Br. J. Pharmacol. (1988), **93**, 932–936

A possible role for prostaglandins in the expression of morphine dependence in guinea-pig isolated ileum

M.A. Johnson, R.G. Hill & J. Hughes

Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Rd, Cambridge, CB2 2QB

1 The effects of the cyclo-oxygenase inhibitors, indomethacin (1.3 μM) and mefenamic acid (10 μM), were investigated on the naloxone-precipitated withdrawal contracture of the morphine-dependent guinea-pig ileum *in vitro*.

2 Both indomethacin and mefenamic acid prevented expression of the withdrawal contracture on naloxone challenge.

3 The effects of indomethacin were reversed by the concurrent application of a low dose of prostaglandin E_1 (10 nM).

4 It was concluded that prostaglandins probably play a role in the expression of morphine withdrawal in this model.

Introduction

A model of dependence can be consistently induced and measured *in vitro* using the guinea-pig isolated ileum preparation (Collier *et al.*, 1981; 1983). When this tissue is incubated with opioid drugs for an extended period a contraction of the longitudinal muscle is seen on the application of the opioid antagonist naloxone.

There is evidence that prostaglandins can interact with the acute action of opiates in both guinea-pig ileum (Ehrenpries *et al.*, 1973) and brain (Collier & Roy, 1974). It was therefore of interest to examine whether prostaglandins were also involved in the chronic effects of opiates. Krakow *et al.* (1986) have shown that indomethacin inhibits the withdrawal contraction seen on the addition of naloxone to ileum from a guinea-pig dosed chronically with morphine. We have now shown that cyclo-oxygenase inhibitors can reduce the naloxone-induced withdrawal response in the guinea-pig ileum exposed to morphine *in vitro*, and have obtained evidence supporting a role for prostaglandins in this model. Some of these results have been briefly presented elsewhere (Hill *et al.*, 1986).

Methods

Preparation of segments of ileum

Male, drug naïve Dunkin-Hartley guinea-pigs of Pirbright origin (250–400 g, Interfauna, Huntington, U.K.) were killed by cervical dislocation. Portions of ileum were removed from a point 10 cm orally from the caecum and placed in a modified Krebs solution (mM): NaCl 118.06, KCl 4.69, $MgSO_4$ 1.18, $CaCl_2$ 2.55, KH_2PO_4 1.18, $NaHCO_3$ 25 and D-glucose 11.1, containing 70 μM hexamethonium.

Incubation procedure

Segments of ileum were incubated for 20–24 h at room temperature (20–25°C) in 10 ml organ baths that were constantly perfused with Krebs solution and gassed with 95% O_2 plus 5% CO_2.

The incubation medium was either Krebs solution or Krebs plus (a) 1 μM morphine, (b) 1 μM morphine and 1.3 μM indomethacin (or 10 μM mefenamic acid), (c) 1.3 μM indomethacin (or 10 μM mefenamic acid). One μM morphine was chosen as a concentration that gave consistent *in vitro* dependence, as seen in experiments in which several concentrations were tested (see Results).

The solution was pumped through the baths at a rate of 1 ml min^{-1} by a Gilson peristaltic pump.

Test procedure

The tissues were mounted for recording on coaxial platinum stimulating electrodes in 10 ml baths maintained at 37°C and gassed continuously with 95%

O_2 plus 5% CO_2. They were set up in Krebs solution of the same composition as the incubation medium. However, some tissues incubated in a solution containing morphine only were tested in Krebs solution containing morphine and indomethacin (1.3 μM) or mefenamic acid (10 μM), allowing 40 min before addition of naloxone. The tissues were stimulated electrically for 30 s using trains of pulses (200 ms duration, 0.1 Hz repetition rate). These trains were made up of 0.2 ms square pulses at 20 Hz. A voltage between 80 and 90 V was found to be supramaximal in most experiments. The tissues were allowed to equilibrate for 30–40 min before they were stimulated for a further 30 s, washed and then challenged with naloxone. The naloxone was left in contact with the tissue for 2 min. The size of the contracture produced by the naloxone challenge is expressed as a fraction of the maximum contraction obtained with the subsequent addition of acetylcholine in the same piece of tissue according to a modification of the method of Collier *et al.* (1981):

$$\frac{\text{Response to naloxone}}{\text{Maximum response to ACh (on same tissue)}} \times 100 = \text{Tension ratio}$$

Testing with prostaglandin E_1

Some tissues, which had been incubated for 20–24 h in medium containing indomethacin and morphine, were exposed to prostaglandin E_1 (PGE_1, 100 nM) 10 min before the naloxone challenge. The tissues were then washed, stimulated for 30 s, washed again and a further dose of PGE_1 (10 nM), which did not elicit a contraction, was added before the addition of 1 μM naloxone to the bath. The withdrawal contracture was measured as described previously.

