Br. J. Pharmacol. (1990), **99**, 186–188

Motor response of the human isolated small intestine and urinary bladder to porcine neuromedin U-8

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1 Porcine neuromedin U-8 produced a concentration (0.3 nM–1 μM)-dependent contraction of the longitudinal muscle of the human isolated ileum, which was unaffected by either atropine (1 μM) or tetrodotoxin (1 μM).

2 By contrast, neuromedin U-8 had only a weak effect on the circular muscle of the human isolated ileum.

3 Neuromedin U-8 also produced a concentration-dependent contraction of mucosa-free muscle strips from the dome of the human isolated urinary bladder, its action being unaffected by either atropine or tetrodotoxin.

4 These findings indicate that neuromedin U-8 exerts a direct contractile effect on smooth muscle of the human intestinal and urinary tract.

Introduction

Porcine neuromedins U are two peptides of 8 and 25 amino acids which share the common C-terminal sequence Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2 (porcine neuromedin U-8, Minamino *et al.*, 1985). In the 25 amino acid form, this sequence is immediately preceded by two Arg-Arg residues suggesting a possible cleavage of the larger molecular form. Originally isolated from extracts of porcine spinal cord, neuromedin U-like immunoreactivities have been detected in the mammalian brain, gastrointestinal and genitourinary tract of various species (Domin *et al.*, 1986; 1987; Augood *et al.*, 1988; Ballesta *et al.*, 1988) as well as in the human cerebral cortex and ileum (Domin *et al.*, 1986).

Thus neuromedins U add to the long list of putative regulatory peptides found in the brain and the gut. Until now, relatively little information has been collected about the biological activity of neuromedin U-8 or U-25. Minamino *et al.* (1985) demonstrated that these peptides are potent stimulants of isolated uterine muscle and increase markedly blood pressure in anaesthetized rats. Sumi *et al.* (1987) showed that both neuromedin U-8 and U-25 exert a potent and selective vasoconstrictor activity at splanchnic area level. In both studies, neuromedin U-25 was more potent than neuromedin U-8, indicating that the C-terminal sequence is probably responsible for the biological activity, whose expression is facilitated in the extended form of the peptide. More recently, Brown & Quito (1988) found that neuromedin U-8 affects ion transport in porcine jejunal mucosa via a neural mechanism while having no effect on motility of pig isolated jejunal muscle. The absence of a motor response to neuromedins U-8 and U-25 was also described by Minamino *et al.* (1985) in the guinea-pig isolated ileum.

In the present study we show that synthetic neuromedin U-8 exerts a potent contractile effect on the human isolated urinary bladder and ileum, the effect on the latter being almost restricted to the longitudinal muscle.

Methods

The effects of neuromedin U-8 were investigated on 24 ileal strips from 10 patients (age 61–77 years) and 15 bladder strips from 7 patients undergoing abdominal surgery for carcinoma of the bladder base (enterocistoplasty).

No patient received radio- or chemotherapy before intervention. In all patients, pre-anaesthetic medication was intramuscular atropine (1 mg) and diazepam (10 mg) 2 h before. Anaesthesia was induced by sodium thiopentone (500 mg i.v.) and maintained with N_2O/O_2 (1/2) and halothane (0.6–1%). The patients received pancuronium bromide (6 mg i.v.) during induction of anaesthesia.

Bladder samples were excised from the dome. All bladder and ileal specimens appeared macroscopically normal with no signs of tumour or inflammation. The tissues were placed in ice-cold Krebs solution within 2–3 min from surgical removal. The specimens were pinned flat on a Petri dish containing Krebs solution and the mucosa was carefully dissected out. Small strips (0.5–0.8 cm long, 2–3 mm wide) of muscle were cut along the longitudinal or circular axis of the ileum or along the longitudinal axis of the bladder.

The strips were stored overnight in ice-cold (4°C), and continuously oxygenated (96% O_2, 4% CO_2) Krebs solution, i.e. functional experiments started 16–20 h after surgery. Either bladder or ileal strips were suspended under a resting tension of 10 mN in an organ bath (5 ml) containing oxygenated Krebs solution at 37°C, as described previously (Maggi *et al.*, 1988a, b; 1989a, b). Isometric tension (resting load 10 mN) was recorded on a Basile 7050 Unirecord.

All experiments commenced after an equilibration period of 90–120 min during which the bathing solution was renewed every 10–15 min. Concentration-response curves to neuromedin U-8 were constructed in a non-cumulative manner because tachyphylaxis developed upon cumulative addition of the peptide to the bath (see Results). Increasing concentrations of neuromedin U-8 were added to the bath at 10–20 min intervals and left in contact until maximal responses developed. A thorough washing out was made between application of doses. In a few experiments on the human intestine, cumulative concentration-response curves were constructed, the next concentration being added to the bath when the effect of the preceding one had reached a steady state.

