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Effect of dopamine on dog distal colon in-vitro

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The effects of dopamine, isoprenaline, adrenaline and noradrenaline in dog isolated colon strips were studied. All these drugs produced in the preparation relaxations which were inhibited by propranolol and sotalol or, in the case of noradrenaline, by a mixture of propranolol and phentolamine. A selective β_1-adrenergic antagonist, practolol, had the same inhibitory effects on the relaxations induced by all the agonists, suggesting the existence of β_1- and β_2-adrenoceptor subtypes in the dog distal colon. There is no evidence for the presence of specific dopaminergic receptors in this preparation.

The presence of specific dopaminergic receptors in the gastrointestinal tract has been demonstrated by various authors. Dopamine (DA) has an inhibitory effect on the oesophagus in the opossum (De Carle & Christensen 1976), and on the intraluminal pressure of the stomach in the dog (Valenzuela 1976), guinea-pig (Van Nueten et al 1978) and man (Lanfranchi et al 1977); however on the opossum duodenal smooth muscle (Anuras 1981) and on human colon (Lanfranchi et al 1978) DA has an excitatory action. In a preliminary study, we observed a stimulatory effect of DA on sigmoid colon in man and in the dog in-vivo (Grivegnée et al 1979) which was specifically antagonized by sulpiride, a drug known for its antidopaminergic effect (O'Connor & Brown 1982). In the present work we have studied the in-vitro effect of DA, in comparison with α- and β-adrenoceptor stimulants, on the dog distal colon.

MATERIALS AND METHODS

Segments of distal colon from mongrel dogs (10–30 kg) under pentobarbitone sodium anesthesia (30 mg kg^{-1}), were excised and immersed in Krebs solution aerated with 95% O_2 and 5% CO_2 and stored overnight at 4 °C. Composition of Krebs solution (mM) was: NaCl 118·1; KCl 4·7; $CaCl_2H_2O$ 2·5; KH_2PO_4 1·2; $MgSO_47H_2O$ 1·2; glucose 5; $NaHCO_3$ 25. For the experiment, the mucosa of the preparation was removed and longitudinal strips (0·5 × 5 cm) were set up in a 50 ml organ-bath containing Krebs solution at 37 °C, gassed with 95% O_2, 5% CO_2, and allowed to equilibrate for at least 60 min. The load on the tissues was 2 g. Recording of the tissue responses were made on a kymograph, using an isotonic lever (×5 magnification).

Two types of experiments were performed: (i) single doses of DA or other amines were added at 15 min intervals. After three reproducible responses, antagonists were incubated for at least 10 min before next addition of the agonist. (ii) Cumulative concentration-response curves for DA or other amines were obtained by contracting the muscle with 2×10^{-2} M KCl and by adding the drugs cumulatively until maximal response was reached (control curve). Antagonists were incubated for 20 min before the agonist was added in the same way as in the control curve. The results were expressed as % of the maximal induced response in the control curves.

Drugs used were: adrenaline bitartrate (Fluka); domperidone, haloperidol (Janssen); dopamine hydrochloride, isoprenaline hydrochloride, noradrenaline bitartrate (Winthrop); phentolamine methanesulphonate (Ciba); practolol hydrochloride, propranolol hydrochloride (ICI); sotalol hydrochloride (Bristol); metoclopramide hydrochloride, sulpiride (Delagrange) and tetrodotoxin (Calbiochem).

RESULTS

Effects of dopamine on dog distal colon

Most colon preparations exhibited spontaneous tone and phasic activity which varied in frequency and amplitude (Fig. 1) while the tone of the preparation gradually fell during the experiment. When DA (10^{-5} M) was applied to the bath, a relaxation occurred which developed slowly and reached its maximum in 12 min (Fig. 1), persisting longer than 20 min. After DA had been washed out (generally after 5 min), a rapid return of the tone of the preparation towards the basal level was observed. Isoprenaline (3×10^{-7} M) had the same activity as

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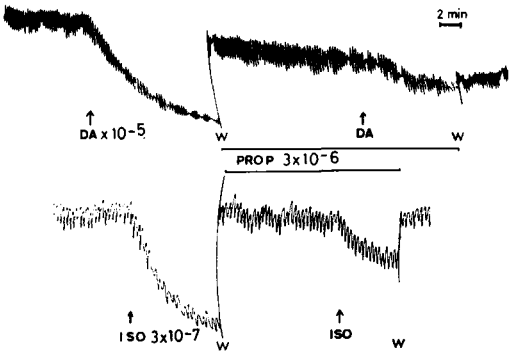

FIG. 1. Relaxation induced by dopamine (DA) and isoprenaline (ISO) in a dog colon preparation in the absence and in the presence of propranolol (PROP). W indicates wash-out.

