Narcotic antagonistic action of cimetidine on the guinea-pig ileum

ISSEI TAKAYANAGI*, YUMIKO IWAYAMA, YUTAKA KASUYA, *Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo* 113, *Japan*

During investigations of actions of cimetidine, a histamine H_2-receptor antagonist (Black, 1976) on isolated smooth muscles, we found that cimetidine antagonized the inhibitory action of morphine and leucine-enkephalin, an endogenous opiate receptor ligand (Hughes, Smith & others, 1975), on the ileum from the guinea-pig. We therefore compared the mode of action of cimetidine on the ileum with that of a narcotic antagonist, naloxone.

Male guinea-pigs, 250 to 350 g, were killed, the ileum isolated and 4–5 cm taken from the middle ileum was suspended in 20 ml organ bath filled with Locke Ringer solution kept at 32° and gassed with 5% CO_2 in oxygen. The Locke Ringer solution used had the following composition (mM): NaCl 154, KCl 5·6, $CaCl_2$ 2·2, $MgCl_2$ 2·1, $NaHCO_3$ 5·9 and glucose 2·8. The pH of the Locke Ringer solution under these conditions was 7·4. Responses of the preparation to drugs were recorded isotonically. In some experiments electrical stimulation was carried out according to Paton (1957). The electrodes were made of platinum and the intraluminal electrode was the anode. Rectangular pulses of 1 ms duration were used at a frequency of 0·1 Hz and strength sufficient to give a maximal response. The responses of the ileum to electrical stimulation were recorded isometrically with an initial tension of 0·5 g.

Twitch responses of the ileum induced by electrical stimulation were inhibited by morphine (10^{-7} M) (Paton, 1957) and leucine-enkephalin (10^{-6} M) (Hughes & others, 1975). This inhibition by morphine and leucine-enkephalin was much reduced by cimetidine (10^{-4} M) and naloxone (3×10^{-8} M) and abolished by higher concentrations of cimetidine (3×10^{-4} M) and naloxone (10^{-7} M). Tetrodotoxin (5×10^{-8} M) and strychnine (10^{-4} M) (Takagi & Takayanagi, 1966) also greatly reduced the twitch responses of the ileum. The inhibition by tetrodotoxin and strychnine was little influenced by naloxone (10^{-6} M) and cimetidine (3×10^{-4} M). These results are shown in Fig. 1.

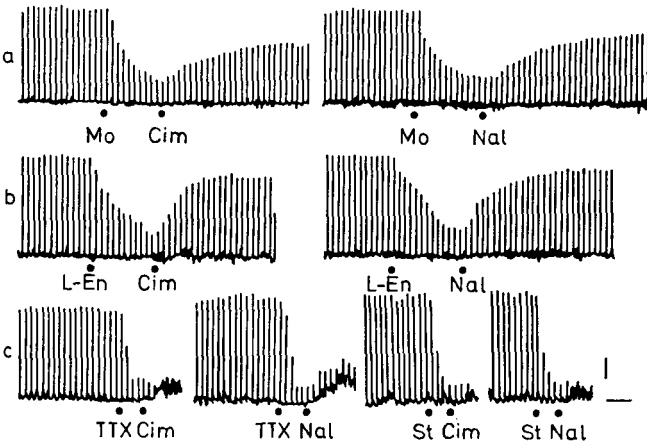

FIG. 1. Effects of cimetidine and naloxone on the inhibition of the twitch responses of the ileum to electrical stimulation induced by morphine, leucine-enkephalin, tetrodotoxin and strychnine. Cim: cimetidine, Mo: morphine, Nal: naloxone, L-En: leucine-enkephalin, TTX: tetrodotoxin, St: strychnine. Concentrations (M) Mo: 10^{-7}; Cim: 10^{-4} (a,b), 3×10^{-4} (c); Nal: 3×10^{-8} (a,b), 10^{-6} (c); L-En: 10^{-6}; TTX: 5×10^{8}; St: 10^{-4}. Horizontal scale: 1 min; vertical scale: 1 g.

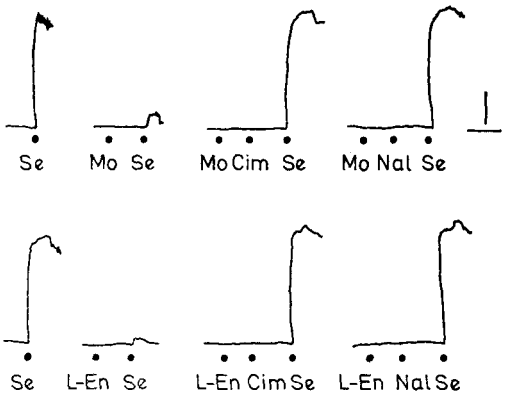

FIG. 2. Reversal of morphine- and leucine-enkephalin-induced block of contractile response of the ileum to 5-HT by cimetidine and naloxone. Se: 5-HT, Mo: morphine, L-En: leucine-enkephalin, Cim: cimetidine, Nal: naloxone. Concentrations (M) Se: 5×10^{-7}; Mo: 10^{-6}; Cim: 10^{-4}; Nal: 10^{-7}; L-En: 10^{-6}. Horizontal scale: 3 min; vertical scale: 1 cm.

