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POSSIBLE MECHANISM OF THE DUAL ACTION OF THE NEW POLYPEPTIDE (ANTHOPLEURIN-B) FROM SEA ANEMONE IN THE ISOLATED ILEUM AND TAENIA CAECI OF THE GUINEA-PIG

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1 Anthopleurin-B (AP-B), a newly isolated polypeptide from a sea anemone (*Anthopleura xanthogrammica*) caused relaxation of the guinea-pig isolated ileum following a transient contraction at concentrations greater than 3×10^{-9} M.

2 AP-B caused a tonic contraction followed by rhythmic relaxation of the guinea-pig isolated taenia caeci at a concentration of 3×10^{-9} M or more.

3 The other polypeptides, anthopleurin-A (AP-A) from the same species or anthopleurin-C (AP-C) from *Anthopleura elegantissima* elicited similar effects but higher concentrations were required in both tissues.

4 These responses induced by AP-B in both tissues were abolished by treatment of each tissue with tetrodotoxin or incubation in a low-Na^+ medium.

5 In the ileum, the AP-B-induced contraction was markedly inhibited by atropine but not by mecamylamine, whereas the AP-B-induced relaxation was not affected by phentolamine or guanethidine.

6 The AP-B-induced contraction of the taenia caeci was also inhibited by atropine, but not mecamylamine. However, in contrast to the ileum, the spontaneous relaxation of this tissue was completely abolished in the presence of phentolamine or guanethidine.

7 These results suggest that the AP-B-induced contractions of both tissues are mainly caused by acetylcholine (ACh) release from the cholinergic nerve terminals and that the AP-B-induced relaxation of the taenia caeci is due to the excitation of adrenergic nerves, while the relaxation of the ileum is mediated through non-adrenergic inhibitory mechanisms.

Introduction

Polypeptides with a potent cardiac stimulant action, anthopleurin-A (AP-A), anthopleurin-B (AP-B), and anthopleurin-C (AP-C), were recently isolated from sea anemones (*Anthopleura xanthogrammica* and *Anthopleura elegantissima*) (Shibata, Dunn, Kuchii, Kashiwagi & Norton, 1974; Shibata, Norton, Izumi, Matsuo & Katsuki, 1976) and their amino acid sequence was determined (Norton, Shibata, Kashiwagi & Bentley, 1976; Tanaka, Haniu, Yasunobu & Norton, 1977; Norton, Kashiwagi & Shibata, 1978).

These polypeptides have almost identical primary chemical structures; AP-A (mol. wt. 5183, amino acid 49), AP-B (mol. wt. 4590, amino acid 42) and AP-C (mol. wt. 4875, amino acid 47). AP-A has a selective, potent, positive inotropic action without any effect on the heart rate, blood pressure and vascular smooth muscle (Shibata *et al.*, 1976; Shibata, Izumi, Seriguchi & Norton, 1978; Blair, Peterson & Bishop, 1978; Scriabine, Van Arman, Morgan, Morris, Bennett & Bohidar, 1979). On the other hand, AP-A, at concentrations higher than that required for a maximal positive inotropic effect, elicited a spontaneous depolarization followed by repolarization in crayfish giant axons (Low, Wu & Narahashi, 1979). More recently, Kudo & Shibata (1980) found that AP-B had a potent excitatory action on the frog spinal cord. However, the effect of these polypeptides on the autonomic nervous system and smooth muscles has not yet been extensively studied. Preliminary experiments indicated that of these polypeptides, AP-B had the most potent pharmacological actions on intestinal preparations. Therefore, the present experiments were undertaken to examine the effect of these polypeptides, in particular AP-B, on the guinea-pig isolated ileum and taenia caeci.

