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DIFFERENTIAL EFFECTS OF THE CALCIUM ANTAGONIST, VERAPAMIL, ON LUMEN SIZES OF TERMINAL ARTERIOLES AND MUSCULAR VENULES IN THE RAT MESENTERIC, PIAL AND SKELETAL MUSCLE MICROVASCULATURES

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The actions of the local perivascular application of verapamil to rat mesenteric, pial and cremaster muscle arterioles and muscular venules (15 to 35 μm i.d.), was examined *in situ*, at the microcirculatory level, by use of a high-resolution closed circuit television microscope recording system. Local application of verapamil (1 to 100 μg) to cremaster muscle arterioles and venules of the rat induces dose-dependent vasodilatation and increased perfusion of capillaries. However, neither arteriolar nor muscular venular lumen sizes and capillary blood flow of the rat mesenteric or pial vasculatures were altered by even high doses of verapamil. Although these direct *in situ* microvascular findings do indicate that verapamil can induced dilatation of microscopic resistance and capacitance vessels in skeletal muscle, our data do not support the concept that verapamil induces non-specific peripheral vasodilatation.

Introduction Calcium (Ca) antagonists, such as verapamil, have been reported to induce relaxation of smooth muscle which is not thought to be mediated by any specific action on receptor sites (Fleckenstein, 1977; Church & Zsotér, 1980). In addition, it has been suggested that Ca antagonists increase blood flow in a number of organ regions, including the mesenteric, femoral, renal and coronary vasculatures, by a direct action on vascular tone by inhibiting Ca^{2+} influx across the vascular smooth muscle cell membranes (Yamagushi, Ikezewa, Takada & Kiyomoto, 1974; Fleckenstein, 1977; Zsotér, 1980). Some workers believe that these Ca antagonists, surprisingly, do not exert any venodilator actions (Zsotér, 1980). Such information has prompted clinical studies with the use of Ca antagonists in the treatment of a variety of cardiovascular disorders, including angina pectoris, arrhythmias, hypertension, and ischaemic heart disease (Sandler, Clayton & Thronicroft, 1968; Schamroth, Krikler & Garrett, 1972; Livesley, Catley, Campbell & Oram, 1973; Hosada & Kimura, 1976; Heupler & Proudfit, 1979; Zsotér, 1980). To our knowledge, there are no direct, *in situ* studies on regional microvasculatures which indicate that Ca antagonists exert direct dilator actions on either terminal arterioles or muscular venules.

By use of direct, *in situ* high resolution microscopy (at magnifications up to 3000 ×) of regional vasculatures of the anaesthetized rat, we have shown that perivascular application of verapamil increases lumen sizes and blood flow in skeletal muscle terminal arterioles and muscular venules in a concentration-dependent manner. However, perivascular verapamil does not alter arteriolar or venular lumen sizes in either the mesenteric or pial microvasculatures.

Methods Male rats (Wistar strain, 120 to 180 g) were lightly anaesthetized with intramuscular pentobarbitone sodium (Nembutal, 25 mg/kg). After induction of anaesthesia, tracheostomies were performed, and catheters were placed in femoral arteries for blood gas (Po_2, Pco_2), pH and blood pressure determinations. *In vivo* quantitative microscopic observations (up to 3,500 ×) were carried out on terminal arterioles and muscular venules in mesentery, cremaster muscle and pial vasculatures by means of an image-splitting television microscope recording system, similar to that described previously for microvessels (Altura, 1971; Altura & Altura, 1974). The rat mesentery, cremaster muscle and pial vasculatures were prepared and kept under physiological conditions according to procedures described previously (Altura, 1971; 1978; Baez, 1973; Altura & Altura, 1974; Altura, Gebrewold & Lassoff, 1980). The mesenteric and cremaster tissues were superfused with a Ringer-gelatin bicarbonate-buffered solution (Altura, 1971), whereas artificial cerebral spinal fluid (CSF) (composition in meq/l: Na^+ 155, Cl^- 137, HCO_3^- 21, K^+ 3.5, Mg^{2+} 1.3, Ca^{2+} 2.2 and glucose 6) was superfused on the pial vasculature (Altura *et al.*, 1980). The Ringer and CSF solutions were maintained at temperatures between 36 to 37.5°C and at a pH of 7.3 to 7.4. The exposed mesenteric, cremaster muscle and brain surface temperatures were kept close to 37.5°C and measured with thermistor probes.

The reactivity of selected arterioles was tested before and after verapamil (Knoll A.G. Ludwigshafen, F.R.G.) by local application of 0.1 ml of a 5% $BaCl_2$ solution. Each dose of verapamil was applied perivascularly for at least 5 to 10 min periods. Arterioles and muscular venules which failed to yield a 30 to 40%

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constriction in response to the standard dose of barium were not used in this study. Verapamil (made up in either Ringer solution or artificial CSF) was applied locally (0.1 to 100 μg) to the vessels in 0.1 ml volumes. Periodically throughout the experiments, plain Ringer solution or artificial CSF in 0.1 ml volumes was also tested for vasoactivity. Reapplication of the test dose of $BaCl_2$ at the end of the experiments, insured that the microvessels had been reactive throughout the testing of verapamil. Systolic blood pressure was measured periodically throughout the experiments (average mean = 115 mmHg). At selected intervals, arterial blood samples were obtained for pH, P_{O_2} and P_{CO_2} measurements. The arterial blood gas values were always found to be normocapnic throughout the experiments (mean P_{CO_2} = 30.2 mmHg). Paired *t* tests were used for statistical analysis of the differences between mean values (±s.e. mean) before and after verapamil and $BaCl_2$ application.

