COMPARATIVE EVALUATION OF THE VASODILATOR EFFECT OF VERAPAMIL, NIFEDIPINE AND DILTIAZEM ON ISOLATED PERFUSED CORONARY ARTERIES OF RABBIT

A. H. KAUR, M. SINGH AND J. SINGH

Department of Pharmacology,
Medical College, Rohtak - 124 001

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Summary : In experiments with isolated perfused rabbit heart, nifedipine (1, 2 and 4 μg) produced a dose-dependent increase in coronary outflow ($P<0.01$). On the other hand effects after verapamil and diltiazem were negligible in such doses, though in high doses (10, 20 and 40 μg) they produced a significant ($P<0.01$) increase in coronary outflow. In experiments where noradrenaline (0.1 $\mu g/ml$) was added in the perfusion fluid, nifedipine (1, 2 and 4 μg), as well as verapamil and diltiazem (10, 20 and 40 μg) produced a dose-dependent, significant increase in coronary outflow ($P<0.01$).

Key words : slow calcium channel blockers nifedipine diltiazem coronary dilatation verapamil

INTRODUCTION

Considering the well proven value of calcium antagonists in treatment of angina pectoris, the association of decreased resistance in coronary circulation with diminished cardiac work assumes obvious importance. Calcium antagonists relax vaxcular smooth muscle but the effect in various vessels differs greatly (3, 13, 20). The three most important calcium antagonists now available are verapamil, nifedipine and diltiazem. Although their exact intracellular actions are not as yet fully understood and may differ from one another (1). they share a common mechanism of action of competitively blocking the cell membrane slow channels, resulting in a decreased influx of Ca^{++} into the cells during active state (7). They relieve and prevent coronary artery spasm, increasing total coronary flow and oxygen delivery (2, 6, 17), smooth muscle relaxation being most evident when the muscle is contracted. These drugs dilate both the large and small coronary arteries (9) and several reports indicate that verapamil, nifedipine and diltiazem can dilate collateral arteries too (8, 10, 16).

There is still a lack of good evidence for superiority of a particular calcium antagonist over the others from view point of coronary vasodilator effect. Himori *et al.* (11)

demonstrated that in dog heart, nifedipine was the most potent and diltiazem was the least potent in increasing coronary blood flow. Further, Taira (18) showed the relative potency of nifedipine, verapamil and diltiazem on anterior septal artery of dog as 1:1/12:1/26 respectively. Spasm produced in peripheral blood vessels by noradrenaline and K^+ was inhibited or abolished by verapamil, nifedipine and diltiazem (3, 20).

There have been only a few reports about the comparative effects of verapamil nifedipine and diltiazem on coronary outflow in experiments using the same preparation, particularly, isolated perfused coronary arteries of the rabbit. Comparative effect of these three calcium slow-channel blockers on isolated perfused rabbit coronary arteries is therefore, reported here.

MATERIAL AND METHODS

Rabbits of either sex (1.5 to 2.0 *kg*) were stunned and bled to death. The hearts were isolated and perfused with Ringer-Locke solution at 37°C through the aorta as described by Langendorff & Pfluger (14). Verapamil, nifedipine, or diltiazem, (1, 2 or 4 μg) were injected in the inflow tube to observe the dose-dependent effect on coronary outflow (n=10, for each drug). The effect of higher doses of verapamil and diltiazem (10, 20 and 40 μg) was also studied.

In another series of experiments, using the same experimental set up as above, after noting the coronary outflow, noradrenaline (0.1 $\mu g/ml$) was added into the reservoir solution to make the preparation 'tonic'. Then the effect of different doses of nifedipine (1, 2 and 4 μg), and verapamil and diltiazem (10, 20 and 40 μg) was ascertained again.

Drugs used were nifedipine (Bayer A. G, Germany), verapamil (Boehringer-Knoll, India), diltiazem (Tanabe Seiyaka, Japan) and noradrenaline bitartrate (Nordrin, Unichem, India). Nifedipine stock solution was made by dissolving 10 *mg* of drug in 1.5 *ml* of 70% ethanol and 1.5 g of prewarmed (35°C) polyethylene glycol. Further dilution of the drug and solutions of all other drugs were made fresh in distilled water. Nifedipine solutions were never exposed to light since the drug is unstable in bright light.