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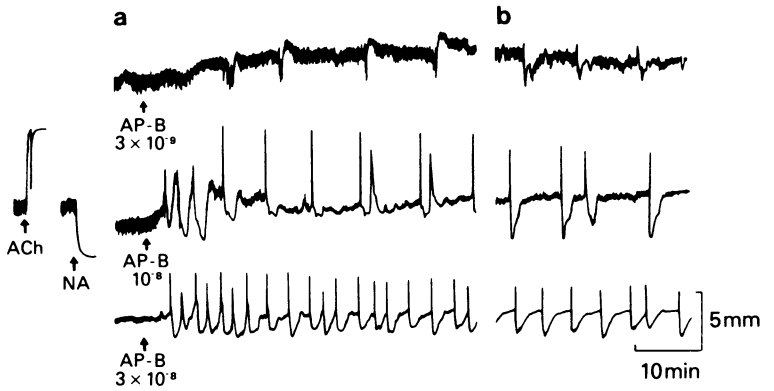

Figure 1 Isotonic spontaneous contraction and relaxation induced by different concentrations of anthopleurin-B (AP-B) in the guinea-pig isolated ileum. AP-B was added at arrows. (a) Record 0 to 50 min after AP-B; (b) 90 to 110 min after AP-B. ACh: acetylcholine 10^{-6} M; NA: noradrenaline 10^{-6} M.

Methods

Guinea-pigs (250 to 350 g) were killed by cervical dislocation. A 2 cm length of the ileum and the taenia caeci was removed. Preparations were suspended in 20 ml organ baths containing Krebs–Ringer bicarbonate solution of the following composition (mM): NaCl 120, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4 \cdot 7H_2O$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.2 and glucose 5.8; pH 7.4. A low-Na^+ solution was made by the replacement of 100 mM NaCl (for the ileum) or 70 mM NaCl (for the taenia caeci) with isotonic sucrose. The solution was gassed with 95% O_2 and 5% CO_2 and temperature was maintained at 32°C for the ileum and 37°C for the taenia caeci. A resting tension of 0.5 and 1.5 g was applied to the ileum and the taenia caeci, respectively. The preparations were equilibrated for 60 min before drugs were added. Responses were recorded isotonically on a pen recorder through an isotonic transducer. Blocking drugs were applied to the bathing medium 15 min before the administration of AP-B. Three to five preparations from different animals were used for each experiment.

Drugs

The following drugs were used in the present study: AP-A, AP-B and AP-C isolated from *Anthopleura xanthogrammica* or *A. elegantissima* were donated by Dr T.R. Norton of the University of Hawaii. These polypeptides were dissolved in distilled water at a concentration of 10^{-4} to 10^{-6} M and kept frozen as stock solution. Other agents included, tetrodotoxin (TTX, Sankyo Co.), procaine hydrochloride (Sigma Co.) acetylcholine chloride (Daiichi-Seiyaku Co.), atropine sulphate (Tokyo Kasei Co.), mecamylamine hydrochloride (Meiji Seika Co.), chlorpheniramine maleate (Sankyo Co.) indomethacin (Merk Co.), noradrenaline bitartrate (Sigma Co.), bretylium tosylate (Burroughs-Wellcome Co.), and guanethidine sulphate (Ismeline, Ciba-Geigy Co.).

Results

Ileum

In the guinea-pig isolated ileum, AP-A, AP-B, and AP-C elicited rhythmic, biphasic responses, consisting of a transient contraction followed by a relaxation, at concentrations above 6×10^{-7} M, 3×10^{-9} M, and 6×10^{-7} M, respectively. These actions lasted for at least 2 h. Washing with normal solution four times for 1 min removed the effects of these polypeptides within 30 min. The response to AP-B (10^{-8} M) was almost unchanged during at least 5 repeated applications for 30 min every hour. Figure 1 shows a representative biphasic response following treatment with different concentrations of AP-B (3×10^{-9}, 10^{-8}, and 3×10^{-8} M). The contractile and relaxant responses induced by AP-B (10^{-8} M) were comparable to the maximal responses to acetylcholine (10^{-6} M) and noradrenaline (10^{-6} M), respectively (Figure 1). The frequency of the biphasic response induced by AP-B increased in a dose-dependent manner, whereas spontaneous mechanical activity of the ileum was decreased with increasing concentrations of AP-B (10^{-8} to 3×10^{-8} M) (Figure 1). The AP-B (10^{-8} m)-induced biphasic response of the ileum was markedly inhibited or abolished by treatment with tetrodotoxin (TTX, 5×10^{-7} M), procaine (6×10^{-5} M) or a low-Na^+

