the present investigation, Pertussis toxin has been used in the guinea-pig ileum to investigate the mechanisms underlying the acute inhibitory effects of normorphine and clonidine on electrically-induced contractions and on the naloxone-precipitated withdrawal contractures seen in tissues chronically exposed to normorphine.

Methods Ileum was obtained from male guinea-pigs (Dunkin Hartley, 300–500 g) killed by cervical dislocation and exsanguination, 2 or 3 days after intraperitoneal injection of 50 μg purified Pertussis toxin or of vehicle. Segments of tissue (2–2.5 cm) were taken from the caecal end of the ileum after discarding the terminal 5–10 cm. Some segments of tissue were used immediately for determinations of acetylcholine sensitivity and the acute effects of normorphine and clonidine on electrically-evoked responses, other segments were prepared for incubation with normorphine.

Acute effects of normorphine and clonidine were determined in segments of ileum set up in Krebs solution at 36°C and gassed with 95% O_2 and 5% CO_2. When appropriate, the ileum was stimulated transmurally with parallel platinum electrodes using a supramaximal voltage (60 V), a pulse duration of 1 ms and a frequency of 0.1 Hz. Responses were recorded isometrically under 1 g tension. After a 20 min equilibration period, the effect of normorphine or clonidine on electrically-induced contractions was determined using a contact time of 1.5 min and a dose interval of 10 min. Segments were used for one concentration-response curve only.

Dependence was induced by incubating ileal segments for 16–21 h in Krebs solution containing normorphine 10^{-6} M at a temperature of 10–11°C. Incubation fluid was pumped over the tissues at a rate of 2.5 ml min^{-1}. Control tissues were incubated in Krebs solution without normorphine. After incubation, tissues were set up in the appropriate Krebs solution. Withdrawal was precipitated with a Krebs solution containing naloxone (10^{-7} M) administered after a 45 min equilibration period. Between the 20th and 30th min of equilibration, reference responses to acetylcholine and to electrical stimulation were obtained. Responsiveness to electrical stimulation was reassessed 3–5 min after exposure to naloxone. The contracture elicited by naloxone was expressed as a percentage of the maximal response to acetylcholine in that tissue.

Purified Pertussis toxin (Irons & Maclennan, 1979) was dissolved in equal parts of glycerol and a buffer solution of 50 mM Tris and 1 M NaCl at pH 8. The toxin was a gift from Dr L. Irons, Public Health Laboratory Service, Porton Down. Statistical significance tests were performed using Student's *t* test.

Results Pertussis toxin pretreatment markedly reduced the acute inhibitory effects of normorphine and clonidine on responses to electrical stimulation (Figure 1a and b). There was a displacement to the right of the concentration-response curve and a

