Br. J. Pharmac. (1979), **66**, 373–375

NALOXONE INHIBITS THE ANTI-DIARRHOEAL ACTIVITY OF LOPERAMIDE

M.F. PIERCEY & M.J. RUWART

CNS Research and Experimental Biology Research, The Upjohn Company, Kalamazoo, Michigan 49001, U.S.A.

1 Subcutaneous prostaglandin E_2 (2.5 mg/kg) produces profuse diarrhoea in fed rats.

2 Pretreatment of rats with subcutaneous loperamide (1.0 mg/kg) completely prevents prostaglandin-induced diarrhoea. If naloxone is administered prior to loperamide injections the activity of the antidiarrhoeal compound is completely destroyed.

3 These data provide strong evidence that the antidiarrhoeal activity of loperamide is mediated via the opiate receptor.

Introduction

Loperamide, a new antidiarrhoeal agent (Van Bever & Lal, 1976), effectively antagonized diarrhoea induced by castor oil in rats (Niemegeers, Lenaerts & Janssen, 1974a; Bianchi & Goi, 1977) or prostaglandin E_2 (PGE_2) in rats (Karim & Adaikan, 1977) and man (Karim & Adaikan, 1977; Lange, Secher, & Amery, 1977). The therapeutic effect of this compound is believed to be due to its anti-motility properties (Van Neuten, Janssen & Fontaine, 1974; Niemegeers, Lenaerts & Janssen, 1974b; Karim & Adaiken, 1977) and antagonism of prostaglandin-induced enteropooling (Karim & Adaikan, 1977).

Loperamide has been shown to have fewer central narcotic effects than diphenoxylate (Niemegeers *et al.*, 1974a; Colpaert, Niemegeers, Lal & Janssen, 1975; Bianchi & Goi, 1977; Heilman, Salvatore & McGuire, 1977). However, in the duodenum of the anaesthetized dog the effect of loperamide on contractile activity of the circular and longitudinal muscles is blocked by the narcotic antagonist, naloxone (Mackerer, Clay & Dajani, 1976). Electrically-induced contractions in guinea-pig ileum are depressed by loperamide; this effect can also be competitively antagonized by naloxone (Mackerer *et al.*, 1976; Wüster, Shulz & Herz, 1976). These data suggest that the antidiarrhoeal activity of loperamide, presumed to be due to its effects on smooth muscle contraction, may be mediated through opiate receptors. This paper describes a study performed to test this hypothesis.

Methods

Sixty male Upjohn rats (200 to 220 g) were restrained in metal tubes and divided into six equal groups. Groups 1, 2, and 5 were given vehicle and Groups 3, 4, and 6 given naloxone (0.5 mg/kg) by subcutaneous injection (s.c.) at zero time and once again 30 min later to ensure drug levels were maintained. Ten min after the first naloxone or vehicle administration, Groups 5 and 6 were given loperamide (1 mg/kg s.c.) and Groups 1–4 were injected with vehicle; 20 min after loperamide or vehicle treatment, subcutaneous PGE_2 (2.5 mg/kg) was administered to Groups 2, 4, 5 and 6 with the remaining groups receiving vehicle. One hour after prostaglandin or vehicle administration, animals were scored for copious (++), mild (+) or lack of (0) diarrhoea. The activity score was calculated by taking the sum of the number of '+' rats and twice the number of '++' rats. Thus, for a group of 10 rats, the maximum score indicating severe diarrhoea was 20. A score of 0 indicated a complete absence of diarrhoea.

Results

Group 1 rats received only vehicle injections; as expected they had virtually no diarrhoea (Table 1). Group 2 treated with prostaglandin produced a maximum score of 20. Naloxone did not alleviate this diarrhoea (Group 4) nor did it produce diarrhoea when given to rats not receiving PGE_2 (Group 3). Loperamide was very effective against prostaglandin-induced diarrhoea, reducing the activity score from 20 to 1 (Group 5). However, pretreatment with naloxone, completely inhibited the beneficial effect of loperamide (Group 6).

Discussion

Naloxone is considered to be a relatively pure opiate receptor antagonist. Since naloxone by itself does not

0007-1188/79/070373-03 $01.00

Table 1 Effects of naloxone on loperamide inhibition of prostaglandin E_2 (PGE_2)-induced diarrhoea

Group	Treatment N	L	PG	Diarrhoea score ++	+	0	Total score (max = 20)
1	—	—	—	0	1	9	1
2	—	—	+	10	0	0	20
3	+	—	—	0	0	10	0
4	+	—	+	10	0	0	20
5	—	+	+	0	1	9	1
6	+	+	+	10	0	0	20

N = naloxone; L = loperamide; PG = PGE_2.
Diarrhoea score: ++ = number of rats with copious diarrhoea; + = number of rats with mild diarrhoea; 0 = number of rats without diarrhoea.
Total score: the number of rats with copious diarrhoea doubled and added to the number of rats with mild diarrhoea.

cause diarrhoea, its antagonism of the antidiarrhoeal actions of loperamide is most probably due to opiate receptor-mediated blockade. Such a conclusion is consistent with the observations that loperamide competes with naloxone for binding to opiate receptors of both brain and gut (Wüster *et al.*, 1976; Mackerer *et al.*, 1976; Clay, Mackerer & Lin, 1977). In view of these *in vitro* binding studies, it is not totally clear why loperamide administered *in vivo* has few central nervous system effects. Possible explanations include poor penetration across the blood-brain barrier (Wüster *et al.*, 1976) as well as differences in the opiate receptor populations at different sites (Clay *et al.*, 1977). However, loperamide is not totally lacking in central narcotic properties since high doses have been reported to produce both analgesia (Mackerer *et al.*, 1976; Dajani, Bianchi, East, Bloss, Adelstein & Yen, 1977) and opiate-like physical dependence (Dajani *et al.*, 1977; Mackerer, Brougham, East, Bloss, Dajani & Clay, 1977). Moreover, loperamide can suppress morphine abstinence in mice (Dajani *et al.*, 1977).

