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THE INFLUENCE OF THE CALCIUM ANTAGONIST VERAPAMIL ON THE EFFECTS OF BIOGENIC AMINES IN INTACT AND ENDOTHELIUM-FREE BOVINE ABDOMINAL AORTA

MILANKA JEZDIMIROVIĆ*, V. M. VARAGIĆ** and MILICA PROSTRAN***

**Department of Pharmacology, Faculty of Veterinary Medicine, and ***Department of Pharmacology, Faculty of Medicine, Belgrade*

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The calcium antagonist verapamil was found to depress the contractile effects of biogenic amines (noradrenaline, 5-hydroxytryptamine, histamine) on the isolated bovine abdominal aorta. This effect was pronounced both in intact and in endothelium-free preparations. The results indicated that the contractile responses of the bovine abdominal aorta to biogenic amines were calcium-dependent. Nevertheless, the effect of verapamil varied from one biogenic amine to the other. The conctractile responses of the abdominal aorta to noradrenaline and to histamine were practically identical in intact and endothelium-free preparations. However, the effects of 5-hydroxytryptamine were more strongly inhibited by verapamil than the effects of the two other biogenic amines. Thus, the effect of 5-hydroxytryptamine was almost completely blocked by verapamil in endothelium free kovine abdominal aorta. The effects of verapamil were highly specific because they were easily reversed by increasing the extracellular concentration of calcium.

Key words: endothelium, abdominal aorta, verapamil, noradrenaline, 5-hydroxytryptamine, histamine.

INTRODUCTION

The stimulant effect of noradrenaline on smooth muscle is known to require sodium, chloride and potassium ions (Bolton, 1979). For the action of noradrenaline on alpha-adrenoceptors Ca^{++} ion is also necessary (Bülbring and Tomita, 1977). On the other hand, production of the endothelium-derived relaxing factor (EDRF), by both receptor-dependent and receptor-independent stimulation, depends on the concentration of extracellular calcium (Lückhoff et al., 1988). Presumably, calcium might be implicated in the modulatory role of endothelium on the responses of vascular smooth muscle to biogenic amines. It was therefore of interest to investigate the action of verapamil, a calcium antagonist, on the effects of biogenic amines in intact and endothelium-free isolated bovine abdominal aorta.

MATERIALS AND METHODS

Portions of the abdominal region of the aorta were obtained from the local abattoir. The aorta was dissected always from the same anatomical region between the last thoracic and the second lumbar vertebra. The prepared rings of the aorta were 0.5 cm wide.

The preparations of isolated bovine abdominal aorta were arranged in an isolated organ bath of 20 ml capacity. The bathing medium was of the following composition (in $mmol.l^{-1}$): NaCl 136.7, KCl 2.81, $CaCl_2$ 1.8, $MgCl_2$ 0.105, NaH_2PO_4 0.417, $NaHCO_3$ 11.9 and glusose 11.1. The medium was aerated with a mixture of oxygen (95%) and carbon dioxide (5%).

When dissecting the aorta particular care was taken not to produce any lesion to the endothelium. If and when necessary, endothelium was removed by rubbing it off with a wooden rod. The temperature of the bath was 37^0C.

The isometric contractions of the isolated aortal rings were recorded by a microdisplacement myograph transducer (F-50, Narco Bio-Systems). The initial preloading of the preparation was 5 g.

The following substances were used: noradrenaline hydrochloride (Serva), 5-hydroxytryptamine-creatine-sulphate (BDH Biochemicals), histamine hydrochloride (Serva) and verapamil (Isoptin, Lek).

Statistical evaluation was made using Student's t-test.

For details see Jezdimirović et al. (1992_a, 1992_b).

RESULTS

The effects of noradrenaline and verapamil in intact and endothelium-free bovine abdominal aorta. – Verapamil ($2.2x10^{-8}$ $mol.l^{-1}$) produced a depression of the contractile responses of the isolated bovine abdominal aorta to increasing concentrations of noradrenaline. This effect of verapamil was pronounced both in intact and in endothelium-free preparations. The result of this depression was that concentration-response curves for noradrenaline were shifted to the right, as shown in Figure 1.)

The effects of 5-hydroxytryptamine and verapamil in intact and endothelium-free bovine abdominal aorta. – In this series of experiments verapamil ($2.2x10^{-8}$ $mol.l^{-1}$) produced a highly significant depression of the contractile effect of 5-hydroxytryptamine on the isolated bovine abdominal aorta. The depressive effect of verapamil was pronounced both in intact and endothelium-free abdominal aorta, but in endothelium-free preparations the response to 5-hydroxytryptamine was almost completely blocked, as shown in Figure 2.

The effects of histamine and verapamil in intact and endothelium-free bovine abdominal aorta. – The same concentration of verapamil ($2.2x10^{-8}$ $mol.l^{-1}$) significantly reduced the effects of histamine on the isolated bovine abdominal aorta, both in intact and endothelium-free preparations. The concentration-response curves for histamine were shifted to the right by verapamil. It should be pointed out that the effect of verapamil was almost equally pronounced both in intact and endothelium-free preparations, as shown in Figure 3.