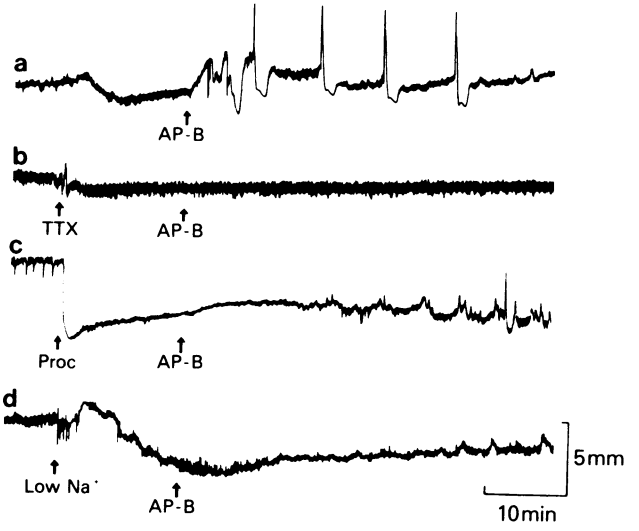

Figure 2 Effect of tetrodotoxin, procaine or the low-Na^+ medium on the contraction and the relaxation induced by anthopleurin-B (AP-B) in the guinea-pig isolated ileum. (a) Control (AP-B 10^{-8} M); (b) tetrodotoxin (TTX 5×10^{-7} M); (c) procaine (Proc 6×10^{-5} M); (d) Low-Na^+-sucrose medium.

medium (Figure 2). Further, the AP-B-induced contraction of the ileum was markedly inhibited in the presence of atropine (10^{-6} M) (Figure 3), but not affected by mecamylamine (3×10^{-5} M), chlorpheniramine (10^{-6} M), methysergide (10^{-6} M) or indomethacin (3×10^{-6} M). Treatment with phentolamine (2×10^{-6} M), bretylium (10^{-4} M) (Figure 3) or guanethidine (10^{-4} M) had no apparent effect on the AP-B-induced relaxation.

Taenia caeci

In the guinea-pig isolated taenia caeci, AP-A, AP-B, and AP-C also elicited a sustained contraction with spontaneous rhythmic, transient relaxation at concentrations above 10^{-7} M, 3×10^{-9} M, and 10^{-7} M, respectively, which lasted for at least 2 h. The contractile responses to these polypeptides returned to the resting level approximately 20 min after washing out with normal solution (four times for 1 min). Tachphylaxis gradually developed during repeated applications of AP-B (10^{-8} M) for 30 min every hour and the response to the fifth application of AP-B was reduced by approximately 40%. Figure 4 shows representative responses of the taenia caeci to different concentrations of AP-B (3×10^{-9}, 10^{-8}, and 3×10^{-8} M). The maximal contractile response of the taenia caeci to AP-B was obtained with a concentration of approximately 3×10^{-8} M, which was as great as the maximal response to acetylcholine (10^{-6} M). AP-B (3×10^{-8} M) induced only relaxation simply because no further contractions could occur (Figure 4, lowest trace). The frequency of spontaneous

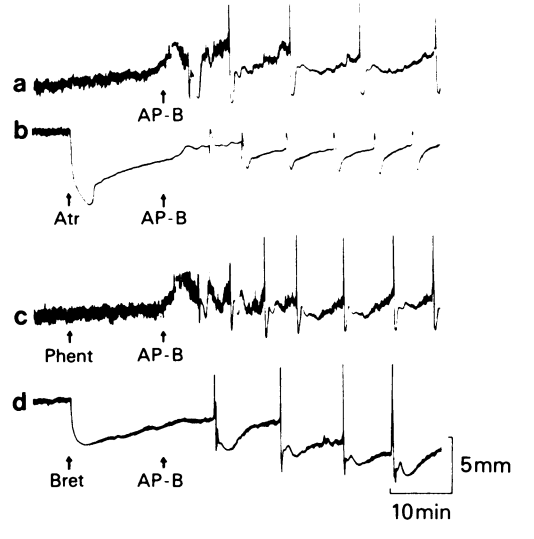

Figure 3 Effect of atropine, phentolamine or bretylium on the contraction and the relaxation induced by anthopleurin-B (AP-B) in the guinea-pig isolated ileum. (a) Control (AP-B 10^{-8} M); (b) atropine (Atr) 10^{-6} M; (c) phentolamine (Phent) 2×10^{-6} M; (d) bretylium (Bret) 10^{-4} M.