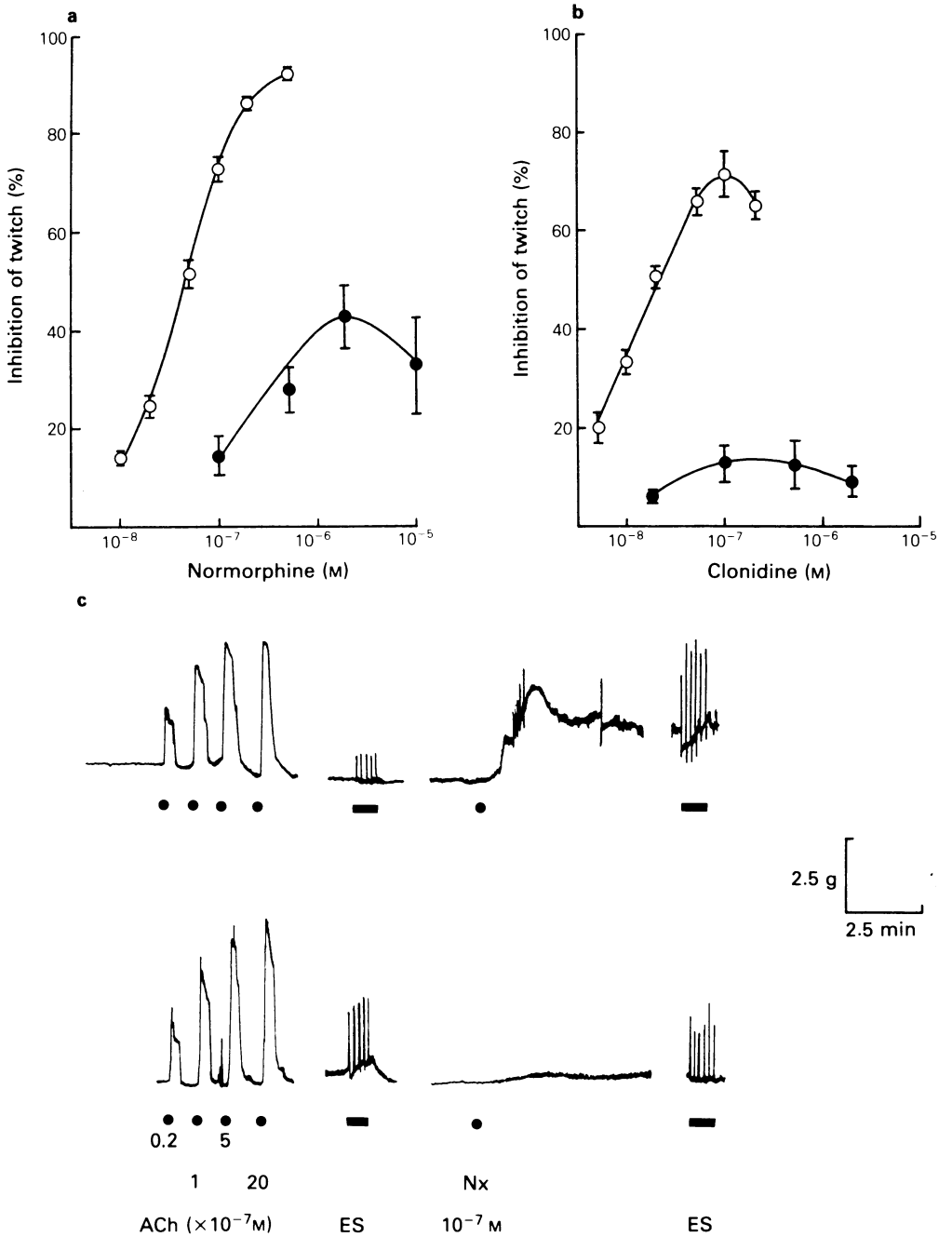

Figure 1 Effect of Pertussis toxin on acute effects of normorphine and clonidine and on naloxone-precipitated normorphine withdrawal in guinea-pig isolated ileum: (a) and (b) show concentration-response curves for normorphine and clonidine, respectively, in the transmurally stimulated ileum, after prior treatment of animals with 50 μg Pertussis toxin (●) or vehicle (○). Responses are expressed as mean inhibition (%) of twitch height; vertical lines indicate s.e.mean; number of experiments not less than 10. In (c) responses of ilea taken from animals pretreated with toxin (lower trace) or vehicle (upper trace) are shown. Both tissues were incubated for 16–21 h at 10–11°C in Krebs solution containing normorphine (10^{-6} M) before being set up in fluid of the same composition at 36°C for recording contractions. Upper trace shows a typical withdrawal contraction in response to naloxone 10^{-7} M (Nx). Lower trace shows that the Nx-precipitated withdrawal contraction of ileum from Pertussis toxin pretreated guinea-pigs is greatly attenuated. Reference responses to acetylcholine (ACh) and electrical stimulation (ES) are also shown.

50–70% reduction in the maximal response. However, the magnitude of responses to acetylcholine and to electrical stimulation was unaltered.

In tissues exposed to 10^{-7} M normorphine for 16–21 h, the naloxone-precipitated withdrawal responses were reduced significantly from 52.5% ± 6.0% (mean ± s.e.mean; $n = 10$) in control tissues, to 11.7% ± 2.9% ($n = 11$) in toxin-treated tissues ($P < 0.001$). An example of the effect of Pertussis toxin on naloxone-precipitated withdrawal is shown in Figure 1c. The small contracture produced by naloxone in tissues incubated in Krebs solution without normorphine was also reduced from 3.25% ± 0.7% to 0.5% ± 0.5% ($n = 10$; $P < 0.01$).

