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Atrial natriuretic peptide inhibits the spontaneous contractions of rabbit isolated ileum

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Abstract—The present study investigates the effects of atriopeptin II on spontaneous phasic contractions of rabbit isolated ileum. Atriopeptin II caused a significant and concentration-dependent decrease in ileum motor activity. This effect was mimicked by 8-Br-cGMP and it was not affected by pretreatment with tetrodotoxin. Verapamil significantly decreased ileum contractions; however, in the presence of this calcium blocker, atriopeptin II further reduced ileal motility. These findings demonstrate that atriopeptin II depresses the motility of rabbit ileum through a cGMP-dependent mechanism and suggest that neither ileal neural networks nor extracellular calcium are involved in this effect.

Atrial natriuretic peptides (ANPs) are a family of structurally related peptides, synthesized, stored and released by atrial cardiocytes (Atlas et al 1984). ANP has potent diuretic, natriuretic, and vasodilatory properties and appears to play a prominent role in fluid and electrolyte homeostasis (Ballerman et al 1985; Genest & Cantin 1988).

Evidence has been provided that the mammalian gastrointestinal tract might be a target organ of ANP physiological activity (Gutkowska & Nemer 1989). Radioimmunological assays have demonstrated the presence of ANP in intestinal homogenates (Vollmer et al 1988; Vuolteenaho et al 1988), and autoradiographic studies have shown the existence of specific binding sites in both small and large intestine (Mantyh et al 1986; Bianchi et al 1989).

The exact role played by ANP in the gut has not been fully established. However, both in-vitro and in-vivo experiments suggest that ANP might be involved in the endogenous neurohumoral regulation of intestinal ions and fluid transport (Kanai et al 1987; Moriarty et al 1990). In addition, ANP was found to exert conflicting actions on intestinal motility, since it increased the amplitude of rat duodenal spontaneous phasic contractions (Baeyens et al 1988), but inhibited cholinergic twitch responses of guinea-pig ileum (Matusak & Kuchel 1989).

Although preliminary experiments have shown the presence of ANP binding sites on rabbit intestine (Bianchi et al 1989), data on the possible influence exerted by ANP on rabbit intestinal motility are still lacking. The aim of the present study was to investigate the effects of atriopeptin II (APII) on spontaneous phasic contractions of isolated rabbit ileum, in order to better characterize the role of this peptide in the regulation of intestinal motility.

Materials and methods

Rabbit isolated ileum. Albino rabbits of either sex, 1·3–1·5 kg, were killed by cervical dislocation and bled. The small intestine was quickly removed and the 9–10 cm segment nearest to the ileo-caecal junction was discarded. After carefully washing out the luminal content with warm (36°C) Tyrode solution, segments of the ileum, 3–4 cm long, were selected from the terminal portion and mounted vertically in a 10 mL organ bath containing Tyrode solution of the following composition (mM): NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·0, NaH_2PO_4 0·4, $NaHCO_3$ 11·9, and glucose 5·6 (pH 7·4 at 37°C). The Tyrode solution was continuously bubbled with 95% O_2 – 5% CO_2. The preparations were connected to an isotonic transducer (Basile 7006) under a tension of 1 g and the amplified (10 times) mechanical activity of the longitudinal muscle was recorded by a Basile 7050 microdynamometer. The ileum was left at rest for a 60 min equilibration period, during which the bath fluid was changed 3 or 4 times. The effects of increasing concentrations of APII or 8-bromoguanosine 3′5′-cyclic monophosphate (8 Br-cGMP) added to the bath were examined on spontaneous phasic contractions. Drugs were given in a small volume which never exceeded 1% of total bath volume (10 mL). In preliminary experiments in which APII was added to the bath in cumulative concentrations, no further increase in the response could be recorded after the effect of the threshold concentration completely developed. However, an interval of 30 min (with intervening washings) between single concentrations of APII completely avoided desensitization. In some experiments, tetrodotoxin or verapamil was added to the bath 20 min before APII. Following the addition of single concentrations of the drugs to the bath, variations of the contraction amplitude were measured and reported as percentage of the resting spontaneous phasic contractions.

In separate experiments, segments of distal ileum were isolated together with the respective portion of mesenterium. After setting up the tissues as described above, the proximal end of the mesenterium was placed in a platinum electrode connected to a Grass S5 stimulator, in order to evoke inhibition of ileum phasic contractions through stimulation of the sympathetic fibres innervating the smooth muscle. The stimulus parameters were square-wave impulses of 10 Hz frequency, 0·5 ms duration and supramaximal voltage for 20 s. Electrical stimulation of sympathetic nerves was carried out in the absence or in the presence of propranolol plus phentolamine or tetrodotoxin added to the bath 20 min before.

Drugs. The drugs used were: rat atriopeptin II, 8-Br-cGMP, tetrodotoxin, (±)-verapamil, (±)-propranolol HCl, phentolamine HCl (Sigma, St Louis, MO, USA). Other reagents were of analytical grade.