Statistical analysis

All data in the text are mean ± s.e.mean. Statistical analysis was performed by means of Student's *t* test for paired or

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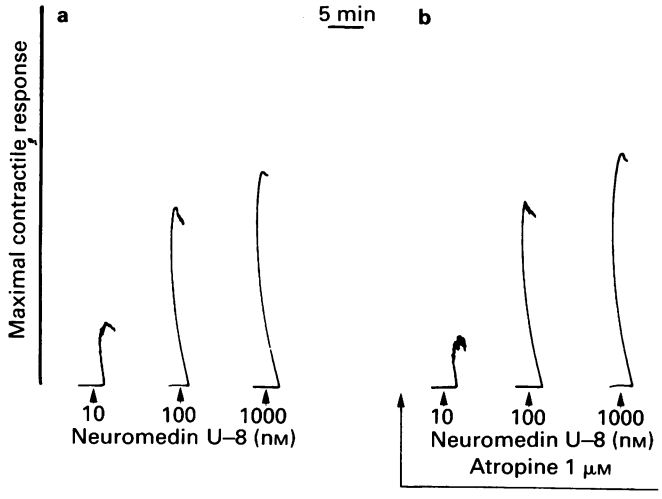

Figure 1 Typical tracings showing the contractile response of the human isolated ileum (longitudinally oriented strips) to neuromedin U-8 in the absence (a) and presence (b) of atropine 1 μM.

unpaired data or by means of the analysis of variance, when applicable.

Drugs

Drugs used were: atropine HCl (Serva) and tetrodotoxin (Sankyo). Neuromedin U-8 was synthesized by Dr P. Rovero, Chemistry Department, Menarini Parmaceuticals, by conventional solid-phase methods.

Results

Human isolated ileum

In longitudinally oriented strips, neuromedin U-8 produced a concentration-dependent contraction (Figures 1 and 2) (threshold concentration 3 nM). The response to the peptide was characterized by tachyphylaxis. In fact, as shown in Figure 2, the cumulative addition of neuromedin U-8 produced a response lower than that observed after non-cumulative addition of the peptide to the bath. This latter approach was therefore used in all subsequent experiments. The maximal response to neuromedin U-8 averaged 53 ± 2% of the response to carbachol (0.1 mM).

The response to neuromedin U-8 was unaffected by either atropine (1 μM, Figures 1 and 2) or tetrodotoxin (1 μM) (Figure 2b) at concentrations which were sufficient to produce marked inhibition or abolition of the primary contraction in response to electrical field stimulation (cf. Maggi *et al.*, 1989a).

In circularly oriented strips (n = 5 from 5 patients), neuromedin U-8 was a very weak stimulant. The threshold concentration was 30 nM and the maximal response did not exceed 15% of that to carbachol (0.1 mM). Even in this case the response to neuromedin U-8 was apparently unaffected by atropine (n = 3, data not shown).

Human isolated urinary bladder

Neuromedin U-8 produced a concentration-dependent contraction (Figure 3, threshold concentration 10 nM, n = 5 strips from 5 patients). Quantitatively, the maximal response to the peptide did not exceed 25% of the maximal response produced by KCl (80 mM, added to the bath). For the bladder, KCl was chosen as the internal standard instead of carbachol because the maximal response to the latter (10 μM) was much larger (150–200%) than that to KCl. The response to neuromedin U-8 was not modified by atropine (Figure 3) at a concentration (1 μM for 15 min) which abolished the contractile

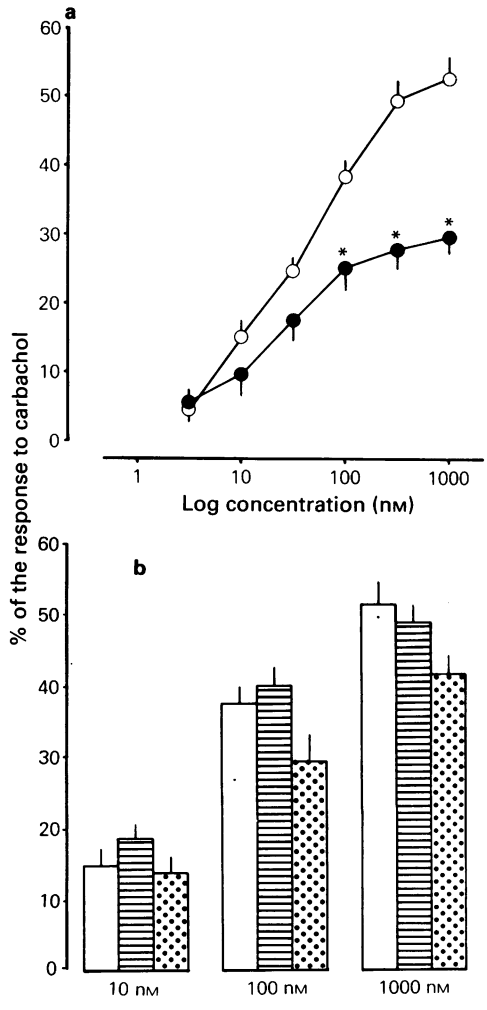

Figure 2 (a) Contractile response of the human isolated ileum (longitudinally oriented strips) to porcine neuromedin U-8. The peptide was added either in a cumulative (●) or non-cumulative (○) manner. Each value is the mean of 5–6 experiments. * Significantly different from the response produced by non-cumulative addition of neuromedin U-8, $P < 0.05$. (b) Contractile response to neuromedin U-8 of longitudinally oriented muscle strips from the human ileum in controls (open columns) and in the presence of atropine (hatched columns) or tetrodotoxin (stippled columns) (1 μM each). Each value is the mean of 5–6 experiments. In (a) and (b), vertical lines indicate s.e.mean.