* Correspondence.

It is well known that 5-hydroxytryptamine in low concentrations contracts the guinea-pig ileum through intramural cholinergic nerve elements (M-receptors) (Rocha e Silva, Valle & Picarelli, 1953; Gaddum & Picarelli, 1957; Kosterlitz & Robinson, 1958; Brownlee & Johnson, 1963). Contraction of the ileum induced by 5-HT (5×10^{-7} M) was inhibited by morphine (10^{-6} M), leucine-enkephalin (10^{-6} M) and tetrodotoxin (3×10^{-7} M). Cimetidine (10^{-4} M) and naloxone (10^{-7} M), the concentrations which did not influence contraction of the ileum induced by 5-HT, abolished the inhibition by morphine and leucine-enkephalin (Fig. 2) but did not influence that by tetrodotoxin. The results thus suggest that cimetidine in high concentrations acts as a narcotic antagonist on the ileum from the guinea-pig.

The authors wish to express their gratitude to Smith Kline—Fujisawa Co. Ltd for the generous gift of cimetidine. November 23, 1977

REFERENCES

BLACK, J. W. (1976). Proceedings of the Sixth International Congress of Pharmacology, Vol. 1, pp. 3–16, *Receptors and Cellular Pharmacology*. Editor: Klinge, E.

BROWNLEE, G. & JOHNSON, E. S. (1963). *Br. J. Pharmac. Chemother.*, **21**, 305–322.

GADDUM, J. H. & PICARELLI, Z. P. (1957). *Ibid.*, **12**, 323–328.

HUGHES, J., SMITH, T. W., KOSTERLITZ, H. W., FOTHERGILL, L. A., MORGAN, B. A. & MORRIS, H. R. (1975). *Nature*, **258**, 577–579.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1958). *Br. J. Pharmac. Chemother.*, **13**, 296–303.

PATON, W. D. M. (1957). *Ibid.*, **11**, 119–127.

ROCHA E SILVA, M., VALLE, J. R. & PICARELLI, Z. P. (1953). *Ibid.*, **8**, 378–388.

TAKAGI, K. & TAKAYANAGI, I. (1966). *Jap. J. Pharmac.*, **16**, 211–216.

Enhancement of the abdominal constriction response of mice to lipopolysaccharides by phosphate

J. R. ROBORGH, *Philips-Duphar B.V., Research Laboratories, Postbox 2, Weesp, The Netherlands*

After intraperitoneal injection of a noxious agent into mice, they respond with repeated waves of constriction and elongation passing caudally along the abdomen ending with extension of the hind limbs. The reaction has been called the 'abdominal constriction response' (Collier, Hammond & others, 1964; Collier, Dinneen & others, 1968). In the course of an investigation of local reactions to parenteral preparations, this response of mice was used as a test model and a remarkable enhancement of the constriction response to bacterial lipopolysaccharides was encountered when these were dissolved in phosphate buffer instead of twice distilled water.

CPB : SE(S) mice (TNO, Zeist, The Netherlands) of either sex 20–25 g were maintained at 21–23°. Coded test solutions were administered intraperitoneally in a dose volume of 0·1 ml per mouse. After the challenge each mouse was separately placed in a translucent cage kept in a quiet environment and the number of constriction responses in the time 10–20 min after the injection counted, as this interval gave the most reproducible results after a challenge. In 4 experiments with different dilutions of acetic acid, in twice distilled water, and using the 10–20 min observation period a fair dose response relation was found (Fig. 1) showing the test model gave a quantitative and reproducible result.

We then used lipopolysaccharides (LPS) from some Gram-negative bacteria. In experiments with the LPS prepared from *Escherichia coli*, *Salmonella typhosa*, *S. typhimurium* or *S. enteritidis* (Difco Laboratories, Detroit, Michigan) it soon became apparent that these polymers in twice distilled water were as slow-acting as acetic acid and elicited only a low frequency of responses. As preparations for parenteral use are sometimes buffered, we then dissolved the LPS in phosphate buffer 0·12 M, pH 7·2 and observed much stronger responses. The results of 9 independent experiments with the four LPS each dissolved in twice distilled water or phosphate buffer at widely different concentrations are given in Fig. 2. It is clear that the use of phosphate buffer has a profound enhancing effect on the frequency of the constriction responses. This effect of phosphate is evident over a dose range extending from 5×10^{-1} to 5×10^{4} ng of LPS per mouse and it is quantitatively the same with all four LPS substances.

In a separate experiment in which 24 mice per group were used, the dose of LPS from *E. coli* was kept constant at 5×10^{2} ng per mouse but dissolved in