Drugs

The following drugs were used: acetylcholine chloride (Sigma), hexamethonium bromide (Sigma), indomethacin (Sigma), mefenamic acid (Sigma), morphine sulphate McCarthy), naloxone hydrochloride (Dupont) and prostaglandin E_1 (Sigma). Stock solutions of acetylcholine, hexamethonium, morphine and naloxone were made up in water, indomethacin in 3% w/v sodium bicarbonate solution, mefenamic acid in 0.1 M NaOH and prostaglandin E_1 in 90% v/v ethanol followed by addition of water. Final dilutions were made in the bathing solution for the tissue.

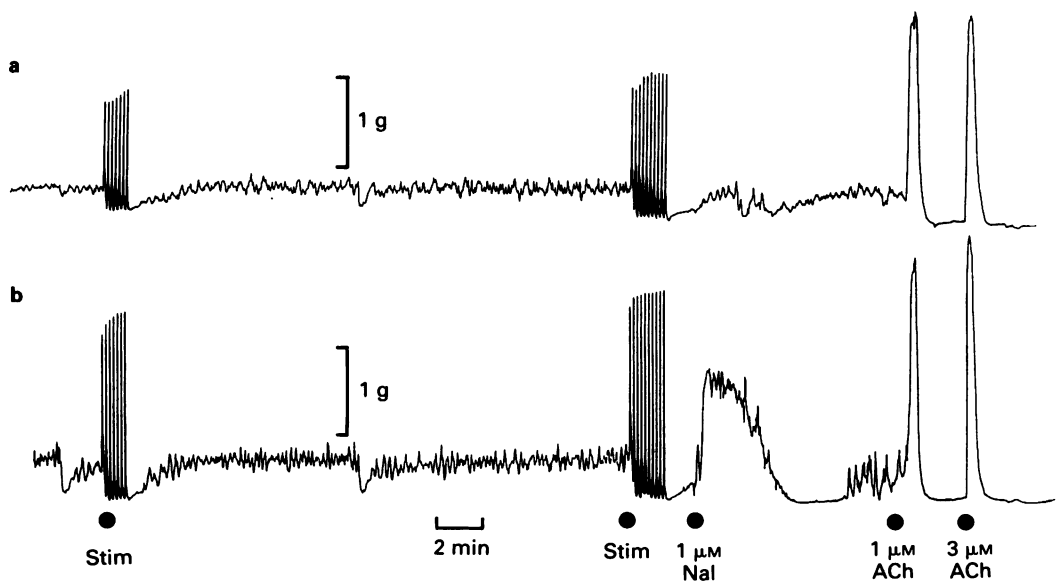

Figure 1 Effect of indomethacin on the contracture elicited by naloxone (Nal) (1 μM). Results are shown from one of six experiments in which segments of ileum were incubated for 20–24 h at 20–25°C in Krebs solution containing morphine (1 μM). These segments were then set up for recording in solution with either (a) morphine (1 μM) and indomethacin (1.3 μM) or (b) morphine (1 μM) alone. The presence of indomethacin markedly reduced the response to naloxone. The acetylcholine (ACh) responses were used to normalize the withdrawal contracture as a fraction of the maximum response of the tissue to this spasmogen. The ability to respond to electrical stimulation (Stim) was unaffected by the different drug treatments used. These results were obtained from segments of ileum from the same guinea-pig tested in parallel in a multiple bath apparatus.

Results

Tissues incubated with morphine

A withdrawal contracture was always seen in tissues which had been incubated overnight with 1 μM morphine solution (Figure 1).

Doses of morphine between 0.1 and 10 μM were tested in pilot experiments and withdrawal contractures, that were dose related, were seen. The response to 1 μM morphine was near maximal and as it was more consistent than that seen with higher concentrations this dose was used in all subsequent studies. Similarly, doses of naloxone between 0.05 and 10 μM were found to be capable of evoking a withdrawal contracture, with the response to 1 μM being maximal and reproducible (Tension ratio 40 ± 4 ($n = 6$) following incubation with 1 μM morphine).

