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Excitatory and inhibitory actions of amrinone on the guinea-pig isolated ileum and vas deferens

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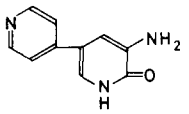
Amrinone, 5-amino-3,4'-bipyridine-6(1H)-one, is a new compound having positive inotropic effects on isolated cardiac muscle preparations, intact experimental animals (Farah & Alousi 1978; Alousi et al 1979), normal volunteers (DeGuzman et al 1978) and patients with congestive



Amrinone

heart failure (LeJemtel et al 1979). The cardiotonic effect of amrinone was not blocked by β-adrenergic blockade or by treatment with reserpine (Alousi et al 1979). Also, unlike cardiac glycosides, amrinone did not affect cardiac Na,K-ATPase activity (Alousi et al 1979). Gaide et al (1980) have recently suggested that amrinone exerts a cardiotonic effect due to increase in the activator Ca^{2+} in a different way from that of isoprenaline or ouabain, Morgan et al (1980) also suggested that amrinone prolongs the liberation of Ca^{2+} into the myoplasm while increasing the rate of Ca^{2+} sequestration. Therefore, amrinone appears to have a cardiotonic effect through mechanism of action different from either β-adrenoceptor stimulants or cardiac glycosides.

On the other hand, Meisheri et al (1980) demonstrated that amrinone inhibited noradrenaline and potassium-induced contractions of rabbit aorta and carbacol- and potassium-induced contractions of guinea-pig taenia caecum. It has also been suggested that the hypotensive action of amrinone is due to relaxation of vascular smooth muscle (Alousi & Helstosky 1980). However, we have observed that amrinone caused a transient contraction of the guinea-pig isolated ileum. Therefore, we have examined its mode of action on intestinal smooth muscle and vas deferens.

Materials and methods

Male guinea-pigs (250–350 g) were killed by a blow on the neck and bleeding via the cervical artery. The procedure used was that described for the ileum by Ohizumi & Shibata (1981) and for the vas deferens by Ohizumi & Shibata (1980). A segment (3 cm) of ileum or vas deferens was suspended in a 20 ml organ bath containing a physiological salt solution with the following composition (mM): NaCl, 120, KCl 4·8, $CaCl_2$ 1·2, $MgSO_4$ 1·3, KH_2PO_4 1·2, $NaHCO_3$ 25·2 and glucose 5·8. The solution was maintained at 37 °C and bubbled with a gas mixture of 95% O_2, 5% CO_2 (pH 7·4).

Mechanical responses of the ileum were recorded isotonically via an isotonic transducer (Natsume, Tokyo) with 0·5 g tension. In the vas deferens, a tension of 1 g was initially applied to the segment and the isometric tension change was recorded via a force-displacement transducer (Toyo-Baldwin, Tokyo).

In some experiments on the vas deferens, transmural stimulation (20 Hz, 0·5 ms, 50 v) was applied for 3 s every 4 min to preparation with a platinum electrode set in a Plexiglas tissue holder. Values were expressed as mean ± s.e.m.

Drugs used. Acetylcholine chloride (Daiichi), atropine sulphate (Sigma), histamine dihydrochloride (Sigma), mecamylamine hydrochloride (Meiji Seika), noradrenaline bitartrate (Sigma), tetrodotoxin (Sankyo). Amrinone was supplied from Sterling-Winthrop Research Institute and was dissolved into 0·5 M lactate (Sigma) to make a high concentration solution (50 mg ml^{-1}). The vehicle had no apparent effect on responses of the ileum and vas deferens.

Results

Application of amrinone to the bath caused a transient contraction of the ileum (Fig. 1a) that was dose-dependent at concentrations greater than 10^{-5} g ml^{-1} with a maximum contraction at 3×10^{-4} g ml^{-1} which was 50·5 ± 6·6% (n = 10) of that induced by acetylcholine (10^{-6} M). This initial maximum contraction was abolished in the presence of atropine (5×10^{-7} M) or tetrodotoxin (5×10^{-7} M) but not of mecamylamine (3×10^{-5} M) (Fig. 1c–d). Subsequently, at the same dose (3×10^{-4} g ml^{-1}) amrinone depressed spontaneous contraction (Figs 1, 2a) and also inhibited the contractile response to acetylcholine (10^{-7} M) (Fig. 2a). Fig. 2a also indicates that after wash-out of drugs the contractile response recovered. Amrinone (10^{-4} and 3×10^{-4} g ml^{-1}) apparently decreased the maximum contraction induced by acetylcholine or histamine, indicating non-competitive inhibition (Fig. 2b). Values of pD'_2, the apparent affinity of the non-competitive inhibitor described by van Rossum (1963), were calculated to be 3·45 ± 0·13 (n = 3) for acetylcholine and 2·97 ± 0·21 (n = 3) for histamine. Amrinone (3×10^{-4} g ml^{-1}) also inhibited the