Statistical analysis. Results are given as percent means ± s.e.m. The significance of differences was evaluated by Student's *t*-test for paired data and P values lower than 0·05 were considered significant; n indicates the number of experiments. IC50 values were calculated by means of a personal computer (IBM PS/2 Model 8565) curve fitting programme (Sigmaplot).

Results

Under basal conditions, rabbit isolated ileum developed a regular phasic activity consisting of spontaneous pendular movements which occurred at a rate of 10–15 min^{-1}.

APII (0·1–10 μM; n = 8 for each concentration) caused a significant and concentration-dependent decrease in spontaneous phasic contractions of rabbit ileum. For each concentration tested, the inhibitory effect was rapid in onset and persistent for about 9–12 min (Fig. 1A). The maximal effect occurred at a

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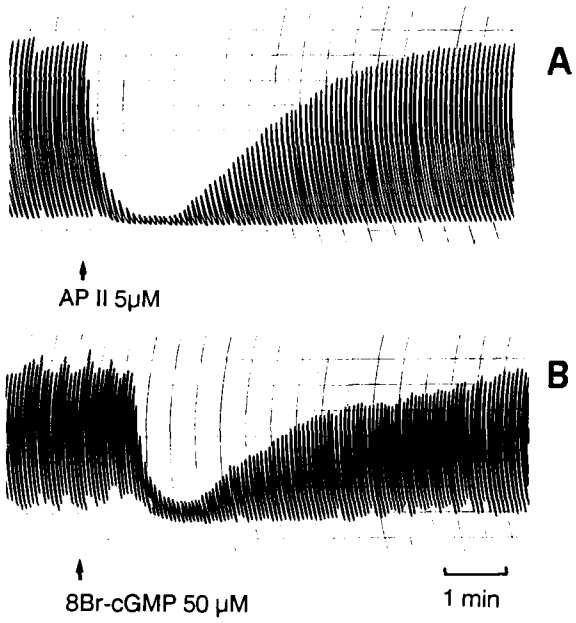

FIG. 1. Typical time-dependent recording of the inhibition of rabbit ileum spontaneous motility following the addition to the bath of APII 5 μM (A) or 8Br-cGMP 50 μM (B).

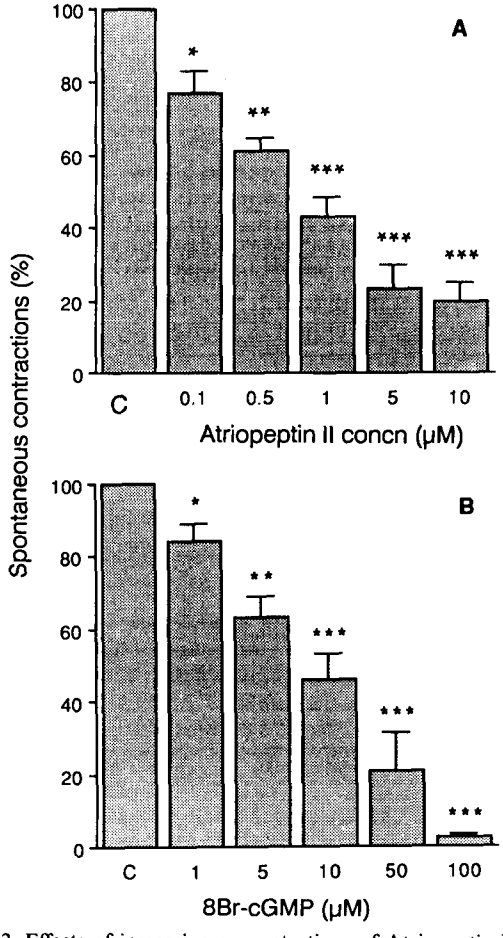

FIG. 2. Effects of increasing concentrations of Atriopeptin II 0·1–10 μM (A) or 8-Br-cGMP 1–100 μM (B) on spontaneous phasic contractions of rabbit ileum. Columns indicate the mean values obtained from six to eight experiments ± s.e.m. (vertical lines). Significant difference from control values (C): $*P<0.05$, $**P<0.01$, $***P<0.001$.

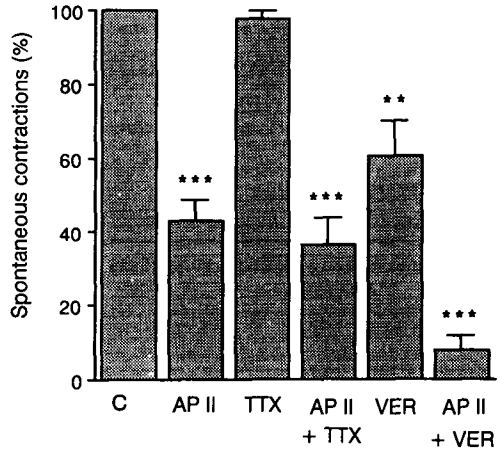

FIG. 3. Effects of APII 1 μM (APII), tetrodotoxin 3 μM (TTX), tetrodotoxin 3 μM plus APII 1 μM (TTX + APII), verapamil 0·3 μM (VER), and verapamil 0·3 μM plus APII 1 μM (VER + APII) on spontaneous phasic contractions of rabbit ileum. Columns indicate the mean values obtained from experiments ± s.e.m. (vertical lines). Significant difference from six to eight control values (C): $**P<0.01$; $***P<0.001$.

concentration of 5 μM, with an IC50 value of 0·82 ± 0·09 μM (Fig. 2A).

