J. Pharm. Pharmacol. 1986, 38: 621–622
Communicated January 13, 1986

Neither morphine nor clonidine influence the non-adrenergic, non-cholinergic inhibitory response in the rat gastric fundus

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Morphine and clonidine are among the few agents reported to modulate non-adrenergic, non-cholinergic (NANC) responses in different tissues. They were therefore tested for their effect on the NANC inhibitory response in the rat gastric fundus. Neither morphine (10^{-5} M), nor clonidine (10^{-5} M in the presence of prazosin 10^{-6} M to avoid its own relaxatory effect) modified the NANC response in the rat gastric fundus.

More and more agents are known to modify neurotransmitter release in the cholinergic and adrenergic systems by interaction with presynaptic receptors. However, only a few substances are known to modify non-adrenergic, non-cholinergic (NANC) neurotransmission. Morphine was reported to depress the NANC inhibitory response in the guinea-pig taenia coli (Shimo & Ishii 1978). Clonidine was found to enhance the NANC excitatory response in the guinea-pig urinary bladder (Muir & Smart 1983), but to inhibit NANC excitatory neurotransmission in the guinea-pig tracheobronchial tree (Grundström & Andersson 1985). We therefore thought it of interest to investigate the effect of morphine and clonidine on the inhibitory NANC response which can be elicited in the rat gastric fundus (Heazell 1977; Lefebvre 1986).

Methods

Female rats (150–300 g) were reserpinized (reserpine 5 mg kg^{-1} intraperitoneally; Hollands & Vanov 1965) 24 h before killing. From the stomach fundus, a longitudinal muscle strip (3 mm–3 cm) was dissected (Vane 1957) and mounted under a load of 1 g between two platinum plate electrodes in an 18 ml organ bath containing Krebs solution (NaCl 118·5, KCl 4·8, $CaCl_2$ 1·9, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, glucose 10·1 mM) at 37 °C, bubbled with 95% O_2–5% CO_2. The Krebs solution also contained 5-hydroxytryptamine (5-HT) 3×10^{-6} M to increase the tone of the strips, and atropine 10^{-6} M. Auxotonic changes in tone (Harvard heart-smooth muscle transducer) were registered on a Beckman type R Dynograph recorder and transmural stimulation was carried out by use of a Grass stimulator (type S88).

Transmural stimulation (supramaximal voltage, 1 ms duration, 5 Hz, during 45 s) was performed 6 times with an interval of at least 20 min. In each strip, morphine 10^{-5} M (10 min incubation), prazosin 10^{-6} M (10 min incubation) or prazosin 10^{-6} M and clonidine 10^{-5} M (respectively 10 and 5 min incubation) were administered in randomized order before the 2nd, 4th and 6th stimulation. Prazosin 10^{-6} M alone was added in only 8 of the 12 tissues. The response in the presence of a drug was expressed in per cent of that before addition of the drugs (control value). Results are given as mean ± s.e.m.

Drugs used were atropine sulphate, clonidine hydrochloride, morphine hydrochloride, prazosin hydrochloride, reserpine, 5-hydroxytryptamine creatinine sulphate. For morphine and reserpine, commercially available ampoules were used. Drugs were dissolved or diluted in isotonic NaCl solution, except for prazosin which was dissolved in distilled water.

Results and discussion

With transmural stimulation (supramaximal voltage, 1 ms, 5 Hz, 45 s), a reproducible relaxatory response was seen. At 5 Hz a maximal or near maximal relaxatory answer is obtained in this preparation (Lefebvre 1986). The relaxation is non-adrenergic, non-cholinergic as the Krebs solution contained atropine in a concentration known to block cholinergic nerve-mediated contractions in this preparation (Lefebvre et al 1983) and as the animals were reserpinized.

Morphine 10^{-5} M did not influence the NANC relaxation (Fig. 1; relaxation in the presence of morphine 101 ± 2% of control value, n = 12). With this concentration of morphine, maximal depression of the NANC inhibitory response in the guinea-pig taenia coli was obtained (Shimo & Ishii 1978) while 10 times lower concentrations produced a decrease of the amplitude of the NANC inhibitory junction potentials in the rabbit proximal and distal colon as seen by Blanquet et al (1982). These authors used preganglionic parasympathetic stimulation and suggested that morphine induces its inhibitory effect at the level of the transmission between the preganglionic parasympathetic fibres and the NANC inhibitory neurons. This could explain the absence of an effect of morphine in our experiments, as the NANC relaxation in the rat gastric fundus is purely postganglionic (Lefebvre 1986). Shimo & Ishii (1978) used transmural stimulation as we did, but it is not clear from their results whether stimulation of preganglionic nerve endings is involved in the response obtained. Small & Yong (1983) were not able to reproduce the results of Shimo & Ishii.