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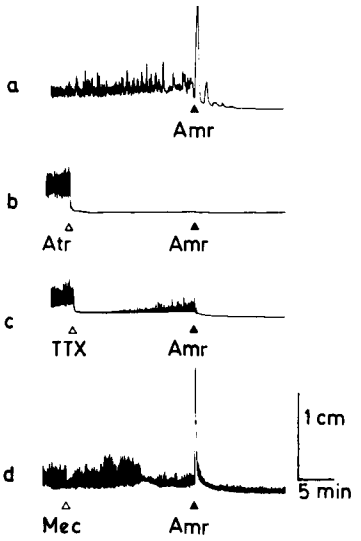

FIG. 1. Effect of various treatments on the response to amrinone in guinea-pig isolated ileum. Atropine (Atr) (5×10^{-7} M), tetrodotoxin (TTX) (5×10^{-7} M) and mecamylamine (Mec) (3×10^{-5} M) was applied to the solution at △ 15 min before application of amrinone (Amr) (3×10^{-4} g ml^{-1}) at ▲.

contraction induced by potassium (40 mM) (data not shown).

In the vas deferens, treatment with amrinone, even at 3×10^{-4} g ml^{-1}, did not cause contraction (Fig. 3), whereas it abolished the contraction induced by noradrenaline (10^{-5} M) (Fig. 3a) and inhibited the contractile response to transmural stimulation (20 Hz, 0·5 ms duration, 40 V for

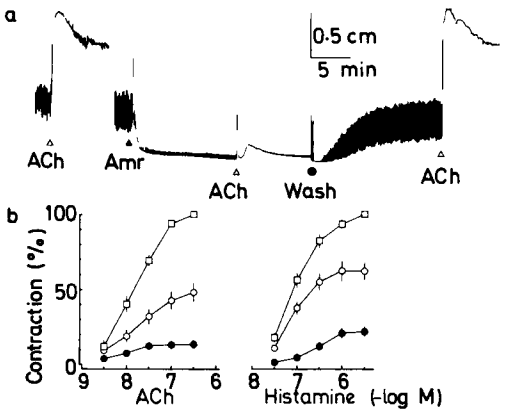

FIG. 2. Inhibitory effect amrinone on contractions of guinea-pig ileum: (a) shows typical result when the tissue was treated with amrinone (Amr) (3×10^{-4} g ml^{-1}) at ▲; (b) is the inhibitory effect of amrinone (10^{-4} g ml^{-1}, ○ and 3×10^{-4} g ml^{-1}, ●) on dose-response curves for acetylcholine and histamine (□ represents each control). In (a), △ represents application of acetylcholine (ACh) (10^{-7} M) and both amrinone and acetylcholine were washed out three times at ●. In (b) vertical bar means standard error of mean (n = 6 for control, n = 3 for amrinone-treated).

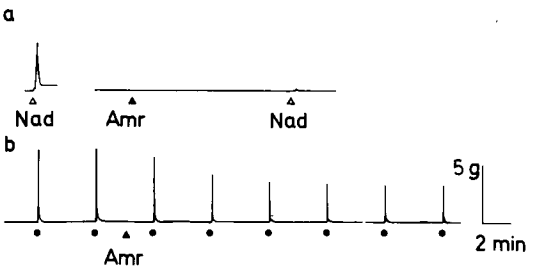

FIG. 3. Inhibitory effect of amrinone on contractile responses to noradrenaline (a) and transmural stimulation (b). Noradrenaline (Nad) (10^{-5} M) and amrinone (Amr) (3×10^{-4} g ml^{-1}) were applied at △ and ▲ respectively. Symbol, ●, represents application of transmural stimulation (20 Hz, 0·5 ms, 50 V, for 3 s every 4 min).

3 s at every 4 min) (Fig. 3b). Amrinone (3×10^{-4} g ml^{-1}) also inhibited the contraction induced by potassium (40 mM) (data not shown). The inhibitory action of amrinone was reversible in the vas deferens as in the ileum.

Discussion

Amrinone caused a transient contraction followed by relaxation of the ileum. The inhibitory actions of atropine and tetrodotoxin on the initial amrinone-induced contraction suggest that it is caused by the release of acetylcholine from cholinergic nerve fibres. Furthermore, mecamylamine, a ganglion blocking agent, failed to inhibit the contraction, indicating that the site of action of amrinone may be on the postganglionic nerve fibres. On the other hand, amrinone had no contractile effect on the vas deferens which is most densely innervated by excitatory adrenergic neurons (Huković 1961; Birmingham & Wilson 1963). Amrinone also caused no contractile response of the rabbit isolated aorta (unpublished data). Therefore, it appears that amrinone has greater stimulatory action on cholinergic nerves in the ileum than on adrenergic nerves in the vas deferens and aorta.

