Br. J. Pharmac. (1986), **89**, 599–602

Evidence against VIP as the inhibitory transmitter in non-adrenergic, non-cholinergic nerves supplying the longitudinal muscle of the mouse colon

J. Fontaine*, A.R. Grivegnee & P. Robberecht**

University of Brussels, Laboratory of Pharmacology* and Department of Biochemistry**, Institute of Pharmacy* and Faculty of Medicine**, C.P. 205/7 – 1050 Brussels, Belgium

1 Vasoactive intestinal peptide (VIP) had two types of effects on the longitudinal muscle of the mouse distal colon.

2 At low concentrations (10^{-8} M) VIP induced a contraction which seemed to be related to the production of prostaglandins as it was abolished after preincubation with indomethacin (10^{-6} M).

3 At higher concentrations (3×10^{-8} and 10^{-7} M) VIP induced relaxations which developed slowly and were related to stimulation of the adenylate cyclase activity of the smooth muscle cells.

4 There is no evidence that VIP is the non-adrenergic, non-cholinergic transmitter released by electrical stimulation in this preparation and responsible for rapid relaxation of the smooth muscle.

Introduction

Non-adrenergic, non-cholinergic (NANC) nerves are widely distributed in peripheral tissues, particularly within the gastrointestinal tract (Burnstock, 1972). Various substances have been proposed as the inhibitory transmitter released by these nerves, including purines (Burnstock *et al.*, 1970) and several peptides. Vasoactive intestinal polypeptide (VIP) is present in neuronal plexuses of the gastrointestinal tract (Furness & Costa, 1982) and it has been suggested that this peptide is released as a neurotransmitter from NANC nerves in the gut (Furness & Costa, 1982; Makhlouf, 1982).

We have previously shown the presence of a NANC inhibitory control in the mouse isolated colon, the nature of which remains to be elucidated (Fontaine *et al.*, 1984). In the present work we have analysed the effects of VIP on this preparation in order to see whether it is a possible candidate for the inhibitory transmitter release by these nerves.

Methods

Organ bath experiments

Swiss Webster mice (30–40 g) were stunned and killed by exsanguination; the terminal colon was dissected out and placed under an initial load of 1 g in an organ bath containing Krebs solution with the following composition (mM): KCl 4.7, NaCl 118.1, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 5. The bath (10 ml) was maintained at 36 ± 1°C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2. The isometric contractions of the longitudinal muscle were measured with a Grass force-displacement transducer. Preparations were allowed to equilibrate in Krebs solution for at least 60 min before drugs were added. Acetylcholine (ACh) 10^{-6} M was added at the beginning of each experiment to test the reactivity of the preparation.

VIP was left in contact with the tissue for periods of 2 or 3 min at intervals of 15 min.

Antagonists were incubated with the tissue for at least 10 min before agonists were tested ($n = 4–6$ for each antagonist).

Adenylate cyclase assay

Terminal colons of Swiss Webster mice were dissected out and incubated for 20 min in a bath containing NaCl (0.24 M) and EDTA (2.5 mM) before the mucosa was carefully peeled off.

Histological controls were made to be sure that all mucosal cells were removed. The longitudinal smooth muscles were then homogenized and stored in liquid nitrogen in aliquots of 3 mg ml^{-1} protein until use. The adenylate-cyclase activity was determined by the procedure of Salomon *et al.* (1974) with minor

modifications as previously described (Waelbroeck *et al.*, 1981).

Drugs

Acetylcholine hydrochloride (Roche), atropine sulphate (Fluka), indomethacin (Merck, Sharp & Dohme), mepyramine maleate (Rhône-Poulenc), methysergide hydrogen maleate (Sandoz), naloxone hydrochloride (Endo), phentolamine methane sulphonate (Ciba), propranolol hydrochloride (ICI), tetrodotoxin (Calbiochem) and synthetic vasoactive intestinal peptide (generous gift from Dr D.H. Coy, Tulane University, New Orleans, LA., U.S.A.) were used. Indomethacin (31 mg) was dissolved in ethanol (4 ml) and diluted with distilled water to give 0.31 mg ml^{-1}.

Doses refer to molar concentrations (M) of salts in the bath.

Results

Effects of VIP on the longitudinal muscle preparation of the mouse colon

VIP (10^{-8} M) induced reproducible contractions of the terminal colon (Figure 1). The responses were rapid in onset, an initial peak being followed by increased phasic activity. At concentrations above 3×10^{-8} M VIP, there was an inhibition of the spontaneous acivity of the colon and a dose-related relaxation of the preparation. This effect developed slowly and reached its maximum in 2–3 min (Figure 1). It was reproducible at 15 min intervals.