Tissues incubated with morphine plus cyclo-oxygenase inhibitors

Tissues which had been incubated in Krebs solution only, morphine (1 μM) plus indomethacin (1.3 μM) or indomethacin (1.3 μM) alone showed a much reduced response to the addition of naloxone (Figures 1 and 2). When tissues were incubated in solutions containing both morphine (1.0 μM) and indomethacin (1.3 μM) and then tested in Krebs solution containing only morphine (1.0 μM), the withdrawal contracture was still suppressed. This suggests that the action of indomethacin in these tissues is prolonged and not easily reversed.

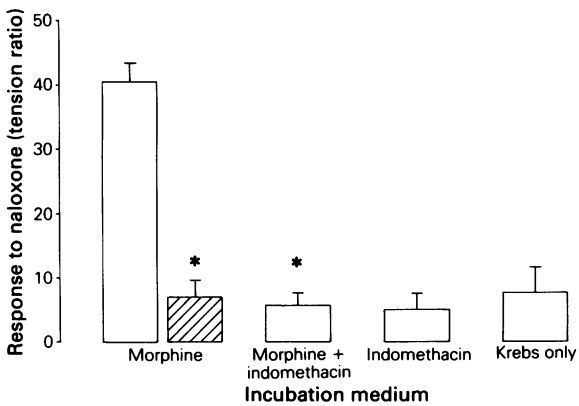

Figure 2 Effect of indomethacin on the contracture response to naloxone. Segments of ileum were incubated for 20–24 h at 20–25°C in Krebs solution with the addition of morphine (1 μM) and/or indomethacin (1.3 μM) as indicated. After incubation the segments were tested in Krebs solution of the same composition as the incubation medium. One group of segments, incubated in a solution containing morphine (1 μM) were set up for testing in a solution containing morphine (1 μM) and indomethacin (1.3 μM) (hatched column). The columns show the mean response to naloxone (1 μM) ($n = 6$ for all treatments); vertical lines indicate s.e. mean. The presence of indomethacin significantly (*$P < 0.01$, Mann-Whitney U-test) reduced the naloxone-elicited contracture to the size of responses seen in non-opiate-treated tissues.

Dependent tissues tested in Krebs solution with morphine and cyclo-oxygenase inhibitors

Tissues which were shown to be morphine-dependent, by testing adjacent segments of tissue in parallel, did not show a withdrawal contracture when challenged with naloxone in Krebs solution containing either 1.3 μM indomethacin or 10 μM mefenamic acid (Table 1, Figures 1 and 2).

Reversal of the effects of cyclo-oxygenase inhibition by prostaglandin E_1

Tissues which were incubated and tested in Krebs solution containing morphine (1 μM) and indomethacin (1.3 μM) did not show a contracture when challenged with naloxone (Tension ratio = 3.8 ± 2.1, $n = 6$). When PGE_1 (10 nM) was added to the bath,

Table 1 The effect of mefenamic acid on the naloxone precipitated withdrawal contracture

Addition to incubation medium	*Addition to testing medium*	*Response to naloxone* (Tension ratio)[a]
Morphine (1 μM)	Morphine (1 μM)	12.5 ± 2.0 (8)
Morphine (1 μM)	Morphine (1 μM) + mefenamic acid (10 μM)	1.9 ± 0.4 (8)*

Segments of ileum were incubated for 20–24 h at 20–25°C in incubation medium containing 1 μM morphine. After incubation the segments were set up for recording in Krebs solution either with the addition of morphine or with the addition of morphine and mefenamic acid (10 μM). The presence of mefenamic acid significantly reduced (*$P < 0.01$, Mann-Whitney U-test) the contracture precipitated by the addition of naloxone (1 μM). The peak control tension ratio seen following morphine incubation and naloxone challenge in this experiment is rather low (cf. values illustrated in Figure 2) but the difference with the cyclo-oxygenase inhibitor-treated tissue is still apparent (see Discussion).

[a] Results shown are mean $\pm$ s.e. mean of n (number in parentheses) observations.

