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Stimulating action of catecholamines on isolated preparations of the rat colon and human and rabbit taeniae coli

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Summary

1. Adrenaline and noradrenaline induced dose related relaxations of the superfused rat colon. The relaxations were progressively transformed into contractions when β-adrenoceptors were blocked with increasing doses of oxprenolol.

2. Since phentolamine or phenoxybenzamine inhibited the contractions induced by adrenaline or noradrenaline in the presence of oxprenolol, it is assumed that the contractions are caused by stimulation of excitatory α-adrenoceptors.

3. Human and rabbit taeniae coli showed responses that were qualitatively similar to those observed with the rat colon.

Introduction

The presence of inhibitory α- and β-adrenoceptors in the intestine was demonstrated by Ahlquist (1948) and confirmed by Ahlquist & Levy (1959). There is, however, evidence for the presence of excitatory adrenoceptors. In particular, Lands, Luduena, Grant & Ananenko (1950), Munro (1951) and Lands (1952) showed that catecholamines can induce contractions of the guinea-pig ileum. More recently, a contractile response to catecholamines in various segments of the gastrointestinal tract of the guinea-pig has been reported by Bailey (1968) and Guimaraes (1969).

Preliminary experiments with a conventional organ bath technique have shown that the relaxation of the isolated rat colon produced by adrenaline or noradrenaline is reversed into a contraction by previous treatment with oxprenolol (Gagnon, 1970). Oxprenolol appears to be more effective than propranolol in reversing the response to catecholamines in the isolated rat colon (Gagnon & Belisle, 1970). These observations raise the question whether the contractile effect of catecholamines on the intestine, treated with a β-blocking agent, depends on the experimental conditions or on the β-blocking agent used, and whether excitatory α-adrenoceptors are present also in the intestine of other species.

The purpose of the present study was to use the technique of cascade superfusion to confirm the results obtained with the conventional organ bath technique. This technique has the advantage of enabling the simultaneous recording of responses induced by an agonist in three different tissues, one being used as control, while the other two are treated with one or more antagonists. The base line of the tissues remains more stable and this greatly facilitates the evaluation of the effects of drugs.

Methods

Rats of either sex were killed by stunning and bleeding from the carotid arteries. The ascending colon was rapidly removed, placed in a Petri dish containing cool Krebs solution and dissected free.

Rabbit taeniae coli were obtained from albino rabbits weighing 1–1·5 kg, which were killed by stunning and bleeding from the carotid arteries. A segment of ascending colon was taken out, the taeniae coli isolated and a strip of 3–4 cm dissected.

Human taeniae coli were isolated by the technique of Bucknell & Whitney (1964) from specimens of human colon freshly resected. Colons obtained from patients suffering from ulcerative colitis or intestinal occlusion were not included. Immediately after resection, the colons were placed in cool Krebs solution, and strips of taeniae coli, 3–4 cm long, dissected.

The tissues or the strips were mounted in an organ bath and immediately superfused with Krebs solution at 37° C, at a rate of 10 ml/minute. The solution was gassed continuously with 95% oxygen and 5% carbon dioxide. The composition of the Krebs solution was as follows (g/l.): NaCl, 6·9 ; KCl, 0·35 ; $CaCl_2$, 0·28 ; KH_2PO_4, 0·16 ; $MgSO_4 \cdot 7H_2O$, 0·29 ; dextrose, 2 ; $NaHCO_3$, 2·1. Tissues were superfused for about 30 min until spontaneous activity fully developed.

The technique of superfusion was basically that described by Gaddum (1953). The tissues were connected to a Harvard Heart and Smooth Muscle Transducer model # 356, counterweighted with 1–2·5 g, and the movements were recorded on an electronic Harvard Recording system with special pens giving a maximal deflection of about 90 mm.

All drug solutions were freshly prepared in Krebs solution. Adrenaline and noradrenaline were always protected against oxidation by addition of ascorbic acid (0·2–0·5 mg/ml). Solutions of agonists and antagonists were infused through a rubber tube into the superfusing fluid at a rate of 0·1 ml/min and the infusions were continued for fixed periods of 4 min for all agonists. The concentration of drugs refers to the final concentration of the free bases in the superfusing fluid. In some experiments, three rat colons taken from different animals or three segments of human or rabbit taeniae coli taken from the same specimen were superfused with the agonist. Thereafter, the first tissue was used as control, while the other two tissues were treated with oxprenolol or with oxprenolol and phentolamine. Oxprenolol was preferred to propranolol since Gulati, Gokhale, Parikh, Udwadia, & Krishnamurty (1969) have shown that propranolol can block α-adrenoceptors also.

Changes of the base line were calculated with a Gelman planimeter, and the results expressed as the area below the tracing. All experimental findings are expressed as means $\pm$ S.E. ; the differences between control and treatment periods were calculated according to Steel & Torrie (1960).