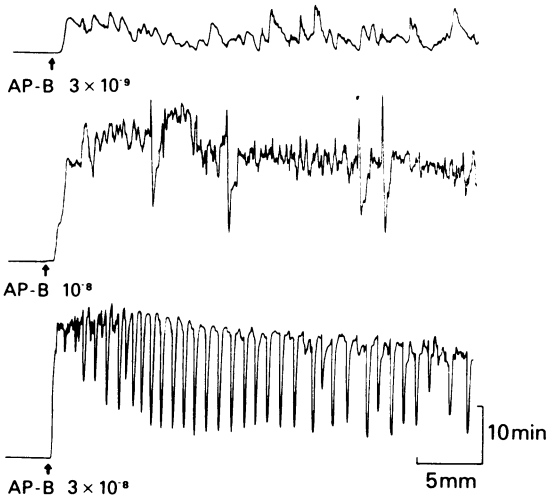

Figure 4 Isotonic contraction and relaxation induced by different concentrations of anthopleurin-B (AP-B) in the guinea-pig isolated taenia caeci. AP-B was added at arrows.

rhythmic tension changes of the taenia caeci was increased with increasing concentrations of AP-B (10^{-8} to 3×10^{-8} M). This AP-B (10^{-8} M)-induced response was inhibited or abolished by treatment with atropine (5×10^{-6} M), TTX (5×10^{-7} M) or a low Na^+ medium (Figure 5). However, mecamylamine (5×10^{-5} M) chlorpheniramine (10^{-6} M), methysergide (10^{-6} M) or indomethacin (3×10^{-6} M) had no apparent effect on the AP-B-induced response of the taenia caeci. Figure 6 shows the effect of phentolamine (10^{-6} M) or guanethidine (3×10^{-5} M) on the mechanical response to AP-B (3×10^{-8} M); neither treatment had an apparent effect on the tonic contraction induced by AP-B (3×10^{-8} M), but both inhibited the rhythmic relaxation. Bretylium also had a similar inhibitory action.

Discussion

In the guinea-pig isolated ileum and taenia caeci, AP-B elicited contractions followed by relaxations. Only in higher concentrations did AP-A and AP-C cause a similar response. The AP-B-induced responses were completely abolished by a specific Na^+-channel blocker (TTX) or the low-Na^+ medium in each tissue. On the other hand, in crayfish giant axon, AP-A elicited spontaneous depolarization with repolarization which was abolished by TTX or low Na^+ medium (Low *et al.*, 1979). Further, in the spinal cord, AP-B caused marked enhancement of the stimulation-induced root potential and L-glutamate-induced depolarizations; these effects were abolished by TTX (Kudo & Shibata, 1980). These observations suggest that the AP-B-induced contraction followed by relaxation of either ileum or taenia caeci is mediated through the activation of neuronal elements. It is assumed that AP-B causes membrane depolarization by increasing Na^+ permeability across the nerve cell

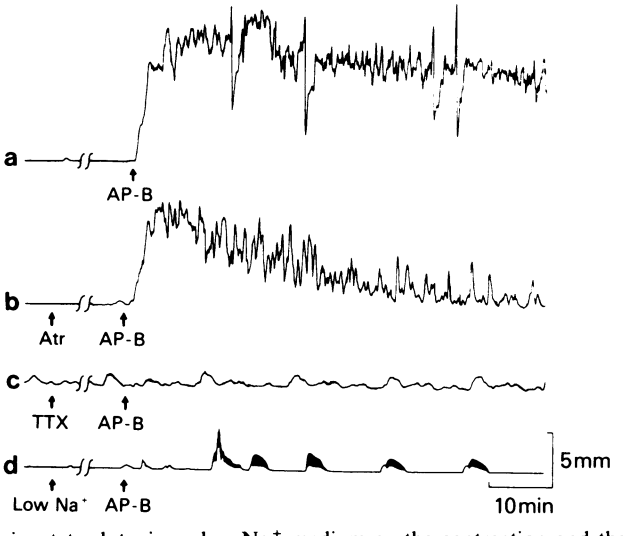

Figure 5 Effect of atropine, tetrodotoxin or low-Na^+ medium on the contraction and the relaxation induced by anthopleurin-B (AP-B) in the guinea-pig isolated taenia caeci. (a) Control (AP-B 10^{-8} M); (b) atropine (Atr) 5×10^{-6} M; (c) tetrodotoxin (TTX) 5×10^{-7} M; (d) low Na^+-sucrose medium (Low-Na^+). AP-B was added at arrows after treatment with Atr or TTX and incubation in the low-Na^+ medium for 15 and 40 min, respectively.