DA on the colon, however, the relaxation developed more quickly.

In the presence of propranolol (3×10^{-6} M), the drug-induced relaxation to DA and isoprenaline was greatly inhibited (Fig. 1). The residual relaxation in the presence of propranolol was not modified by tetrodotoxin (3×10^{-7} M), phentolamine (3×10^{-6} M), metoclopramide, sulpiride, domperidone or haloperidol (each 10^{-5} M).

Concentration-response curves for dopamine

DA (10^{-6}–10^{-4} M) was able to fully relax a colon preparation that was first contracted by a large concentration of KCl. The IC50 value (concentration needed to develop 50% of the maximal response), determined from concentration response curves was 5×10^{-6} M (n = 78). Phentolamine (3×10^{-6} M) did not affect the concentration-response curve of DA while propranolol (10^{-6} and 3×10^{-6} M) displaced it to the right with diminished maximal induced relaxation (Fig. 2). Both practolol (10^{-6} and 10^{-5} M) and sotalol (10^{-6} and 10^{-5} M) displaced the curve of DA to the right and lowered the maximal induced relaxation at the highest concentration used (Fig. 3).

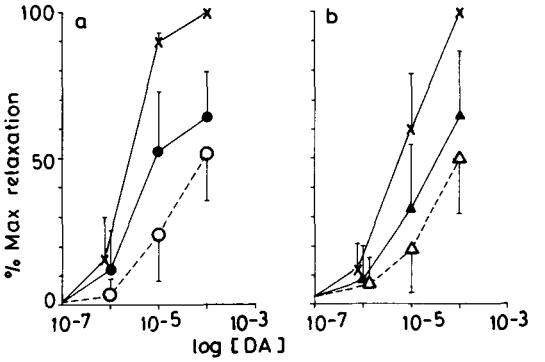

FIG. 3. Cumulative concentration-response curves for the relaxing effect of dopamine on dog KCl-contracted colon. Each point is the mean value ± s.e.m. of 5–8 experiments in the absence (×) and in the presence of (a) practolol (●) 10^{-6} M and (○) 10^{-5} M or (b) sotalol (▲) 10^{-6} M and (△) 10^{-5} M.

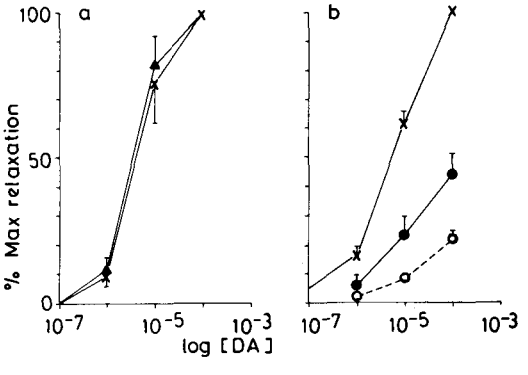

FIG. 2. Cumulative concentration-response curves for the relaxing effect of dopamine on dog colon. These curves were obtained after contracting the muscle preparation with 2×1^{-2} M KCl. Each point is the mean value ± s.e.m. of 4–7 experiments in the absence (×) and in the presence of (a) phentolamine (▲) 3×10^{-6} M or (b) propranolol (●) 10^{-6} M and (○) 3×10^{-6} M.

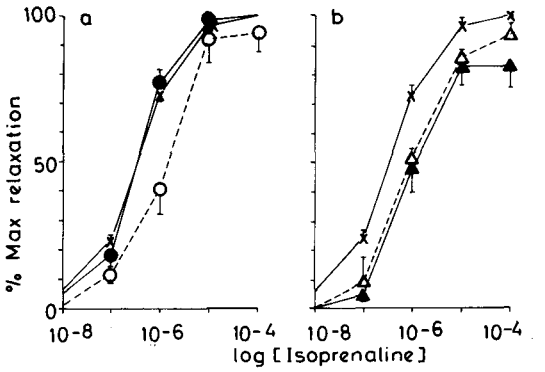

FIG. 4. Cumulative concentration-response curves for the relaxing effect of isoprenaline on dog KCl-contracted colon. Each point is the mean value ± s.e.m. of 4 experiments in the absence (×) and in the presence of (a) propranolol (○) 10^{-6} M and phentolamine (●) 3×10^{-6} M or (b) practolol (△) 10^{-5} M and sotalol (▲) 10^{-5} M.

