Octopamine relaxes rabbit jejunal smooth muscle by selective activation of dopamine D₁ receptors

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Summary. The effect of octopamine on intestinal smooth muscle of rabbit isolated jejunum has been studied. Octopamine induced a dose-dependent decrease of muscle tone and this reproducible relaxation was not modified by tetrodotoxin or by agents that acted on adrenergic nerve terminals. Adrenoceptor antagonists, at concentrations sufficient to block each adrenoceptor type, did not reduce the actions of octopamine. On the other hand, octopamine-induced relaxations were affected by agents that have the ability to change cyclic AMP (cAMP) content; such as alloxan (an adenylate cyclase inhibitor), imidazole (a stimulator of phosphodiesterase), and isobutyl methylxanthine (an inhibitor of phosphodiesterase). Direct stimulation of adenylate cyclase by octopamine was demonstrated using radioimmunoassay of cAMP. Furthermore, haloperidol and perphenazine at concentration required to block dopamine receptor sites attenuated both smooth muscle relaxation and the formation of cAMP induced by octopamine. The effect of octopamine was totally blocked by SCH 23390, an antagonist of dopamine D-1 receptors. The lack of effect of domperidone and sulphiride, antagonists of dopamine D-2 receptors, on the actions of octopamine excludes the involvement of dopamine D-2 receptors. These results suggest that octopamine acts on intestinal dopamine D-1 receptor sites to produce relaxation of rabbit jejunum through an increase of cAMP.

Key words: Octopamine — Dopamine receptors — Cyclic AMP — Jejunum of rabbits

Introduction

Octopamine, the phenol analogue of noradrenaline, is an important neurohormone and neurotransmitter in invertebrates (Robertson and Juorio 1976; Axelrod and Saavedra 1977; David and Coulon 1985). Octopamine has been identified in mammalian brain (Harman and Horn 1976; Duffield et al. 1981) and a deficiency has been found to be associated with depressive states (Sandler et al. 1979). Octopamine has been reported to increase locomotor activity (Hicks 1977) and avoidance conditioning (Delacour et al. 1983). In peripheral tissues, this amine has sympathomimetic effects on blood vessels (Kerol et al. 1968) and it has been proposed to be a partial agonist at adrenoceptors (Williams et al. 1987). However, the role(s) of octopamine and related molecules in vertebrates are still unclear.

The present study was designed to examine the effects of octopamine on muscle tone of mammalian small intestine. The results suggest that octopamine acts selectively on dopamine D₁ receptors and not on adrenoceptors to produce relaxation of rabbit jejunum.

Methods

New Zealand white rabbits of either sex (3–4 kg body weight) were killed by a blow on the head and exsanguinated. A segment of jejunum was quickly removed and placed in a 20 ml organ bath (37 ± 1°C) containing aerated (5% CO₂ in O₂) Tyrode solution (mM: NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.0, NaHCO₃ 11.9, NaH₂PO₄ 0.4 and D-glucose 5.5).

Measurement of muscle tone. The tissues were placed under 1 g of resting tension and equilibrated for more than 1 h with repeated rinsing every 20 min. Isometric responses of the longitudinal muscle were measured through a transducer connected to a polygraphic recorder (Gould 2400S). Relaxation was recorded as a decrease in the amplitude of spontaneous contraction of the preparations (Fig. 1A). Octopamine was allowed to act on the intestine until a stable relaxation was obtained. Responses were terminated by rinsing twice with 20 ml Tyrode solution. The tissues were incubated with the agonist for 5 min every 30 min repeatedly until uniform responses to a given concentration were obtained. In a preliminary study, 5 min preincubation was found to be a sufficient time for the actions of antagonists at the concentrations used to block responses to the standard agonists; antagonists were thus added 5 min before the introduction of agonist. Responses to the agonist in the presence of antagonists were compared with control, the responses obtained without the treatment with antagonists.

The effects of putative antagonists were expressed as a percentage of the control mean values that were generated by two separate applications of the agonist.