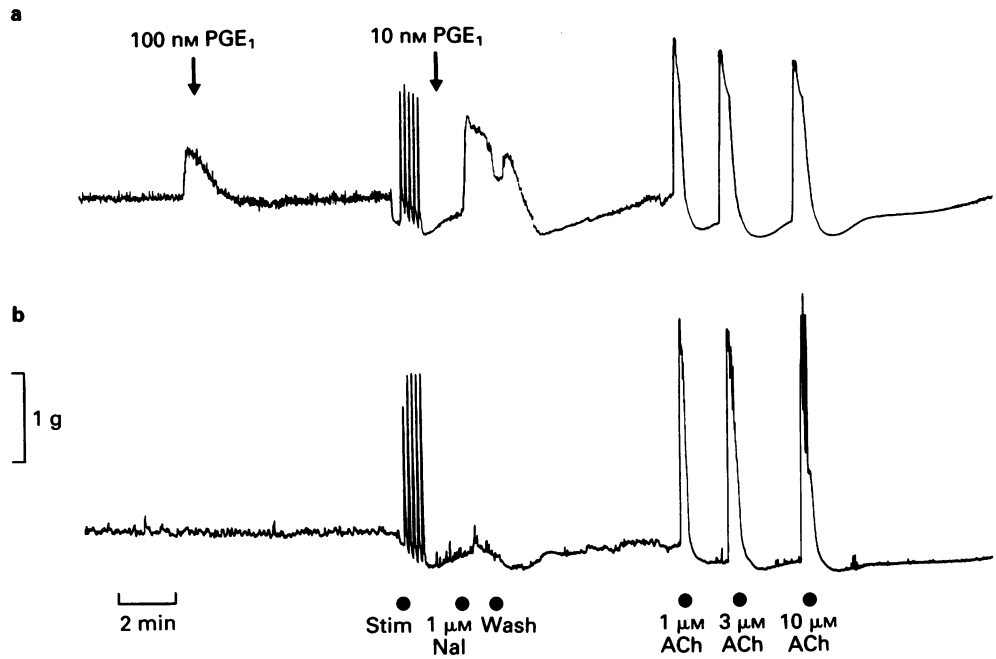

Figure 3 Effect of prostaglandin E_1 (PGE_1) on the naloxone-induced contracture in the presence of indomethacin. Results are shown from one of six experiments in which segments of ileum were incubated and tested in Krebs solution with morphine (1 μM) and indomethacin (1.3 μM). PGE_1 was added to the bath in trace (a) at a concentration that elicited a contraction (100 nM) to confirm the activity of the prostaglandin. Then PGE_1 was added to the bath at a concentration that did not elicit a contracture (10 nM) prior to the addition of naloxone (Nal, 1 μM). The addition of PGE_1 caused a significant increase in the size of contracture ($P < 0.01$, Mann-Whitney U-test) elicited by naloxone. (b) Shows a parallel experiment in which no PGE_1 was added, using a segment of ileum from the same guinea-pig, incubated and tested in parallel with that segment providing the result shown in (a).

however, a naloxone precipitated contracture was observed (Tension ratio = 32.0 ± 6.9, $n = 6$. Figure 3).

Discussion

These results suggest that the presence of prostaglandins is necessary for the ileum to exhibit a withdrawal contracture to naloxone following incubation in the presence of morphine. It seems likely that prostaglandins are necessary for the manifestation of the withdrawal response rather than for the induction of dependence. This is demonstrated by the observation that whereas tissues that have been incubated with morphine alone display a pronounced withdrawal response on naloxone challenge, paired tissues from the same length of ileum and also incubated with morphine show a reduced response to naloxone after a short exposure to the cyclo-oxygenase inhibitors prior to the naloxone challenge (Table 1, Figure 2). We have found that a lower dose of indomethacin (0.5 μM) also inhibits the naloxone contracture (result from a single experiment). However, it has been shown that tissues incubated with morphine and indomethacin together will show a withdrawal response in the presence of added PGE_1 (Figure 3). The size of the withdrawal contracture recorded in these experiments, *per se*, does not seem to influence the action of the cyclo-oxygenase inhibitors used (e.g. compare Figure 2 with Table 1).