Discussion Until recently, investigations into the role of cyclic nucleotides in neurotransmission in tissues such as the guinea-pig ileum have been restricted to studies of the effects of alterations in cyclic AMP levels. The availability of Pertussis toxin allows a new approach to the problem. Islet-activating protein, a pertussis toxin, has been shown to attenuate receptor-mediated inhibition of adenylate cyclase by selective inactivation of a guanine nucleotide regulatory protein (Katada & Ui, 1982). Since in the present experiments the presynaptic inhibitory effects of normorphine and clonidine in the ileum were markedly attenuated by Pertussis toxin pretreatment, it is likely that these actions were mediated through inhibition of adenylate cyclase. This mechanism of action has been proposed to explain the action of clonidine and opiates in other tissues (Costa *et al.*, 1983; Martinez-Olmedo & Garcia-Sainz, 1983). Similar results with morphine and clonidine in preparations of guinea-pig ileum have also recently been reported by Lujan *et al.* (1984). In the present experiments, dependence was measured by the size of the naloxone-precipitated withdrawal contracture in normorphine-dependent tissues (Collier *et al.*, 1981); it was markedly reduced by treatment with Pertussis toxin. It should be noted that neighbouring segments of ileum were used in the experiments to demonstrate the effects *in vitro* of Pertussis toxin on normorphine dependence and on the acute inhibitory effect of normorphine. Thus, it is probable that a similar mechanism is involved in both the acute and chronic actions of normorphine.

In an earlier study with Pertussis vaccine as a source of toxin, it was reported that vaccine treatment reduced normorphine withdrawal contractures in the ileum without altering the acute effects of normorphine (Collier *et al.*, 1983). A likely explanation for this apparent discrepancy is the reported significant variation between batches in the toxin content of Pertussis vaccine (Lujan *et al.*, 1984).

In view of the effects of Pertussis toxin described here, it is likely that the common mechanism proposed to explain the acute and chronic effects of normorphine involves inhibition of adenylate cyclase. The findings also support the cyclic AMP hypothesis of dependence which implicates adenylate cyclase in the adaptive changes underlying opiate dependence.

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References

COLLIER, H.O.J., CUTHBERT, N.J. & FRANCIS, D.L. (1981). Model of opiate dependence in the guinea-pig isolated ileum. *Br. J. Pharmac.*, **73,** 921–932.

COLLIER, H.O.J., PLANT, N.T. & TUCKER, J.F. (1983). Pertussis vaccine inhibits the chronic but not acute action of normorphine on the myenteric plexus of guinea-pig ileum. *Eur. J. Pharmac.*, **91,** 325–326.

COLLIER, H.O.J. & ROY, A.C. (1974). Morphine-like drugs inhibit the stimulation by E prostaglandins of cyclic AMP formation by rat brain homogenate. *Nature*, **248,** 24–27.

COSTA, T., AKTORIES, G., SCHULTZ, G. & WUSTER, M. (1983). Pertussis toxin decreases opiate receptor binding and adenylate cyclase inhibition in a neuroblastoma x glioma hycrid cell line. *Life Sci.*, **33,** 219–223.

IRONS, L.I. & MACLENNAN, A.P. (1979). Isolation of the lymphocytosis promoting factor-haemagglutinin of Bordetella Pertussis by affinity chromatography. *Biochim. biophys. Acta*, **580,** 175–185.

KATADA, T. & UI, M. (1982). Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. *Proc. natn, Acad. Sci.*, U.S.A., **79,** 3129–3133.

MARTINEZ-OLMEDO, M.A. & GARCIA-SAINZ, J.A. (1983). Effect of Pertussis toxin on the hormonal regulation of cyclic AMP levels in hamster fat cells. *Biochim. biophys. Acta.*, **760,** 215–220.

LUJAN, M., LOPEZ, E., RAMIREZ, R., AGUILAR, H., MARTINEZ-OLMEDO, M.A. & GARCIA-SAINZ, J.A. (1984). Pertussis toxin blocks the action of morphine, norepinephrine and clonidine on isolated guinea-pig ileum. *Eur. J. Pharmac.*, **100,** 377–380.

PELAYO, F., DUBOCOVICH, M.L. & LANGER, S.Z. (1978). Possible role of cyclic nucleotides in regulation of noradrenaline release from rat pineal through presynaptic-adrenoceptors. *Nature*, **274,** 76–78.

SHARMA, S.K., KLEE, W.A. & NIRENBERG, M. (1975). Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. *Proc. natn. Acad. Sci., U.S.A.*, **72,** 3092–3096.

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