Assay of adenylate cyclase activity. The activity of adenylate cyclase in the jejunal longitudinal muscle was determined by measuring the amount of cAMP formed using unlabelled ATP as the substrate (Albano et al. 1973). The isolated jejunum was cleaned of mucosa and longitudinal strips were prepared as described previously (Cheng et al. 1987). The strips were suspended in Tyrode solution (1:3, w/v) and homogenized in a ground glass polytron at 4°C for 30 s.
After removal of the pellet formed by 800 x g x 10 min centrifugation, a membrane-enriched pellet fraction was separated at 20,000 x g x 30 min (Sorvall refrigerated centrifuge, 4°C). The standard assay solution, in a final volume of 75 μl, contained: 50 μl ATP buffer (mM: 2 ATP, 3 MgCl₂, 10 NaCl, 10 KCl, 2 EDTA, pH 7.4) and 25 μl assay buffer (mM: 60 Tris-base, 8 theophylline, pH 7.4). Solution of test compound or blank control was incorporated in the assay buffer. The reaction was started by the addition of the membrane suspension. The mixture was incubated in a shaking water bath (65 strokes/min) at 30°C for the desired period of time. Reactions were terminated by placing the tubes containing the mixture in a water bath at 110°C for 3 min; they were then stored at -20°C overnight. The samples were then thawed and resuspended in 1 ml assay buffer and centrifuged at 1600 x g x 10 min; 50 μl samples were taken for the determination of cAMP by radioimmunoassay (Steiner et al. 1982) using commercial kits (Amersham, Buckinghamshire, UK). Values presented are the averages of duplicate measurements and the intra-assay coefficient of variation was less than 5%. Recovery was 90-93% and the appropriate corrections were made. Protein content was determined using bovine serum albumin as the standard (Lowry et al. 1951).

Data analysis. All values are expressed as mean ± SEM and N indicates the number of individual determinations. Statistical differences between two means (P < 0.05) were determined by the Student's t-test for paired and/or unpaired observations (Sokal and Rohlf 1969). Where two or more treatment means were compared to one control mean, determinations of the differences (P < 0.05) were carried out with Dunnett's multiple comparison (Dunnett 1955). The pD₂ values (van Rossum 1963) calculated from the concentration-response curves were defined as the negative logarithm of the molar ED₅₀ value.

Drugs. The drugs used were as follows: phentolamine (Regitine, Ciba-Geigy, Basel, Switzerland), SCH23390 (RBI, Natick, MA, USA) and guanethidine (Wako, Osaka, Japan). Alloxan, desipramine, haloperidol, imidazole, 3-isobutyl-1-methylxanthine (IBMX), isoprenaline, methoxamine, octopamine, perphenazine, propranolol, sulpiride and yohimbine were purchased from Sigma (St. Louis, MO, USA). Domperidone was a gift from Janssen Pharmaceutics (Beerse, Belgium). Tetrodotoxin (TTX) was supplied by Sankyo (Tokyo, Japan).

Results

Effect of octopamine on the motility of rabbit jejunum

In the isolated jejunum of rabbits, spontaneous motility of longitudinal muscle can be observed. Octopamine produced reproducible inhibition of pendular movement at concentrations of 0.1-10 μmol/l (Fig. 1A, B). This effect of octopamine appeared quickly and was easily washed out. By dosing at 30 min intervals, repetitive doses resulted in almost identical responses. The rhythmic rate (number/min) of jejunal spontaneous motility was not modified by the treatment with octopamine. Neither the rhythmic rate nor the muscle amplitude were modified by the vehicle.

Inhibition of muscle contraction amplitude induced by octopamine was dose-dependent (Fig. 1B) and reached a maximum (Eₘₓ) at 10 μmol/l. The ED₅₀ obtained from cumulative concentration-response curves was 2 μmol/l (Fig. 1B). The maximal relaxation induced by octopamine was 87.8 ± 3.6% (N = 8) of the relaxations induced by 0.1 μmol/l isoprenaline. In the experiments described below, 5 μmol/l of octopamine (an approximate ED₅₀ concentration) was employed.

Effect of neurone-blocking agents on the action of octopamine

In the presence of tetrodotoxin (1 or 2 μmol/l; sufficient to block the neuronal sodium channels; Gershon 1967; Katz and Miledi 1967), relaxations induced by octopamine (5 μmol/l) were not modified (Table 1). Similarly, guanethidine (1 or 5 μmol/l) had no effect on the octopamine-induced relaxation, despite the concentration being sufficient to block the release of noradrenaline (Starke 1972; Table 1).

Desipramine, at a concentration sufficient to block the uptake of tyramine into adrenergic neurones (Cheng et al. 1987), did not influence relaxations induced by octopamine (Table 1). Spontaneous contractions were not modified by