Collier & Roy (1974) have shown that opiates specifically inhibit prostaglandin E-stimulated cyclic AMP formation in rat brain preparations *in vitro*. It is therefore possible that a similar mechanism could be operating in the neurones of the myenteric plexus, such that a balance is maintained between inhibition of the system by the opiate and stimulation by prostaglandins. Thus when naloxone is added to the system the balance would be tipped in favour of stimulation by prostaglandins and hence result in the production of a withdrawal contracture. In the presence of indomethacin (or mefenamic acid) the

balance shifts in favour of inhibition so that when naloxone is added the excitability of the tissue is not sufficient to result in a withdrawal contracture.

It has been suggested by Ehrenpreis *et al.* (1973) that prostaglandins can reverse the inhibition of the electrically evoked twitch of guinea-pig ileum produced by morphine and this is consistent with the results of Kadlec *et al.* (1974) who showed that prostaglandins can increase the electrically stimulated release of acetylcholine in guinea-pig ileum. Okpako & Taiwo (1984) have demonstrated that cyclo-oxygenase inhibitors can inhibit electrically evoked release of acetylcholine from myenteric plexus longitudinal muscle strip preparations. However, at the concentration of indomethacin we were using we found no inhibition of electrically evoked twitch. This is therefore unlikely to explain the 80% decrease in the naloxone-elicited contracture seen in the presence of indomethacin. One possibility would be that other neurotransmitters are involved in the withdrawal contracture. It has been shown that a large proportion of the contracture is due to acetylcholine release since it is reversed by atropine or hyoscine, although there is a hyoscine resistant component of the contracture which is likely to be operated by substance P (Tsou *et al.*, 1982; Chahl 1983). It is possible that a variety of interneurones releasing a range of transmitters are involved and the role of prostaglandins may be one of modulating the sensitivity of the final cholinergic neurone to these various inputs. In view of the recent discovery that lipoxygenase metabolites of arachidonic acid act as second messengers for presynaptic inhibition in *Aplysia* (Piomelli *et al.*, 1987), it would be of interest to study the action of lipoxygenase inhibitors on the withdrawal contracture.

References

CHAHL, L.A. (1983). Contracture of guinea pig ileum on withdrawal of methionine enkephalin is mediated by substance P. *Br. J. Pharmacol.*, **80**, 741–749.

COLLIER, H.O.J. (1983). Cellular aspects of opioid tolerance and dependence. In *Opioids Past, Present and Future.* ed. Hughes, J., Collier, H.O.J., Tyers, M.B. & Rance, M. pp. 109–125. London: Taylor & Francis.

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

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*, **246**, 24–27.

EHRENPRIES, S., GREENBERG, J. & BELMAN, S. (1973). Prostaglandins reverse inhibition of electrically induced contractions of guinea pig ileum by morphine, indomethacin and acetylsalicylic acid. *Nature*, **245**, 280–283.

KADLEC, O., MASEK, K. & SEFERNA, I. (1974). A modulating role of prostaglandins in contractions of guinea-pig ileum. *Br. J. Pharmacol.*, **51**, 565–569.

KRAKOW, M.S., EHRENPRIES, E.D. & EHRENPRIES, S. (1986). Inhibition by indomethacin of naloxone-precipitated opiate withdrawal contraction in isolated guinea pig myenteric plexus-longitudinal muscle preparation. *Life Sci.*, **39**, 243–247.

HILL, R.G., HUGHES, J. & JOHNSON, M.A. (1986). Cyclo-oxygenase inhibitors affect morphine dependence in the guinea-pig isolated ileum. *Br. J. Pharmacol.*, **87**, 170P.

OKPAKO, D.T. & TAIWO, Y.O.O. (1984). Cyclo-oxygenase inhibitors antagonize indirectly evoked contractions of the guinea-pig isolated ileum by inhibiting acetylcholine release. *Br. J. Pharmacol.*, **82**, 577–582.

PIOMELLI, D., VOLTERRA, A., DALE, N., SIEGELBAUM, S.A., KANDEL, E.R., SCHWARTZ, J.H. & BELARDETTI, F. (1987). Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplysia* sensory cells. *Nature*, **328**, 38–43.

TSOU, K., LOUIE, G. & WAY, E.L. (1982). Manifestations of gut opiate withdrawal contracture and its blockade by capsaicin. *Eur. J. Pharmacol.*, **81**, 377–383.

(Received June 24, 1987
Revised October 3, 1987
Accepted October 24, 1987)