The following drugs were used: oxprenolol hydrochloride and phentolamine mesylate (Ciba Co. Ltd., Dorval) ; (−)-adrenaline bitartrate (K & K Laboratories, Plain View, N.Y.) ; (−)-noradrenaline hydrochloride (Schuchardt Co., München, Germany) ; phenoxybenzamine hydrochloride (Smith, Kline and French, Montreal) ; acetylcholine chloride and atropine sulphate (Sigma Chemical Co., St. Louis, U.S.A.) ; 5-hydroxytryptamine creatinine sulphate (B.D.H., Montreal) ; methysergide bimaleate (Sandoz, Montreal).

Results

Rat colon

Figure 1 shows an experiment in which three isolated rat colons were superfused and tested for their sensitivity to adrenaline (30 and 100 ng/ml). The relaxations induced by these doses of adrenaline were similar in all three tissues. Thereafter, oxprenolol was applied to the second and the third tissues, and allowed to act for 12–15 minutes. On the first colon, adrenaline had the same effect as before, while on the second and third tissues, it produced a contraction instead of a relaxation. When phentolamine was applied to the third tissue in the continued presence of oxprenolol, adrenaline induced three different responses: relaxation of the first colon, contraction of the second colon, and a much reduced contractile response of the third colon.

The results obtained in a large series of experiments are summarized in Fig. 2. The responses to adrenaline and noradrenaline were measured before and after applying oxprenolol in different concentrations. Adrenaline and noradrenaline (3–100 ng/ml) produced dose related relaxations. Although the differences were not significant statistically, the changes induced by noradrenaline were usually slightly greater than those caused by adrenaline, especially when low concentrations were used.

The addition of a low concentration of oxprenolol (0·5 μg/ml) to the superfusing fluid reversed the effects of the lower concentrations of adrenaline (3–30 ng/ml), while the higher concentration (100 ng/ml) still had a relaxing effect. Apparently, the concentration of oxprenolol was too low to block the effect of the large dose of adrenaline. The concentration of oxprenolol was then doubled (1 μg/ml) and, in a large number of experiments, it was further increased to 7·5 μg/ml. This latter

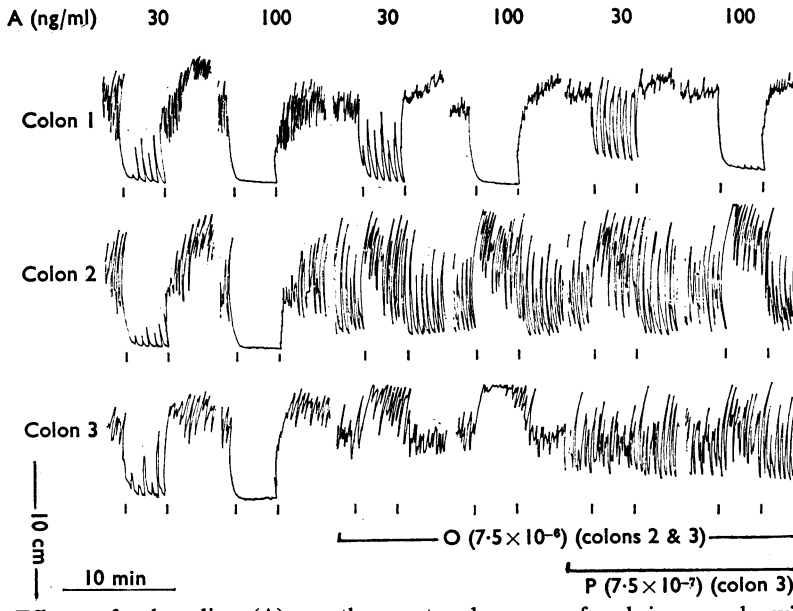

FIG. 1. Effects of adrenaline (A) on three rat colons superfused in cascade with Krebs solution. Colon 1, always relaxed; colon 2, relaxed first but contracted after oxprenolol (O) (7·5 μg/ml); colon 3, relaxed first, contracted after oxprenolol and finally showed little effects after phentolamine (P) 0·75 μg/ml). Vertical bars indicate the period of infusion of adrenaline.

concentration was sufficient to block the β-adrenoceptors completely and adrenaline now produced dose dependent contractions.

Similar results were observed with noradrenaline (3–100 ng/ml). The excitatory effect of noradrenaline was smaller than that observed with adrenaline, especially with low doses (3, 10 and 30 ng/ml). The difference was statistically significant ($P<0.02$; $P<0.05$; $P<0.05$, respectively).