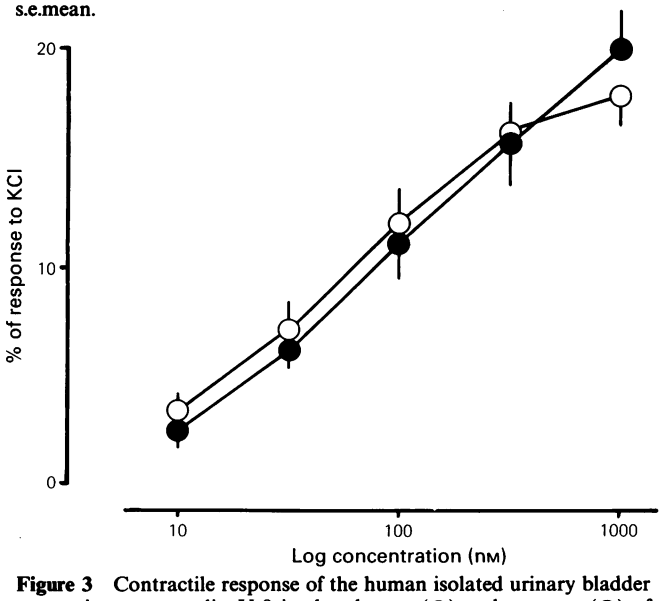

Figure 3 Contractile response of the human isolated urinary bladder to porcine neuromedin U-8 in the absence (○) and presence (●) of atropine (1 μM). Each value is the mean of 5–6 experiments; vertical lines indicate s.e.mean.

response to carbachol ($n = 4$ from 4 patients). In addition, the contractile response to neuromedin U-8 (30 nM–1 μM) was unaffected by tetrodotoxin (1 μM 15 min before) which abolished the response to electrical field stimulation (10 Hz, pulse width 0.25 ms, 60 V for 5 s, $n = 3$ from 3 patients).

Discussion

The present findings indicate that neuromedin U-8 exerts a potent contractile effect on the human isolated ileum and urinary bladder muscle. The action of neuromedin U-8 was atropine- and tetrodotoxin-resistant in both preparations, suggesting that activation of intramural nerves does not play a significant role. In the ileum, the action of neuromedin U-8 was markedly selective for strips cut along the longitudinal axis, because small and inconsistent effects were observed in strips cut along the circular axis. Marked differences in the response of longitudinally compared to circularly cut strips from the human ileum was also observed for other peptides: as an example, the neuropeptide galanin is a potent stimulant in longitudinal strips but produces weak and inconsistent responses in circular strips (Maggi *et al.*, 1989a; Maggi *et al.*, unpublished observations). By contrast, both longitudinal and circular strips responded strongly to both carbachol and tachykinins indicating that both preparations are suitable for functional studies (Maggi *et al.*, 1989a; Maggi *et al.*, unpublished observations.

Although we made no attempt to separate the two muscular layers, it appears reasonable to hypothesize that neuromedin U-8 receptors are selectively located on the longitudinal muscle of the human ileum. Indeed, the contention that the weak responses to neuromedin U-8 observed in circularly cut strips might have been produced by concomitant contraction of the longitudinal muscle cannot be excluded.

Neuromedin U-8 and U-25 have been found in the gut and urinary tract of several species (see Introduction for references) and their functional role as regulatory peptides has been proposed. In the rat intestine, intramural neurones and nerves containing neuromedin U-8-like immunoreactivity were found in both myenteric and submucosal plexus (Augood *et al.*, 1988; Ballesta *et al.*, 1988).

However, no nerve fibres were demonstrated in the muscle layers while abundant fibres were seen in the mucosa (Augood *et al.*, 1988; Ballesta *et al.*, 1988). Neuromedin U-8 was found to be ineffective in producing contractions of the guinea-pig ileum or porcine jejunum (Minamino *et al.*, 1985; Brown & Quito, 1988) while it affected markedly intestinal ion transport (Brown & Quito, 1988) and splanchnic circulation (Sumi *et al.*, 1987). On the basis of these results, it could be hypothesized that neuromedin U-8 is not a motor transmitter in the gut, being selectively involved in other functions. The present findings indicate that this is not necessarily the case and underline the importance of functional studies on the human isolated gut, in view of the relevant species-related differences which may be encountered with regard to motor response to exogenous transmitters and intramural nerve activation (Maggi *et al.*, 1988a; 1989a).

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(Received July 11, 1989
Revised August 29, 1989
Accepted September 1, 1989)