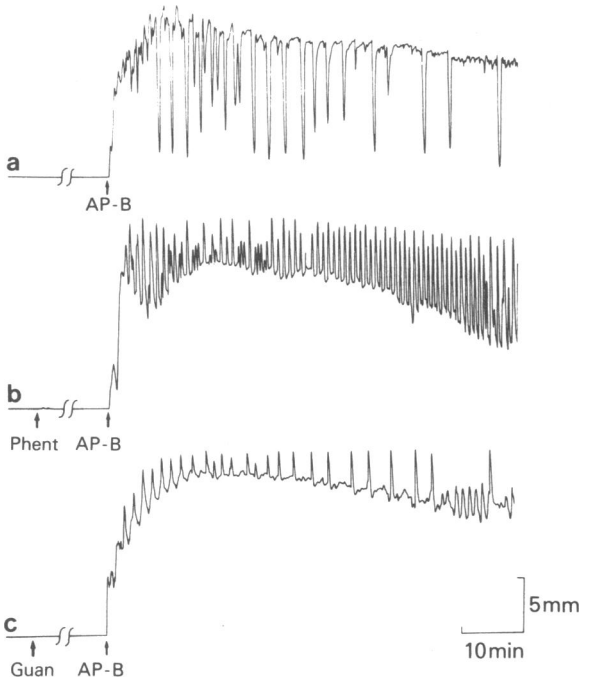

Figure 6 Effect of phentolamine or guanethidine on the contraction and the relaxation induced by anthopleurin-B (AP-B) in the guinea-pig isolated taenia caeci. (a) Control (AP-B 3×10^{-8} M); (b) phentolamine (Phent) 10^{-6} M; (c) guanethidine (Guan) 3×10^{-5} M. AP-B was added at arrows after treatment with Phent or Guan for 15 min.

membrane, which probably plays an important role in the dual action of AP-B.

The AP-B-induced contractions of the ileum and the taenia caeci were inhibited by treatment with a muscarinic blocker, but were not affected after treatment with a nicotinic, histamine, or tryptamine blocking agent or a prostaglandin synthesis blocking agent, suggesting that the AP-B-induced contractions of both tissues were mainly mediated through endogenous ACh released from the cholinergic nerve terminals. Since either α-adrenoceptor or adrenergic neurone blocking agents inhibited the relaxant response of taenia caeci to AP-B, the rhymic relaxation may be due to the release of noradrenaline by the excitation of adrenergic nerves. However, these adrenergic blocking agents had no influence on the AP-B-induced relaxation of the ileum. It has been proposed that non-adrenergic inhibitory nerves, as well as adrenergic inhibitory mechanisms, are present throughout the mammalian gastrointestinal smooth muscle preparations (Burnstock, 1972). This leads us to speculate that the AP-B-induced relaxation of the ileum may be attributed to excitation of the non-adrenergic inhibitory nerves.

It was previously reported that AP-A had a potent, selective positive inotropic effect without any apparent effect on the heart rate and blood pressure and vascular smooth muscle (Shibata *et al.*, 1976; 1978; Blair *et al.*, 1978; Scriabine *et al.*, 1979). It was also suggested that the cardiostimulant action of AP-A is not attributable to adrenergic mechanisms since a β-adrenoceptor blocking agent and reserpine-pretreatment had no influence on the effect of AP-A (Shibata *et al.*, 1976; Blair *et al.*, 1978). AP-B and AP-C with selective, positive inotropic action also had no apparent effect on the rabbit aorta and portal vein (unpublished data). These observations suggest that these polypeptides have no apparent effect on the adrenergic nerves in cardiovascular tissues. However, these polypeptides caused an increased endogenous noradrenaline release through excitation of the adrenergic nerves in the guinea-pig vas deferens, resulting in a potent contraction (unpublished data). At present, although the different effects of these polypeptides on the adrenergic tissues in different organs is not known, it would be interesting to elucidate such mechanisms.

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