It has been previously reported that the antimotility effects of loperamide are inhibited by naloxone (Mackerer *et al.*, 1976; 1977; Dajani *et al.*, 1977). Naloxone has also been shown to antagonize inhibition by loperamide of the electrically stimulated guinea-pig ileum (Mackerer *et al.*, 1976, Wüster *et al.*, 1976). While correlation between opiate receptor binding and anti-diarrhoeal activity has been elegantly demonstrated by Stahl, Van Bever, Janssen & Simon (1977), data bearing directly on this question have been lacking. Our results represent a direct validation of the hypothesis that opiate receptor stimulation represents the underlying mechanism for the antidiarrhoeal effects of loperamide.

The authors wish to thank Ortho Pharmaceutical Corporation, New Jersey, and Endo Laboratories Inc., New York, for their generous gifts of loperamide and naloxone, respectively. The technical assistance of B. D. Rush and M. S. Klepper is gratefully acknowledged.

References

BIANCHI, C. & GOI, A. (1977). On the antidiarrheal and analgesic properties of diphenoxylate, difenoxine and loperamide in mice and rats. *Arzn.-Forsch.*, **27**, 1040–1043.

CLAY, G.A., MACKERER, C.R. & LIN, T.K. (1977). Interaction of loperamide with [^{3}H]-naloxone binding sites in guinea-pig brain and myenteric plexus. *Mol. Pharmac.*, **13**, 533–540.

COLPAERT, F.C., NIEMEGEERS, C.J.E., LAL, H. & JANSSEN, P.A. (1975). Investigations on drug produced and subjectively experienced discriminative stimuli. 2. Loperamide, an antidiarrheal devoid of narcotic cue producing actions. *Life Sci., Oxford*, **16**, 717–728.

DAJANI, E.Z., BIANCHI, R.G., EAST, P.F., BLOSS, J.L., ALDELSTEIN, G.W. & YEN, C.H. (1977). The pharmacology of SC-27166: A novel antidiarrheal agent. *J. Pharmac. exp. Ther.*, **203**, 512–526.

HEILMAN, R.D., SALVATORE, A.T. & MCGUIRE, J.L. (1977). Ethanol-induced sleep: lack of potentiation by loperamide. *Fedn Proc.*, **36**, 1019.

KARIM, S.M.M. & ADAIKAN, P.G. (1977). The effect of loperamide on prostaglandin-induced diarrhea in rat and man. *Prostaglandins*, **13**, 321–331.

LANGE, A.P., SECHER, N.J. & AMERY, W. (1977). Prostaglandin-induced diarrhea treated with loperamide or diphenoxylate. *Acta med. scand.*, **202**, 449–454.

MACKERER, C.R., BROUGHAM, L.R., EAST, P.F., BLOSS, J.L., DAJANI, E.Z. & CLAY, G.A. (1977). Antidiarrheal and central nervous system activities of SC-27166 (2-[3-(5-methyl-1,3,4-oxadiazol-2-yl)-3,3-diphenylpropyl]-2-

azabicyclo[2.2.2]octane), a new anti-diarrheal agent, resulting from binding to opiate receptor sites of brain and myenteric plexus. *J. Pharmac. exp. Ther.*, **203**, 527–538.

MACKERER, C.R., CLAY, G.A. & DAJANI, E.Z. (1976). Loperamide binding to opiate receptor sites of brain and myenteric plexus. *J. Pharmac. exp. Ther.*, **199**, 131–140.

NIEMEGEERS, C.J.E., LENAERTS, F.M. & JANSSEN, P.A.J. (1974a). Loperamide (R-18553), a novel type of anti-diarrheal agent. Part I: *In vivo* oral pharmacology and acute toxicity. Comparison with morphine, codeine, diphenoxylate and difenoxine. *Arzn.-Forsch.* **24**, 1633–1636.

NIEMEGEERS, C.J.E., LENAERTS, F.M. & JANSSEN, P.A.J. (1974b). Loperamide (R-18553), a novel type of anti-diarrheal agent. Part 2: *In vivo* parenteral pharmacology and acute toxicity in mice. Comparison with morphine, codeine and diphenoxylate. *Arzn.-Forsch.*, **24**, 1636–1641.

STAHL, K.D., VAN BEVER, W., JANSSEN, P. & SIMON, E.J. (1977). Receptor affinity and pharmacological potency of a series of narcotic analgesic, antidiarrheal and neuroleptic drugs. *Eur. J. Pharmac.*, **46**, 199–205.

VAN BEVER, W. & LAL, H. (1976). *Synthetic Antidiarrheal Drugs.* New York: Marcel Dekker.

VAN NUETEN, J.M., JANSSEN, P.A.J. & FONTAINE, J. (1974). Loperamide (R-18553), a novel type of antidiarrheal agent. Part 3: In vitro studies on the peristaltic reflex and other experiments on isolated tissues. *Arzn.-Forsch.*, **24**, 1641–1644.

WÜSTER, M., SCHULZ, R. and HERZ, A. (1976). Opiates compared with anti-diarrheals concerning their affinity to the morphine receptor and action on the isolated guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmac.*, **293** (Suppl.), R35.

(Received August 14, 1978.
Revised November 7, 1978.)