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MODULATORY EFFECTS OF DILTIAZEM ON INOTROPIC RESPONSES TO AMRINONE ON RABBIT ISOLATED ATRIA

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Abstract : The effect of pretreatment with graded concentration of diltiazem on the inotropic responses to amrinone were studied on isolated atria of rabbit. The responses to amrinone were modified by diltiazem in a biphasic manner; initial potentiation followed by inhibition. The potentiation is proposed to be due to synergistic rise in cytosolic calcium ion concentration by diltiazem and amrinone. The inhibition by diltiazem in higher concentration may be due to blockade of calcium ion influx and depletion of intracellular calcium ion from storage sites.

Key words : phosphodiesterase inhibitor, calcium ion, sarcoplasmic reticulum, diltiazem, concentration, amrinone

INTRODUCTION

Phosphodiesterase inhibitors (PDEI) are known to exert inotropic effect on myocardium (1). It has been proposed that PDEI increase calcium ion (Ca^{++}) recirculation within myocytes (2). Holmbergh et al showed that these drugs stimulate release of Ca^{++} from sarcoplasmic reticulum (SR) (3). Haikala et al (4) reported that simendon, a new PDEI causes positive inotropic effect probably due to increased sensitivity of contractile protein to Ca^{++} rather than Ca^{++} influx.

Parhate et al (5) have reported that diltiazem (DZM), a calcium channel blocker potentiates the agonist induced contraction due to augmented release of Ca^{++} from SR.

Based on above reports that PDEI and DZM increase cytosolic Ca^{++}, we investigated the influence of DZM pretreatment on the inotropic responses to amrinone(AMN) on rabbit isolated atria preparation.

METHODS

Overnight fasted albino rabbits weighing between 1.5–2 kg were sacrificed and atria isolated by method of Clark (6) and was mounted by method of Heffter (7) in oxygenated Ringer–Locke at 37°C. Basal amplitude of contraction was recorded on smoked kymograph paper. The inotropic responses to AMN in graded concentration (conc) starting from 0.25 µg to the conc producing the ceiling effect (64 µg) were recorded by adding the drug to the organ

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bath of 20 ml capacity. The contact time for each conc was 2 min. Wash was given after each response recording and preparation was allowed to return to the baseline value. The conc–response curve of AMN was plotted on graph and interpolate was drawn at 20 µg. Since this fell over 25% of response, this conc was selected for further study.

After recording the basal contraction, the *per se* effect of DZM (0.25 µg) on basal contraction was recorded. Without wash, AMN 20 µg was added to the organ bath and the effect of DZM pretreatment on the inotropic response to AMN was recorded. Then wash was given and the preparation was allowed to return to baseline. The same procedure was repeated with DZM pretreatment in graded conc from 0.5 to 16 µg, keeping AMN conc of 20 µg constant.

The amplitude of contraction was measured in mm. Results were expressed as mean ± SEM and were statistically analysed for significance by paired `t' test. Drugs used were: –Inj. amrinone lactate (manufactured by Win Medicare Ltd., New Delhi) prepared as 20 µg/0.5 ml in distilled water; Diltiazem powder (Sigma Chemicals Co, St Louis, USA, product No. 2521) was dissolved in distilled water and then prepared in dilution from 0.25 to 16 µg serially out of stock solution. All the solutions were freshly prepared and used within 3 hours.

RESULTS

Amrinone (20 µg) produced increase in the amplitude of contraction. The basal amplitude of contraction was reduced by 25% from the beginning to the last conc probably due to reduction in the sensitivity of the preparation. Diltiazem *per se* (0.25 and 0.5 µg) caused increase in the amplitude of contraction, wheareas 8 and 16 µg conc produced reduction. The inotropic responses

TABLE I : Showing the effects of different concentrations of diltiazem *per se* and diltiazem+amrinone (20 µg) on the amplitude of contraction of rabbit isolated atria.
(n = 6 in each group of experiments)

Basal amplitude at the begining of cycle. Height in mm ± SEM	*Diltiazem*		*Amrinone in the absence and presence of diltiazem.*
	µg/bath	*per se effect*	
5 ± 0.52	–	–	b9** ± 1.4
5 ± 1.3	0.25	a6* ± 0.37	c15. ± 0.73
4 ± 0.9	0.5	a5* ± 0.58	c10* ± 0.58
4 ± 0.37	1	a5 ± 0.58	c9 ± 0.58
4 ± 0.37	2	a5 ± 0.52	c8.5 ± 0.43
4 ± 0.37	4	a5 ± 0.58	c8* ± 0.63
4 ± 0.37	8	a3* ± 0.37	c3* ± 0.22
4 ± 0.37	16	a2** ± 0.37	c2** ± 0.34

*P < 0.05 ** P < 0.01
a = compared with baseline value b = compared with baseline value c = compared with b.

to AMN were found accentuated following DZM pretreatment of 0.25 and 0.5 µg conc; however, there was reduction with 1 to 16 µg conc, when the values of AMN without pretreatment with DZM are compared with those of AMN after DZM pretreatment. Results are shown in Table I.