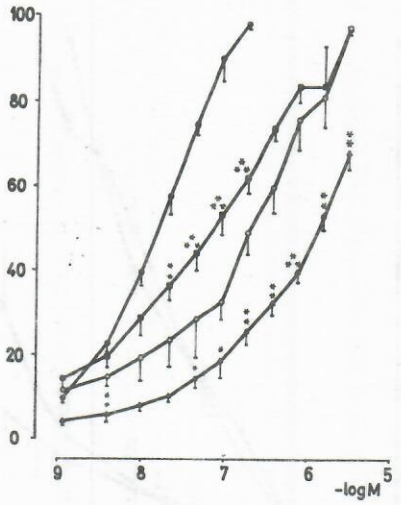

Figure 1. Concentration-response curves for increasing concentrations of noradrenaline alone (●) and of noradrenaline in the presence of verapamil (■), in intact (●■) and in endothelium-free preparations (OΔ) of the isolated bovine abdominal aorta. Abscissa: concentrations of noradrenaline in the bath. Ordinate: percentage increase of the isometric contraction of the isolated bovine abdominal aorta (xP<0.05; xxP<0.01; xxxP<0.001). Every point is the mean value of 6 experiments ± s. e. m.

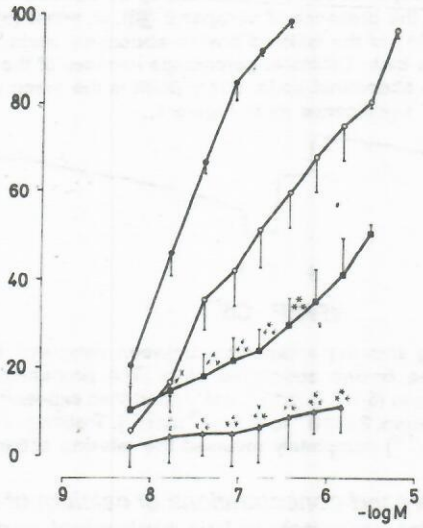

Figure 2. Concentration-response curves for increasing concentrations of 5-hydroxytryptamine alone (●) and of 5-hydroxytryptamine in the presence of verapamil (■), in intact (●■) and in endothelium-free preparations (OΔ) of the the isolated bovine abdominal aorta. Abscissa: concentrations of 5-hydroxytryptamine in the bath. Ordinate: percentage increase of the isometric contraction of the isolated bovine abdominal aorta. Every point is the mean value of 6 experiments ± s. e. m. Statistical significance as in Figure 1.

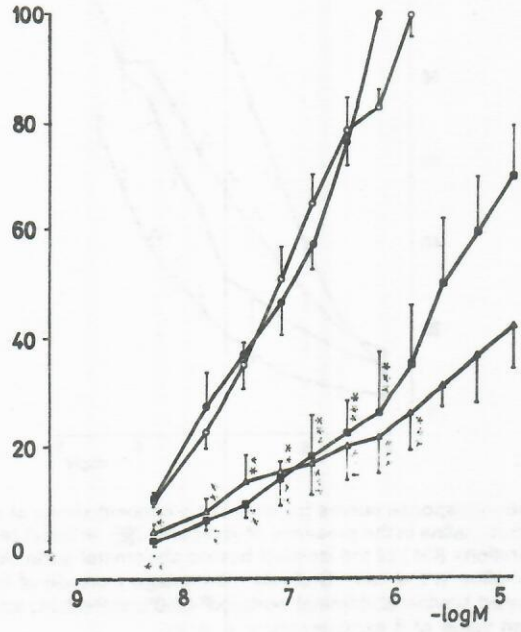

Figure 3. Concentration-response curves for increasing concentrations of histamine alone (●) and of histamine in the presence of verapamil (■), in intact (●■) and in endothelium-free preparations (OΔ) of the isolated bovine abdominal aorta. Abscissa: concentrations of histamine in the bath. Ordinate: percentage increase of the isometric contraction of the isolated bovine abdominal aorta. Every point is the mean value of 6 experiments ± s. e. m. Statistical significance as in Figure 1.

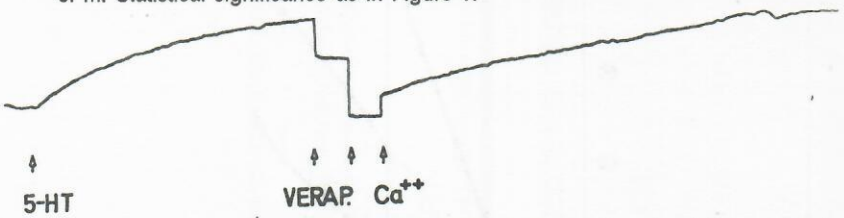

Figure 4. Original tracing showing antagonism between verapamil and calcium on the isolated endothelium-free bovine abdominal aorta. The preparation was precontracted by 5-hydroxytryptamine (5-HT, 5.7×10^{-8} mol.l^{-1}) and then exposed to increasing concentrations of verapamil (Verap, 2.2×10^{-8} to 4.3×10^{-8} mol.l^{-1}). Trebling of the concentration of calcium in the bath (Ca^{++}) completely reversed the relaxing action of verapamil.