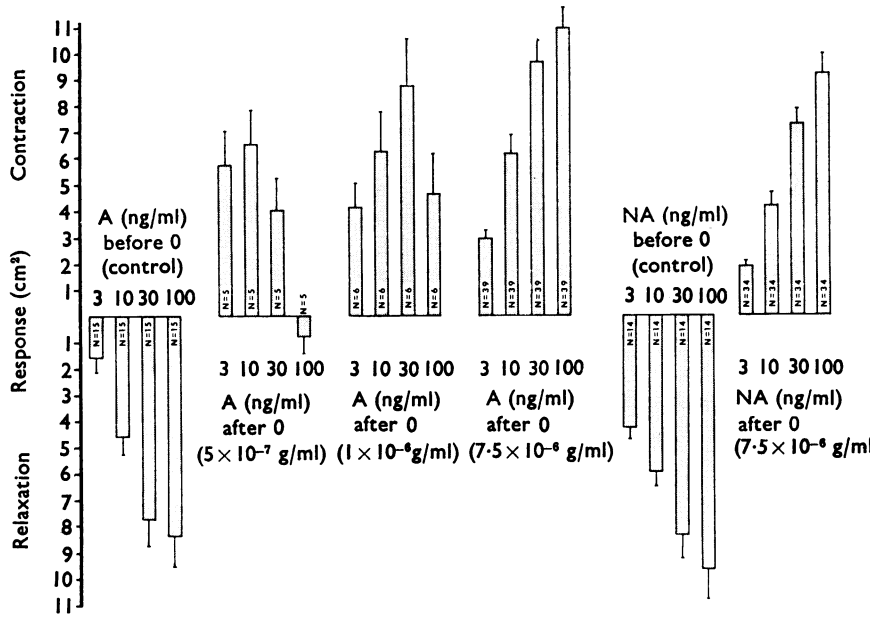

FIG. 2. Histogram showing the effects of different concentrations of oxprenolol on the responses of the rat colon to different concentrations of adrenaline. Abscissae: concentrations (ng/ml) of adrenaline (A) or noradrenaline (NA). Ordinates: responses expressed as area (cm^2) of the tracing above base-line ; upward columns, contractions ; downward columns, relaxations. Oxprenolol (O) was used in three concentrations (0·5, 1 and 7·5 μg/ml) in the adrenaline experiments (group of columns 2–4) and in one concentration (7·5 μg/ml) in the experiments with noradrenaline (group 6). N, number of observations ; vertical bars, S.E. of mean.

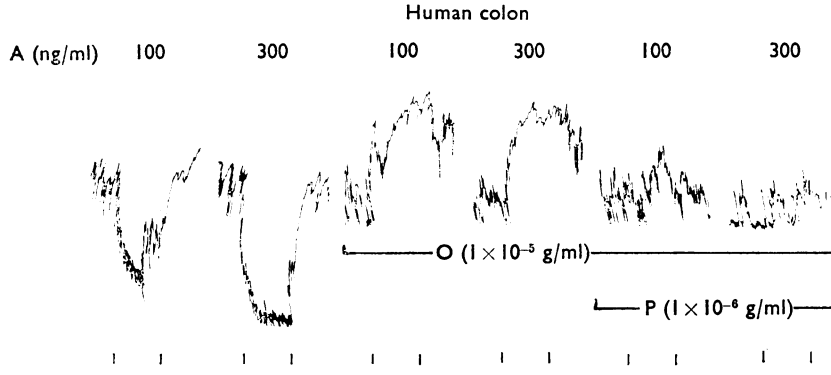

FIG. 3. Effects of adrenaline (A) upon the human taeniae coli. Oxprenolol (O) converted the relaxations caused by adrenaline to contractions which were inhibited by phentolamine (P). Vertical bars indicate the period of infusion of adrenaline.

These data indicate that both adrenaline and noradrenaline induced contraction of the isolated rat colon after the β-adrenoceptors had been blocked by oxprenolol. Since the contractile responses to catecholamines are blocked by phentolamine (Fig. 1) and phenoxybenzamine, the contractions can be assumed to be due to stimulation of excitatory α-adrenoceptors.

In some experiments, concentrations of atropine (0·75 μg/ml) and methysergide (1 μg/ml) sufficient to block the effects of acetylcholine (10 ng/ml) and 5-hydroxytryptamine (1 μg/ml) respectively were shown not to alter significantly the contractile responses induced by adrenaline (30 and 100 ng) after treatment with oxprenolol (7·5 μg/ml). These results suggest that the contractions induced by the catecholamines are not due to a release of either acetylcholine or 5-hydroxytryptamine, or to a non-specific action of adrenaline and noradrenaline upon acetylcholine or 5-hydroxytryptamine receptors.

Human and rabbit taeniae coli

Human taeniae coli showed spontaneous activity comparable to that of the rat colon but were less sensitive to the action of adrenaline. The effects produced by adrenaline before and after treatment with β- and α-adrenoceptor blocking agents did not differ qualitatively from those observed on the rat colon. Oxprenolol converted the catecholamine induced relaxations into contractions which were almost completely inhibited by phentolamine.

