ISOLATED TERMINAL GUINEAPIG ILEUM - AN *IN VITRO* MODEL TO DEMONSTRATE DALE'S REVERSAL PHENOMENON

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The inhibitory effect of sympathomimetic amines on the gastrointestinal smooth muscles of mammals is a well known phenomenon and is known to be mediated through both α-and β-adrenoreceptors (1). However, adrenaline and noradrenaline stimulate to contract pig oesophagus (2), terminal oesophagus of cat (3) and terminal ileum of dog and cat (5). On the other hand, adrenaline has both excitatory and inhibitory actions on the guineapig terminal ileum (8) which prompted us to explore the possibility of demonstrating the Dale's adrenaline reversal phenomenon *in vitro* (4).

Adult guineapigs of either sex (250-300 *g*) were used. All experiments were done on pieces obtained from the last 6 cm portion of the terminal ileum adjacent to the ileo-caecal junction. The tissue pieces, 2-3 cm long were suspended in 10 *ml* bath containing Tyrode solution kept at 37°C and bubbled continuously with oxygen. Isotonic contractions against 0.5 *g* tension and magnified 6-fold, were recorded on a slow moving kymograph.

Adrenaline hydrochloride (0.01-3 μg/*ml*), 5-hydroxytryptamine creatinine sulphate (0.1-0.3 μg/*ml*) and carbachol (0.05-0.1 μg/*ml*) were used as agonists. Phenoxybenzamine (0.1-1 μg/*ml*), dihydroergotamine (0.1-1/μg/*ml*), pronethalol (0.5-1 μg/*ml*), propranolol (0.5-1 μg/*ml*), cyproheptadine (0.5 μg/*ml*) and atropine (1 μg/*ml*) were used as blockers.

Smaller doses (0.01-0.03 μg/*ml*) of adrenaline relaxed the intestine (Fig. I-A). Subsequent exposure to the same dose either resulted in tachyphylaxis or produced contraction. However, doses from 0.1 μg/*ml* and above elicited dose-dependent contractions (Fig. I-A). Contractile responses were also evident with 5-hydroxytryptamine and carbachol.

The α-blockers, phenoxybenzamine or dihydroergotamine completely blocked, in about 15 min, the contractile responses to adrenaline and usually reversed them to

relaxation (Fig. I-B); in this respect, phenoxybenzamine was more potent than dihydroergotamine. The resultant relaxant effect could be blocked by β-adrenoreceptor blockers, propranolol or pronethalol (Fig. I-B). Though cyproheptadine completely abolished the excitatory effect of 5-hydroxytryptamine, in the doses used, it had no effect on the response to adrenaline. Also, phenoxybenzamine and dihydroergotamine did not alter the 5-hydroxytryptamine-induced contractile responses. Further, neither cyproheptadine nor phenoxybenzamine modified the responses of the tissue to carbachol. Atropine in concentrations sufficient to abolish the contractile responses to carbachol was without effect on the responses to adrenaline and 5-hydroxytryptamine.