Concentration-response curves for adrenaline, noradrenaline and isoprenaline

Adrenaline, noradrenaline and isoprenaline from 10^{-7}–10^{-5} M induced concentration-related relaxation of the dog colon, contracted by KCl (Figs 4, 5).

IC50 values determined from concentration-response curves were 4×10^{-7} M for adrenaline (n = 15), 3×10^{-7} M for noradrenaline (n = 12) and $3{\cdot}3 \times 10^{-7}$ M for isoprenaline (n = 16).

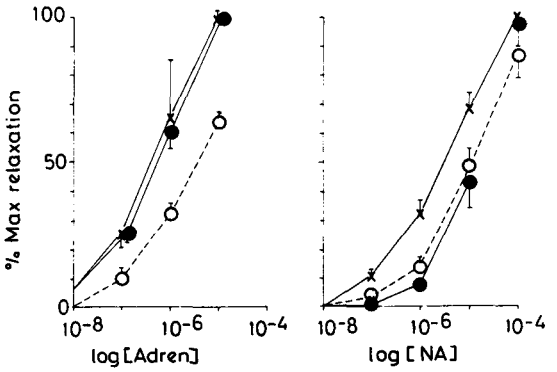

FIG. 5. Cumulative concentration-response curves for the relaxing effect of adrenaline and noradrenaline on dog KCl-contracted colon. Each point is the mean value ± s.e.m. of (4–10) experiments in the absence (×) and in the presence of propranolol (○) 3×10^{-6} M or phentolamine (●) 3×10^{-6} M.

Phentolamine (3×10^{-6} M) did not affect the concentration-response curves for adrenaline (Fig. 5) and isoprenaline (Fig. 4), but shifted the curve for noradrenaline to the right (Fig. 5) while propranolol (3×10^{-6} M) shifted the curves to the right for the three sympathomimetics (Figs 4, 5). Practolol (10^{-5} M, n = 4) and sotalol (10^{-5} M) had the same inhibitory effects on isoprenaline-induced relaxations (Fig. 4). Metoclopramide (10^{-5} M, n = 4) and sulpiride (10^{-5} M, n = 4) had no effect on the concentration response curves for the sympathomimetics.

DISCUSSION

DA relaxes the rabbit isolated intestine by stimulating both α- and β-adrenoceptors (Heilman & Lum 1971; Gupta et al 1970). Our results show that DA at high concentration exerts a relaxing effect on the dog distal colon in-vitro by stimulating β-adrenoceptors, since the responses are antagonized by propranolol and not by phentolamine.

As practolol, a selective β_1-adrenoceptor antagonist, has the same inhibitory effect on DA-induced relaxation as propranolol and sotalol, which are non-selective β-adrenoceptor blocking drugs, both β_1- and β_2-adrenoceptors seem to be stimulated by DA. There is no evidence for the presence of specific DA receptors in the smooth muscle of the dog colon, as metoclopramide, sulpiride, domperidone and haloperidol (10^{-5} M), all known for their antidopaminergic activity (see Creese et al 1983), had no effect on the relaxations observed.

Isoprenaline and adrenaline had the same activity as DA on dog colon, inducing relaxations of the preparation by stimulating a mixture of β_1- and β_2-adrenoceptors, though, on a molar basis, these amines were about 10 time more potent than DA. Noradrenaline induced relaxations by stimulating α-, β_1- and β_2-adrenoceptors.

Catecholamines relax the intestine of many mammalian species (Ahlquist & Levy 1959; Furchgott 1960; Bowman et al 1970) by acting on α- and β-adrenoceptors. The β_1-, or β_2-subtypes of adrenoceptors (Lands et al 1967) mediate the relaxation of rabbit ileum (Wagner et al 1981) and cat colon (Ek & Lundgren 1982) and our results are in agreement indicating the coexistence of β_1- and β_2-adrenoceptor subtypes in the dog colon.

However, in our experiments, the inhibitory effect of propranolol, sotalol and practolol in the sympathomimetic-induced relaxations are weak, even at high concentrations (10^{-6} and 3×10^{-6} M) and the maximal induced responses to DA are lowered by these drugs. Noradrenaline at high concentrations induced relaxations of rabbit intestine which were resistant to α- and β-adrenoceptor-blocking drugs (Wikberg 1977) and probably due to sympathomimetic-induced non-specific spasmolytic actions.

In conclusion, DA, like isoprenaline and adrenaline, induces relaxation of the longitudinal muscle of the dog distal colon by stimulating β_1- and β_2-adrenoceptors, whilst noradrenaline stimulates both α- and β-adrenoceptors.

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