In the rabbit taeniae coli, adrenaline induced relaxations during the control periods ; after treatment with oxprenolol, the relaxations were reversed to contractions which were almost completely inhibited by phentolamine.

Discussion

The sensitivity of the large intestine to the relaxing effects of adrenaline and noradrenaline varies from species to species, the human and rabbit taeniae coli being less sensitive than the rat colon. Moreover, in the rat colon, noradrenaline has a somewhat greater inhibitory effect than adrenaline. This finding is not in agreement with the general concept that adrenaline is more potent than noradrenaline in stimulating β-adrenoceptors, but there are other reports of similar observations in the rat colon (Gaddum, Peart & Vogt, 1949) and the guinea-pig ileum (Lands *et al.*, 1950).

The relaxing effects of both catecholamines on the intestinal smooth muscle are prevented by the administration of a β-adrenoceptor blocking agent. In addition, the occupation of β-adrenoceptors reveals a stimulating effect of catecholamines on the intestinal smooth muscle in all three species. These results confirm and extend the observations of Regoli & Vane (1964) on the rat isolated colon, of Bailey (1968) and Guimaraes (1969) on the guinea-pig isolated stomach, and of Bucknell & Whitney (1964) on the human taeniae coli. It seems reasonable to conclude that once the β-adrenoceptors are occupied, the catecholamines can act on excitatory α-adrenoceptors and induce a contraction. This view is supported by the finding that the catecholamine induced contractions are almost abolished by concentrations of phentolamine or phenoxybenzamine which block the α-adrenoceptors.

The results obtained with human and rabbit taeniae coli confirm the observations made on the rat colon and show the same pattern of responses.

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REFERENCES

AHLQUIST, R. P. (1948). A study of the adrenotropic receptors. *Am. J. Physiol.*, **153,** 586–600.

AHLQUIST, R. P. & LEVY, B. (1959). Adrenergic receptive mechanism of the canine ileum. *J. Pharmac. exp. Ther.*, **127,** 146–149.

BAILEY, D. M. (1968). Some effects of sympathomimetic amines on isolated smooth muscle preparations from the stomach of the guinea-pig. *Br. J. Pharmac.*, **34,** 204*P*.

BUCKNELL, A. & WHITNEY, B. (1964). A preliminary investigation of the pharmacology of the human isolated taenia coli preparation. *Br. J. Pharmac. Chemother.*, **23,** 164–175.

GADDUM, J. H. (1953). The technique of superfusion. *Br. J. Pharmac. Chemother.*, **8,** 321–326.

GADDUM, J. H., PEART, W. S. & VOGT, M. (1949). The estimation of adrenaline and allied substances in blood. *J. Physiol., Lond.*, **108,** 467–481.

GAGNON, D. J. (1970). Intestinal smooth muscle: Demonstration of catecholamines-induced contraction mediated through alpha-adrenergic receptors. *Eur. J. Pharmac.*, **10,** 297–300.

GAGNON, D. J. & BELISLE, S. (1970). Stimulatory effects of catecholamines on the isolated rat colon after beta-adrenergic blockade with oxprenolol and propranolol. *Eur. J. Pharmac.*, **12,** 303–309.

GUIMARAES, S. (1969). Alpha excitatory, alpha inhibitory and beta inhibitory adrenergic receptors in the guinea-pig stomach. *Archs int. Pharmacodyn. Ther.*, **179,** 188–201.

GULATI, O. D., GOKHALE, S. D., PARIKH, H. M., UDWADIA, B. P. & KRISHNAMURTY, V. S. R. (1969). Evidence for a sympathetic alpha receptor blocking action of beta receptor blocking agents. *J. Pharmac. exp. Ther.*, **166,** 35–43.

LANDS, A. M., LUDUENA, F. P., GRANT, J. T. & ANANENKO, E. (1950). The pharmacologic action of some analogs of 1-(3,4 dihydroxyphenyl)-2-amino-1-butanol (ethyl-norepinephrine). *J. Pharmac. exp. Ther.*, **99,** 45–56.

LANDS, A. M. (1952). Sympathetic receptor action. *Am. J. Physiol.*, **169,** 11–21.

MUNRO, A. F. (1951). The effect of adrenaline on the guinea-pig intestine. *J. Physiol., Lond.*, **112,** 84–94.

REGOLI, D. & VANE, J. R. (1964). A sensitive method for the assay of angiotensin. *Br. J. Pharmac. Chemother.*, **23,** 351–359.

STEEL, R. G. D. & TORRIE, J. H. (1960). *Principles and Procedures of Statistics.* New York, Toronto, London: McGraw-Hill Book Co.

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