However, Meisheri et al (1980) have reported that amrinone inhibited contractile responses to carbachol and potassium in guinea-pig taenia caecum and to noradrenaline and potassium in rabbit aorta. In the present experiments, after the transient contractions it produced in the guinea-pig ileum, amrinone also produced a relaxation, it also inhibited, non-competitively, the contractions induced by acetylcholine and histamine in the ileum and also the contractile responses to noradrenaline and transmural stimulation in the vas deferens. Tissue treated with amrinone recovered rapidly from the relaxation following its wash-out, indicating the action to be readily reversible. Therefore, it is concluded that amrinone causes a non-specific inhibition of smooth muscle contraction besides which it induces a transient contraction of the guinea-pig ileum through a release of acetylcholine from cholinergic nerve terminals.

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Use of a lipid emulsion as a novel carrier for corticosteroids

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Liposomes are easily taken up by reticuloendothelial systems (RES) and some inflammatory cells and because of this corticosteroids have been incorporated into them and have been found to inhibit inflammation, for example arthritis in animals (Dingle et al 1978) and man (De Silva et al 1979), more intensively than a corresponding amount of free drug. Problems with liposomal therapy are instability of the liposomes and lack in clinical experience. We incorporated dexamethasone palmitate in a lipid emulsion (intralipid) that is taken up readily by phagocytic cells (Hallberg 1965) and is very stable and in widespread use for parenteral nutrition in man. We now report the tissue distribution patterns of dexamethasone-lipid emulsion and its anti-inflammatory activity in rats.

Dexamethasone (Merk) and [6,7-^{3}H(N)]dexamethasone (NEN) were used. [^{3}H]Dexamethasone palmitate and [^{3}H]dexamethasone disodium phosphate were synthesized from the [^{3}H]dexamethasone. Unlabelled salts were prepared from dexamethasone in the same way. They were identified by t.l.c., i.r. and n.m.r. The [^{3}H]dexamethasone were mixed with the unlabelled drug in an appropriate ratio before use. The synthesis of [6,7-^{3}H]dexamethasone palmitate was carried out as follows: [6,7-^{3}H]dexamethasone 0·107 μmol, in 5 ml of pyridine and 0·2 μmol of palmitoyl chloride in 0·5 ml of ether were mixed at 0 to 4 °C and then, reacted at 17 °C for 24 h. The solvents were evaporated and the residual dissolved in a small volume of ethanol applied to a silica gel column (2·5 cm × 30 cm) and the dexamethasone palmitate eluted with butyl acetate–benzene (1:1 by volume).

[6,7-^{3}H]Dexamethasone palmitate was obtained by completely evaporating the solvent. The chemical and radiochemical purities were 99·8% and higher than 99·9%, respectively t.l.c.: butyl acetate–benzene 1:1 v/v.

The [6,7-^{3}H]dexamethasone disodium phosphate was synthesized by the method of Chemerda et al (1960) using [6,7-^{3}H]dexamethasone derived from [6,7-^{3}H]dexamethasone iodide via methyl sulphonyl dexamethasone, and the compound obtained reacted with phosphoric acid in acetonitrile in the presence of silver phosphate. The resultant [6,7-^{3}H]dexamethasone phosphate, was purified by cation exchange column (Amberlite 120B, H type) and converted to the disodium phosphate by neutralizing with sodium hydroxide. The chemical and radiochemical purities were 99·5% and 99·9%, respectively (t.l.c.: chloroform–ethanol 9:1 v/v).

The lipid emulsion containing dexamethasone palmitate was prepared as follows: Dexamethasone palmitate (482 mg) was dissolved in 22·4 g of soybean oil containing 2·4 g of yolk phospholipids which consisted of 79% phosphatidylcholine, 17% phosphatidylethanolamine and 4% other phospholipids, such as sphingomyelin, lysolecithin. The mixture was emulsified with a Manton Goulin homogenizer at a pressure of 100 kg cm^{-2} under nitrogen until no particles larger than 1 μm were detected by light microscopy. The solution was poured into 90 ml of water.

The average particle size of the lipid emulsion was 0·25 μm all particles being less than 1 μm in diameter. One hundred ml of the lipid emulsion contained 4·82 mg dexamethasone palmitate (3 mg as dexamethasone), 10 g of soybean oil, 1·2 g yolk phospholipids and 2·25 g of glycerol, and the labelled preparation had a radioactivity of 3 μCi ml^{-1}.

Male rats of Wistar strain, 140 to 160 g were injected with 0·1 ml of 1% solution of λ-carrageenan (Sigma) into the hind paw. One ml of either the lipid emulsion containing [^{3}H]dexamethasone palmitate or the solution of

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