The VIP-induced contractions were not modified by atropine (3×10^{-7} M), mepyramine (2.5×10^{-7} M), methysergide (5×10^{-7} M), propranolol (10^{-6} M), phentolamine (10^{-6} M), naloxone (4×10^{-7} M) or tetrodotoxin (2×10^{-7} M). However, after preincuba-

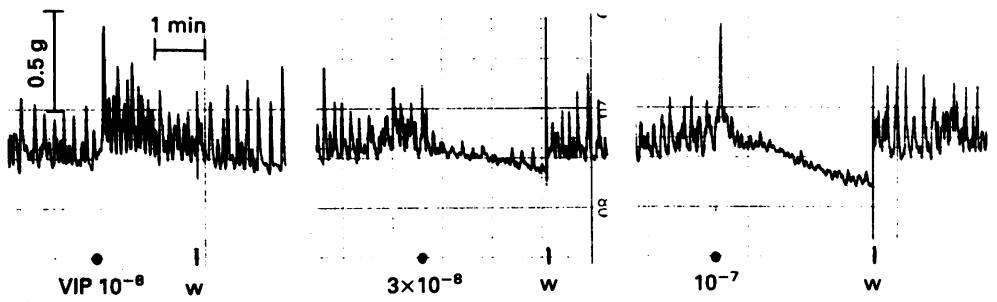

Figure 1 Effect of vasoactive intestinal peptide (VIP, 10^{-8}, 3×10^{-8} and 10^{-7} M) on the mouse colon given at 15 min intervals. W = washout.

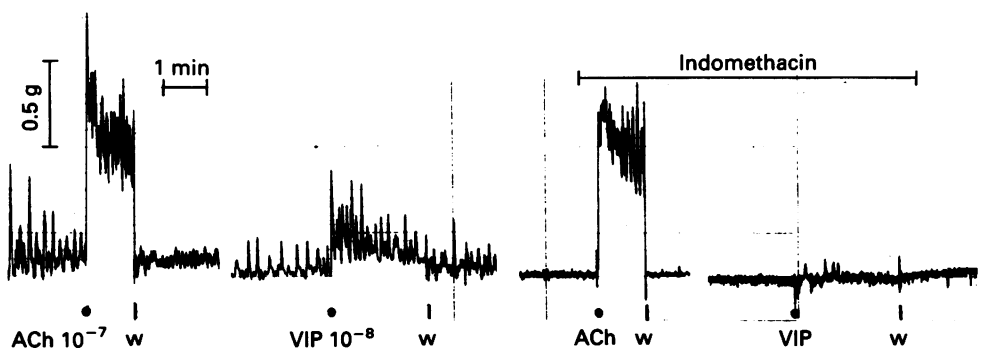

Figure 2 Inhibitory effects of indomethacin (10^{-6} M) on the response of the mouse colon to VIP (10^{-8} M). The acetylcholine (ACh)-induced response is not modified. W = washout.

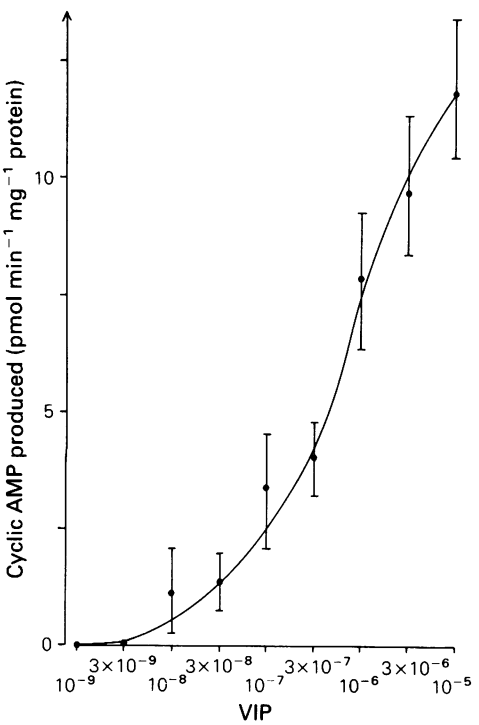

Figure 3 Concentration-effect curve for the production of cyclic AMP of mouse colonic smooth muscle homogenates in the presence of increasing concentrations of VIP. Mean values are shown, vertical lines indicate s.e.mean; $n = 6$.

tion with indomethacin (10^{-6} M) for 30 min ($n = 6$) the contractions to VIP 10^{-8} M were abolished while the acetylcholine (10^{-7} M)-induced responses were not modified (Figure 2). Under the same experimental conditions, the solvent for indomethacin (40% ethanol in water) had no effect.