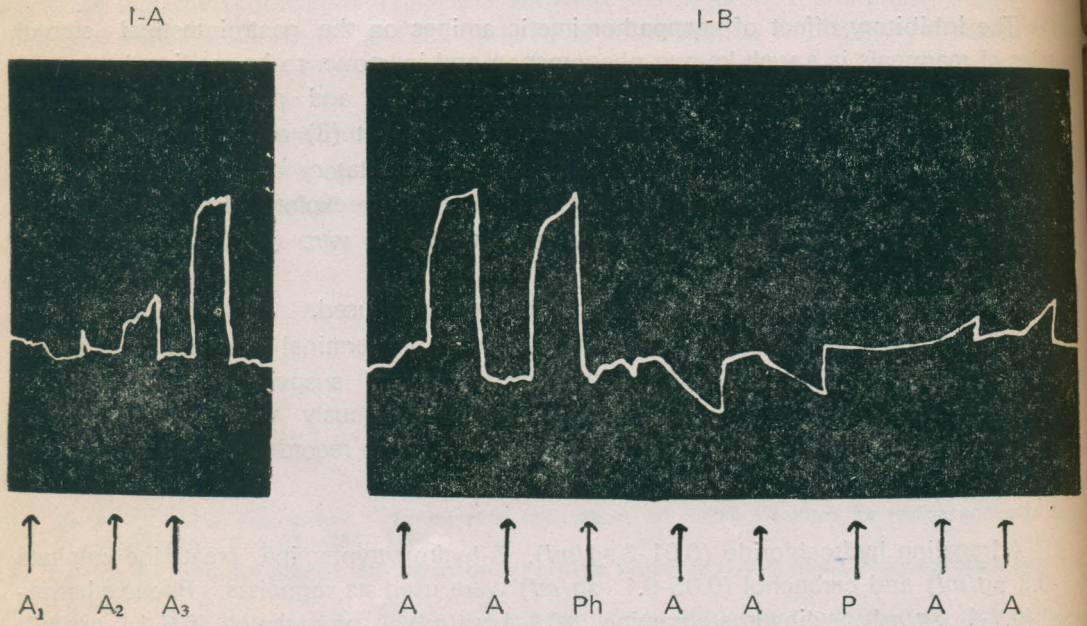

Fig. 1-A : Isolated guineapig ileum. Adrenaline was added at arrows. A_1 –0·03 μg/ml induced relaxation; A_2 (0.1 μg/ml) and A_3 (0.3 μg/ml) produced dose-dependent contraction.

Fig. 1-B : Isolated guineapig ileum showing Adrenaline Reversal. Drugs were added at arrows. A-adrenaline (0.3 μg/ml); Ph-phenoxybenzamine (1 μg/ml); P-propranolol (1 μg/ml).

The excitatory response to high doses of adrenaline and its blockade/reversal by phenoxybenzamine or dihydroergotamine indicate that this response is mediated through α-adrenoreceptors. The abolition of subsequent relaxation by β-blockers (propranolol or pronethalol) suggest the β-inhibitory receptor involvement. The initial relaxant responses to smaller doses of adrenaline could not be explained in terms of its β-receptors mediation because of the development of quick tachyphylaxis. However, no such

tachyphylaxis was observed with the relaxant response to high doses of adrenaline following α-blockade.

The involvement of 5-hydroxytryptamine in the mediation of the excitatory effect of adrenaline in guineapig ileum has been suggested (6,7). However, in the present study, though cyproheptadine could effectively block the responses to 5-hydroxytryptamine, it did not alter those of adrenaline. Similarly, the doses of phenoxybenzamine which reversed the adrenaline responses did not modify the 5-hydroxytryptamine-induced contractions. This, therefore, negates the involvement of tryptaminergic receptor and strongly supports the specific involvement of α-excitatory and β-inhibitory receptors in mediating the adrenaline responses.

The results of the present study on guineapig ileum demonstrate a close resemblance to those with spinal cat blood pressure preparation in relation to reversal of responses to adrenaline. The greater sensitivity of β-adrenoreceptors on the vascular smooth muscle which results in fall in blood pressure to small doses of adrenaline is well known. Likewise, our findings suggest that probably the β-receptors present in this part of the ileum are much sensitive to low doses of adrenaline, resulting in relaxation; however, as the dose of adrenaline is increased, the contraction mediated by α-component is superimposed upon the former.

Thus, the terminal portion of the guineapig ileum is suitable for use as an *in vitro* model to demonstrate the Dale's adrenaline reversal phenomenon.

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REFERENCES

1. Ahlquist, R.P. and B. Levy. Adrenergic receptive mechanisms of the canine ileum. *J. Pharmac. Exp. Ther.*, **127** : 146–149, 1959.

2. Burnstock, G. Membrane potential changes associated with stimulation of smooth muscle by adrenaline. *Natu* **186:** 727, 1960.

3. Christensen, H.D. and E.E. Daniel. Electric and motor effects of autonomic drugs on longitudinal oesophag smooth muscle. *Am. J. Physiol.*, **211:** 387–394, 1966.

4. Dale, H.H. On some physiological actions of ergot. *J. Physiol. Lond.*, **34:** 163–206, 1906.

5. Gazet, J.C. and R.J. Jarrett. The ileocaeco colic sphincter studies *in vitro* in man, monkey, cat and dog. *Br. Surg.*, **51:** 368–370, 1964.

6. Innes, I.R. Action of dexamphetamine on 5-hydroxytryptamine receptors. *Br. J. Pharmac. Chemother.*, **21:** 422 435, 1963.

7. Munro, A.F. The effect of adrenaline on the guineapig intestine. *J. Physiol.*, **112:** 84–94, 1951.

8. Kohli, J.D. Receptors for sympathomimetic amines producing contraction of the guineaping ileum. *Abst. 23r Int. Congr. Physiol. Sci.*, 483, 1965.