RESULTS

Nifedipine, in doses of 1,2 and 4 μg, produced a marked increase in coronary outflow, in a dose-dependent manner. The mean control (n=10) coronary outflow was 6.50 ± 0.29 *ml/min*. Injections of nifedipine (1, 2 and 4 μg) produced a mean coronary outflow 7.23 ± 0.13, 7.84 ± 0.11 and 8.47 ± 0.11 *ml/min* respectively. The increase in coronary outflow was significant ($P<0.01$) at each dose level. On the other

hand similar doses of verapamil and diltiazem produced a slight or insignificant ($P>0.05$) increase in coronary outflow, while in high doses (10, 20 and 40 μg) both verapamil and diltiazem produced a significant ($P<0.01$) increase in coronary outflow (Table I).

TABLE : *Effect of verapamil and diltiazem (10, 20 and 40 μg) on isolated perfused rabbit coronary arteries (n=10)

	Coronary outflow ml/min			
	Control	*After drug*		
	(n=10)	*10 μg*	*20 μg*	*40 μg*
Verapamil	6.1±0.22	6.91±0.14*	7.55±0.16*	8.77±0.25*
Diltiazem	5.9±0.21	6.63±0.19*	7.37±0.17*	8.19±0.27*

*Value significantly differs ($P<0.01$) from control.

In another series of experiments, before making the preparation tonic with noradrenaline, the mean control coronary outflow was 6.61±1.23 *ml/min* and after adding noradrenaline (0.1 μg/ml) in perfusion fluid it was 5.77±0.25 *ml/min*. In this preparation nifedipine (1, 2 and 4 μg) produced a mean coronary outflow 7.43±0.18, 8.02 ±0.20 and 8.73±0.23 *ml/min*, respectively. In the preparations where control coronary outflow was 6.73±0.32 and after adding noradrenaline it was 5.60±0.17 *ml/min*, verapamil (10, 20 and 40 μg) changed the mean coronary outflow to 7.46±0.22, 8.15±0.19 and 9.26±0.14 *ml/min* respectively. In third sets of experiments, the mean control coronary outflow before and after adding noradrenaline was 7.18±0.25 and 5.93±0.16 *ml/min* respectively. Diltiazem (10, 20 and 40 μg) changed the mean outflow to 8.04±0.29 8.55±0.10 and 9.14±0.18 *ml/min*, respectively. The effects of nifedipine, verapamil and diltiazem on coronary outflow were dose-dependent and the increase in coronary outflow was significant ($P<0.01$) at each dose level.

DISCUSSION

In the present study verapamil, nifedipine and diltiazem produced a marked increase in coronary outflow in a dose-dependent manner, though in different doses. The increase in coronary outflow might be due to a direct smooth muscle relaxant effect of these drugs on the coronary blood vessels.

Coronary spasm is due to strong contractions of coronary vascular smooth muscle cells, which is triggered by an increase of intracellular Ca^{++} (17, 21, 22). Several investi-

gators have suggested that the source of calcium involved in the noradrenaline induced contracture might be mainly intracellular (3, 12, 15, 20). The contractile response of isolated smooth muscle to noradrenaline consists of an initial rapid and ensuing slow component (5). The initial phase of noradrenaline-contraction in rabbits aorta was shown to be essentially unaffected by blockade of Ca^{++} entry or removal of extracellular calcium. However, the slow phase seemed to be dependent on a Ca^{++} influx (4). The noradrenaline induced Ca^{++} release was also demonstrated (5) in isolated rings of rabbit aorta preloaded with ^{45}Ca. In our study, nifedipine, verapamil and diltiazem produced a dose-dependent increase in coronary outflow in tonic preparations where noradrenaline was added to perfusion fluid, which indicates that verapamil, nifedipine and diltiazem also inhibit the tonic response of noradrenaline probably by interfering with Ca^{++} at the intracellular sites. Our findings are in agreement with those who reported inhibition of noradrenaline induced contractions in human peripheral arteries and veins (15) and in isolated vascular preparations from different animal species (3, 12, 20). Further, these reports suggest that calcium antagonistic drugs suppress or interfere in the release of Ca^{++} from storage sites or intracellular binding sites, during noradrenaline induced contractions.

Results of our study indicate that dose to dose, nifedipine is a more potent coronary vasodilator than verapamil and diltiazem. These results are in agreement with those of Himori *et al.* (11) and Taira (18) obtained with perfused dog heart. In our study verapamil and diltiazem were found to be equally effective as coronary vasodilators though Himori *et al.* (11) and Taira (18) found diltiazem to be less potent than verapamil. High potency of nifedipine can be due to its high lipid solubility (18) as opposed to that of diltiazem and verapamil, and nifedipine may readily reach intracellular sites so as to interfere with an increase in intracellular calcium concentration (18). These drugs could counter the noradrenaline induced coronary spasm by an inhibitory effect on the release of intracellular Ca^{++} from the storage sites or by affecting the binding of Ca^{++} in the intracellular pools as suggested by other workers (3,12,15,20).

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