Reversal by increased concentrations of calcium of the effect of verapamil. – In preparations of the endothelium-free abdominal aorta, the increasing concentrations of verapamil (2.2×10^{-8} to 4.3×10^{-8} mol.l^{-1}) produced relaxation of the aorta which was precontracted with 5-hydroxytryptamine (5.7×10^{-8} ml.l^{-1}). This effect of verapamil was easily reversed by trebling the concentration of calcium in the medium, as shown in Figure 4. The same results were obtained with noradrenaline and histamine (not shown in the Figure).

DISCUSSION

It was found in the present experiments that verapamil, a calcium antagonist, depressed the contractile responses of the isolated bovine abdominal aorta to all biogenic amines used (noradrenaline, 5-hydroxytryptamine, histamine). This effect was pronounced both in intact and endothelium-free preparations. This indicates that the contractile responses of the bovine abdominal aorta to biogenic amines are calcium-dependent. In general terms, this finding is in accordance with previous results indicating extracellular calcium dependence of contraction and endothelium-dependent relaxation of the aorta and its branches (Tayo and Bevan, 1987).

It is considered that spontaneously released endothelium-derived relaxing factor is a functional antagonist to noradrenaline-induced contractions presumably by reducing the stimulated influx of extracellular calcium (Auch-Schwelk and Vanhoutte, 1991). Our experiments with verapamil indicate that the contractile effects of noradrenaline, both in intact and endothelium-free preparations, are equally dependent on extracellular calcium.

The effect of verapamil varied from one biogenic amine to the other. The contractile responses of the aorta to noradrenaline were almost identical in intact and endothelium-free preparations. The same holds true for histamine. Meanwhile, the effects of 5-hydroxytryptamine were strongly inhibited by verapamil, more than the effects of the other two biogenic amines. 5-hydroxytryptamine has been found to stimulate calcium uptake by human umbilical vein endothelial cells. This action takes place at the level of the plasma membrane rather than in intracellular stores of calcium (Gill et al., 1992).

In the isolated mesenteric and femoral arteries of the rat the contractile responses to 5-hydroxytryptamine were potentiated after removal of the endothelium. Still, the response to neuronally released 5-hydroxytryptamine was not affected by endothelium (Urabe et al., 1991). An enhanced arterial constrictor response to 5-hydroxytryptamine has been demonstrated following endothelial damage in vivo, suggesting that endothelial injury or dysfunction may play a role in the pathophysiology of arterial spasm (Orlandi et al., 1990). On the other hand, verapamil is an inhibitor of 5-hydroxytryptamine uptake in a variety of cell types (Hill et al., 1990).

In our experiments the contractile response of the aorta to 5-hydroxytryptamine was almost completely blocked in endothelium-free preparations. Evidently, in comparison with noradrenaline and histamine, 5-hydroxytryptamine is much more dependent on the extracellular calcium concentration in order to produce a contractile response in the isolated bovine abdominal aorta.

The observed effects of verapamil on contractile effects of biogenic amines were specific, because they were easily reversed by increasing the extracellular calcium concentration in the bath.

Our results also indicate the possible significance of different distribution of the specific receptors for biogenic amines between endothelium and the vascular smooth muscle. This is particularly evident in the case of 5-hydroxytryp-

tamine. The effect of this amine is almost completely blocked in endothelium-free preparations, thus indicating the predominant distribution of 5-HT receptors, dependent on calcium channels, in the endothelium of the aorta.

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DEJSTVO KALCIJUMSKOG ANTAGONISTA VERAPAMILA NA EFEKTE BIOGENIH AMINA NA IZOLOVANU GOVEĐU ABDOMINALNU AORTU SA I BEZ ENDOTELA

MILANKA JEZDIMIROVIĆ, V. M. VARAGIĆ i MILICA PROSTRAN

SADRŽAJ

Kalcijumski antagonist verapamil deprimira kontraktilne efekte biogenih amina (noradrenalin, 5-hidroksitriptamin, histamin) na izolovanoj goveđoj abdominalnoj aorti. Ovaj efekt postoji kako na intaktnoj aorti, tako i na onoj sa

koje je uklonjen endotel. Dobijeni nalazi pokazuju da je kalcijum neophodan za kontraktilne efekte biogenih amina na abdominalnoj goveđoj aorti. Ipak, u ovom pogledu ima razlike među ispitivanim biogenim aminima. Kontraktilni efekti noradrenalina i histamina su skoro identični na intaktnoj i deendotelijalizovanoj aorti. Međutim, efekti 5-hidroksitriptamina su visoko značajno smanjeni pod dejstvom verapamila. Dejstvo ovog amina na deendotelijalizovanim preparatima je skoro kompletno blokirano verapamilom. Opisani efekti verapamila su specifični jer se vrlo lako mogu isključiti povećanjem koncentracije kalcijuma u hranljivom medijumu.