8-Br-cGMP (1–100 μM; n = 6 for each concentration) produced similar results to those obtained with APII. Following the addition of 8-Br-cGMP to the bath, an inhibition of ileal spontaneous activity could be observed after 1–2 min latency and a duration of about 10–15 min (Fig. 1B). The maximal inhibitory effect occurred at a concentration of 100 μM, with an IC50 value of 8·21 ± 1·13 μM (Fig. 2B).

Tetrodotoxin (3 μM; n = 6) did not modify spontaneous ileum motility and failed to affect the inhibitory action induced by APII (1 μM) (Fig. 3). Verapamil (0·3 μM; n = 6) significantly reduced ileum contractions; however, in the presence of this drug, APII (1 μM) caused a further inhibition of ileal phasic contractions (n = 6; Fig. 3).

To further elucidate the nature of APII effects, separate experiments were performed on rabbit ileum provided with mesenteric sympathetic nerves. Under these conditions, electrical stimulation completely abolished ileum contractile activity (n = 6), but failed to cause any effect after 20 min exposure of the preparations to propranolol (10 μM) plus phentolamine (10 μM) (n = 6) or tetrodotoxin (3 μM) (n = 6) (data not shown).

Discussion

The results of the present investigation show that APII induced a concentration-dependent decrease in spontaneous phasic contractions of the rabbit isolated ileum. The threshold concentration for this inhibitory response was 0·1 μM, suggesting that, in addition to its effects on neurohumoral regulation of water and electrolyte transport in mammalian intestine (Kanai et al 1987; Moriarty et al 1990), ANP may play a physiological role in the control of intestinal motility. Similar findings have been reported by Matusak & Kuchel (1989), who found that ANP inhibited cholinergic twitch responses and histamine-induced tonic contractions from the guinea-pig isolated ileum. By contrast, ANP enhanced the amplitude of the spontaneous phasic contractions of rat duodenal longitudinal muscle (Baeyens et al 1988). Although the different animal species or intestinal segments employed might explain these discrepancies, the possibility that differences in the sources of ANP (namely, man or rat ANP) might evoke opposite responses from the intestinal muscle should be taken into account.

The actions of ANP are thought to be mediated by specific high affinity receptors (Napier et al 1984), which are present on target tissues, where they are coupled to a particulate guanylate cyclase (Hamet et al 1984; Waldman et al 1984). In particular, ANP was found to promote vascular smooth muscle relaxation via an extracellular calcium-independent mechanism, at concentrations which produced a parallel and marked increase in cGMP levels (Winquist et al 1984; Fiscus et al 1985). In the present study, 8-Br-cGMP, which has been shown to mimic the inhibition of Na-K-Cl co-transport by ANP in the teleost intestine (O'Grady et al 1985), reduced ileum phasic contractions with a pattern similar to that observed for APII. In addition, following relaxation induced by the calcium blocker verapamil, APII caused a further decrease in the spontaneous motor activity, the extent of which was very close to that induced by APII alone. Taken together, these results suggest that the inhibitory action of APII on rabbit ileal motility is mediated by the cGMP pathway, through mechanisms which seem not to involve extracellular calcium. In support of this view, evidence has been provided that ANP stimulated cGMP accumulation in rat ileal cell cultures (Crane et al 1990). Moreover, it was found that intravenous injection of APII in dogs caused an intestinal release of cGMP into venous effluent (Ito et al 1988) and that ANP-induced enhancement of rat duodenal motility was associated with a marked increase in cGMP levels and activation of particulate guanylate cyclase (Baeyens et al 1988).

Conflicting findings have been reported on the possible participation of neural pathways in ANP-evoked responses. Indeed, ANP was found to inhibit both phasic and sustained contractions induced by phenylephrine on rat isolated aorta (Delaflotte et al 1989), while reducing noradrenergic neurotransmission in rat mesenteric arteries through a presynaptic mechanism (Nakamaru & Inagami 1986). Moreover, ANP-elicited electrolyte secretion from rat large intestine was prevented by both atropine and tetrodotoxin, suggesting an involvement of the cholinergic enteric nervous system (Moriarty et al 1990). In our experiments, pretreatment of the tissues with the neuronal blocker tetrodotoxin failed to affect the inhibitory response of APII, indicating that this effect is caused by a direct action on ileum muscle cells rather than by neural conduction blockade. In addition, tetrodotoxin completely prevented ileum relaxation induced by electrical stimulation of mesenteric sympathetic nerves, whereas basal phasic contractile activity was unaffected. These results confirm that, at the concentration of tetrodotoxin used in the present study, neural networks within the intestinal wall were adequately inhibited and that they did participate in the spontaneous contractions displayed by rabbit isolated ileum.

In conclusion, the findings of the present investigation suggest that APII reduces the motility of rabbit ileum through a cGMP-dependent mechanism following a direct interaction with ileal smooth muscle cells.

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