The VIP-induced relaxations (10^{-7} M) were not modified by tetrodotoxin (2×10^{-7} M), phentolamine (10^{-6} M), propranolol (10^{-6} M), methysergide (5×10^{-7} M), mepyramine (3×10^{-7} M), or after preincubation with indomethacin (10^{-6} M).

Effects of VIP on adenylate cyclase activity

From 3×10^{-8} M to 10^{-5} M, VIP induced a dose-related stimulation of the adenylate cyclase activity in homogenates of colonic smooth muscle cells. These stimulatory effects are illustrated in Figure 3.

Discussion

The present results show that, according to the concentration used, VIP has two types of effect on the longitudinal muscle of the mouse distal colon. (1) At low concentrations (10^{-8} M) this peptide induced a small contraction of the preparation by a direct effect on the smooth muscle cells as it was not abolished in the presence of tetrodotoxin. This stimulatory effect seems to be related to the production of endogenous prostaglandins by the preparation as it was abolished after preincubation with indomethacin, a drug known to inhibit prostaglandin synthesis (Vane, 1971). We have previously shown that prostaglandins play a role in the regulation of the muscle tone of the mouse colon (Fontaine *et al.*, 1984). The contractions developed in the presence of low concentrations of VIP could be due either to sensitization of the muscle to the effect of endogenous prostaglandins, or, alternatively, to a greater release of prostaglandins in the presence of the peptide. This remains to be demonstrated. However, there is no evidence of release of prostaglandins by VIP in other gastrointestinal preparations.

(2) At 3×10^{-8} M and 10^{-7} M VIP induced slowly developing relaxations of the colonic smooth muscle cells. This effect is clearly related to a stimulatory effect on the adenylate cyclase activity of these cells which has been described in other intestinal smooth muscles (Cohen & Landry, 1980).

There are numerous studies showing VIP-induced relaxations of gastrointestinal muscle, mainly in circular muscles, whereas contractile effects of cholinergic origin have been described in the longitudinal muscle of the small intestine of guinea-pig and rabbit (Furness & Costa, 1982). More recently, Bennett *et al.* (1984) showed that VIP contracted the longitudinal muscle and relaxed the circular muscle of the guinea-pig colon. These authors suggest that NANC inhibitory nerves may be different in circular and longitudinal muscle strips.

The results of the present study do not support the hypothesis that VIP is an inhibitory neurotransmitter in the isolated longitudinal muscle of the mouse colon. Indeed, VIP does not mimic the effects of stimulating the enteric inhibitory nerves in this preparation (Fontaine *et al.*, 1984).

The authors thank Mr J. Sveska for his technical assistance, Mrs J. Gilbert for her secretarial help and the Belgian 'Fonds National de la Recherche Scientifique' for financial support (Grant No. 34524.80).

References

BENNETT, A., BLOOM, S.R., CH'NG, J., CHRISTOFIDES, N.D., PEACOCK, L.E. & RENNIE, J.A. (1984). Is vasoactive intestinal peptide an inhibitory transmitter in the circular but not the longitudinal muscle of guinea-pig colon? *J. Pharm. Pharmac.*, **36**, 787–788.

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.*, **24**, 509–581.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D.G. & SMYTHE, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmac.*, **40**, 668–688.

COHEN, M.L. & LANDRY, A.S. (1980). Vasoactive intestinal polypeptide: increased tone, enhancement of acetylcholine release and stimulation of adenylate cyclase in intestinal smooth muscle. *Life Sci.*, **26**, 811–822.

FONTAINE, J., GRIVEGNÉE, A. & REUSE, J. (1984). Adrenoceptors and regulation of intestinal tone in the isolated colon of the mouse. *Br. J. Pharmac.*, **81**, 231–243.

FURNESS, J.B. & COSTA, M. (1982). Enteric inhibitory nerves and VIP. In *Vasoactive Intestinal Peptide*. ed. Said, S.I., pp. 391–406. New York: Raven Press.

MAKHLOUF, G.M. (1982). Role of VIP in the function of the gut. In *Vasoactive Intestinal Peptide*. ed. Said, S.I. pp. 425–446. New York: Raven Press.

SALOMON, Y., LONDOS, C. & RODBELL, M. (1974). A highly sensitive adenylate cyclase assay. *Anal. Biochem.*, **58**, 541–548.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. *Nature, New Biol.*, **231**, 232–235.

WAELBROECK, M., ROBBERECHT, P., DE NEEF, PH, CHATELAIN, P. & CHRISTOPHE, J. (1981). Binding of vasoactive intestinal peptide and its stimulation of adenylate cyclase through two classes of receptors in rat liver membranes. Effects of 12 secretin analogues and 2 secretin fragments. *Biochim. biophys. Acta.*, **678**, 83–90.

(Received May 13, 1986.
Revised June 23, 1986.
Accepted June 30, 1986.)