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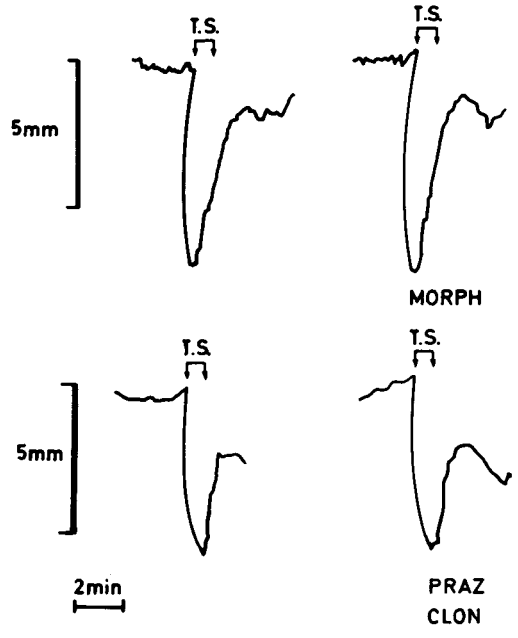

FIG. 1. Longitudinal muscle strip of the rat gastric fundus: relaxatory response to transmural electrical stimulation (T.S., supramaximal voltage, 1 ms, 5 Hz, 45 s). Upper panel: response before and in the presence of morphine 10^{-5} M; lower panel: response before and in the presence of prazosin 10^{-6} M and clonidine 10^{-5} M. After stopping transmural stimulation, the bathing medium was changed.

The influence of the α_2-adrenoceptor selective agonist clonidine on the NANC relaxation was studied in the presence of the α_1-adrenoceptor antagonist prazosin. Indeed, preliminary experiments showed that clonidine 10^{-5} M induced a relaxation of the strips themselves, possibly because in high concentrations, it interacts with the postjunctional α_1-adrenoceptors, present in the rat gastric fundus (Verplanken et al 1984). Prazosin itself, in a concentration of 10^{-6} M, had no influence on the NANC inhibitory response to transmural stimulation (relaxation in the presence of prazosin 96 ± 1% of control value, n = 8). After addition of prazosin, clonidine no longer produced relaxation. In the presence of prazosin and clonidine, a small reduction of the NANC relaxation induced by transmural stimulation was seen in some strips but not in all (Fig. 1; mean relaxation in the presence of prazosin and clonidine 87 ± 3% of control value, n = 12). This is very different from the marked enhancing effect of clonidine on the NANC excitatory response in the guinea-pig urinary bladder (maximal enhancement by clonidine 10^{-6} M of the response at 20 Hz 200%, Muir & Smart 1983). The effect we see is also much smaller than the 70–85% reduction of the NANC excitatory response in the guinea-pig airways in-vivo (Grundström & Andersson 1985); in an in-vitro study with guinea-pig tracheobronchial material the same group observed a very pronounced reduction of the NANC excitatory response with noradrenaline and B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro[4*H*]thiazolo[5,4-*d*]azepine dihydrochloride), but clonidine was not tested (Grundström et al 1984). The observation that in the rat gastric fundus clonidine does not have the same effect as in the guinea-pig urinary bladder or airways, cannot be due to the presence of the α_1-adrenoceptor blocker prazosin, as the enhancing effect of clonidine on the NANC excitatory response in the guinea-pig urinary bladder was reported to be independent of its α-adrenoceptor agonistic activity (Muir & Smart 1983), and as the inhibitory effect of the NANC excitatory response in the guinea-pig tracheo-bronchial tree is due to interaction wth α_2-adrenoceptors (Grundström & Andersson 1985).

We can conclude that within the conditions used, neither morphine nor clonidine influence the NANC inhibitory response in the rat gastric fundus. Their modulating effect on NANC transmission is thus not general and seems tissue related.

This study was supported by Grant No. 3.0019.82 from the Fund for Medical Scientific Research. R. Lefebvre is a Research Associate of the National Fund for Scientific Research (Belgium).

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