DISUCSSION

This study demonstrates that AMN produced positive inotropic response on rabbit isolated atria. The mechanisms have been said to be due to increased cyclic AMP conc (1), Ca^{++}, circulation (2) and Ca^{++} release from SR (3). DZM, a calcium channel blocker has been reported to inhibit Ca^{++} influx via receptor and potential operated Ca^{++} channels in myocardium, thus causing negative inotropic and chronotropic effects (8). It is also proposed that soon after exposure to these drugs, there can be some Ca^{++} influx, but as the channels become saturated, no Ca^{++} is liable to enter through them (9). Moreover it is said that the lipophilic property of DZM allows it to cross the cell membrane and enter cytosol (10) and this may trigger Ca^{++} release from SR (11). In our study, DZM per se in lower conc increased the amplitude of atrial contraction, which may be due to Ca^{++} influx (9) and co–release of Ca^{++} from SR (11). Lower conc of DZM caused accentuation of the inotropic responses to AMN, suggesting an additive response, which may be due to synergistic rise in cytosolic Ca^{++} by DZM (9, 11) and AMN (1, 2, 3). Furthermore, higher conc of DZM diminished the amplitude of contraction as *per se* effect and also blocked the inotropic responses to AMN, suggesting that the level of intracellular Ca^{++} has been diminished probably due to cumulative blockade of Ca^{++} channel (12).

To conclude, on rabbit isolated atria preparation, the inotropic responses to AMN were modified in a dual manner by DZM pretreatment. The initial additive response to AMN with lower conc of DZM could be due to Ca^{++} influx and augmented release of Ca^{++} from SR by DZM and AMN in a synergistic manner. The inhibition of the responses to AMN with higher conc of DZM may be due to Ca^{++} channel blockade and exhaustion of Ca^{++} from storage sites.

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REFERENCES

1. Leyen V Der H, Schmitz W, Scholz H, Scholz J, Steinfath M. New positive inotropic agents acting by phosphodiesterase inhibition on alpha 1 adrenergic stimulation. *Pharmacol Res* 1984; 21: 329–337.
2. Morner SEJN. Phosphodiesterase inhibitors increase calcium recirculation within myocytes. In: phosphodiesterase inhibitors and heart, ed Abingdon JE. The Medicine Group (UK) ltd, 1990: p67.
3. Holmbergh SRM, Williams AJ. Phosphodiesterase inhibitors and cardiac sarcoplasmic reticulum calcium release channel; differential effects of milrinone and anoximone. *Cardiovas Res* 1991; 25: 537–545.
4. Haikala H, Lindane IB, Pohto P. Simendon, a novel calcium sensitiser is as potent and efficient in skinned fibres as in electrically paced papillary muscle. *J Mol Cell Cardiol* 1992; 24 (suppl. 1): 260.

5. Parhate SM, Shembekar AG, Jagtap MK. Effects of diltiazem on calcium ion translocation; a study on frog isolated rectus abdominis muscle. *Indian J Physiol Pharmacol* 1995; 39 (I): 83–85.

6. Clark A. Pharmacological experiments on isolated preparations. *J Physiol London* 1926; 61: p547.

7. Heffter E. In: Experiments with heart muscle; isolated preparation, ed Perry WLM, Livington ltd, London, 1968: 104–07.

8. Godfraind A. Calcium entry blockade and excitation contraction coupling in cardiovascular system. *Acta Pharmacol Toxicol* 1986; 18 (Suppl II); 5–30.

9. Godfraind T, Miller RC. Action of prostaglandin Fzalpha and norepinephrine on calcium exchange and contraction in rat mesentric artery and its sensitivity to calcium entry blockers. *Br J Pharmacol* 1982; 75: 229–236.

10. Wang T. Tsali LI, Schwartz A. The effect of calcium antagonist on sarcoplasmic reticulum. *Eur J Pharmacol* 1984; 100: 253–261.

11. Volpe P, Salviat G, Di Virgilio F. Inositol triphosphate induces release of calcium from sarcoplasmic reticulum of skeletal muscle. *Nature* 1985; 316 : 347–349.

12. Bayer R, *Ehara T.* Comparative studies on calcium antagonists. *Progress Pharmacol* 1978; 12: 31–37.