Results The perivascular application of either Ringer solution or of the artificial CSF failed to exert any significant effect on the diameters of arterioles and muscular venules in the mesenteric, cremaster muscle or pial microvasculatures. Table 1 indicates that, irrespective of the dose applied perivascularly, none of the mesenteric or pial arterioles and muscular venules demonstrated any significant alteration in response to the verapamil. Perivascular application of verapamil to the cremaster muscle vasculature did result in a dose-dependent increase (i.e., 7 to 24%) in arteriolar and venular lumen sizes; numerous 'true' capillaries (3 to 5 μm) previously devoid of blood flow now simultaneously exhibited active, rapid blood flow. Although not shown, it should be noted that even though application of 100 μg of verapamil did not alter arteriolar and venular lumen sizes in the mesenteric or pial vasculatures, by itself, this dose of verapamil could inhibit completely constrictions of these microvessels induced by 1% $BaCl_2$.

Table 1. Influence of verapamil on mesenteric, pial and cremaster muscle terminal arteriolar and muscular venular diameters in intact rats

		Arterioles			*Muscular venules*		
Vasculature, test agent	*Dose*	*Control diameter size* (μm)	*Diameter size after test agent* (μm)	*% change**	*Control diameter size* (μm)	*Diameter size after test agent* (μm)	*% change**
Mesenteric							
$BaCl_2$, 5% (initial)		18.2 ± 0.9	8.0 ± 0	−56**	27.2 ± 0.3	9.3 ± 0.8	−66**
Verapamil	10 μg	15.7 ± 1.6	17.3 ± 1.4	+10	27.2 ± 1.9	28.6 ± 1.7	+5
	100 μg	16.6 ± 1.6	16.2 ± 2.1	−2	25.3 ± 1.1	23.8 ± 3.8	−6
$BaCl_2$, 5% (final)		16.8 ± 0.8	8.9 ± 0.9	−47**	25.2 ± 0.9	10.1 ± 0.9	−60**
Pial							
$BaCl_2$, 5% (initial)		29.8 ± 0.8	16.3 ± 0.7	−45**	26.8 ± 1.6	16.0 ± 0.6	−40**
Verapamil	10 μg	27.3 ± 2.1	29.1 ± 2.8	+6	25.3 ± 2.0	25.3 ± 2.3	0
	100 μg	27.2 ± 1.1	26.8 ± 4.6	−1	26.1 ± 3.8	26.1 ± 3.8	0
$BaCl_2$, 5% (final)		27.3 ± 1.2	15.5 ± 1.5	−43**	26.0 ± 1.7	16.5 ± 0.9	−37**
Cremaster							
$BaCl_2$, 5% (initial)		16.6 ± 0.6	7.8 ± 0.5	−53**	18.8 ± 0.6	10.0 ± 0.1	−47**
Verapamil	1.0 μg	16.3 ± 0.6	16.8 ± 0.6	+3	19.2 ± 0.3	20.5 ± 0.3	+7**
	10 μg	15.6 ± 0.6	18.4 ± 0.9	+18**	19.4 ± 0.2	22.2 ± 0.8	+14**
	100 μg	16.6 ± 0.4	20.6 ± 2.2	+24**	19.6 ± 0.3	23.2 ± 1.1	+18**
$BaCl_2$, 5% (final)		16.6 ± 0.5	8.0 ± 0.6	−52**	19.5 ± 0.5	10.6 ± 0.3	−46**

Values are given as means ± s.e. mean; n = 20 different rats.
* Minus sign signifies vasoconstriction; plus sign signifies vasodilatation.
** Significantly different from control ($P < 0.01$).

Discussion These direct, *in situ* microscopic observations clearly show that verapamil can, dose-dependently, dilate both arterioles and muscular venules in the skeletal muscle microvasculature. However, despite the ability of the Ca antagonist to dilate resistance and capicitance microvessels in the muscle circulation, even high doses of verapamil could not influence either arteriolar or venular lumen sizes and blood flow in the mesenteric and pial (cerebral) vasculatures. In view of our observations, it is difficult to conclude that verapamil is a non-specific peripheral vasodilator (Fleckenstein, 1977). It is, however, possible that since (1) skeletal muscle constitutes more than 40% of the body weight of mammals, and (2) 90 to 95% of skeletal muscle, arterioles and capillaries are normally closed at any one moment, the hypotensive action of verapamil (Fleckenstein, 1977; Zsotér, 1980) may be due to a primary action on skeletal muscle arterioles. This notion lends support to the observed potent femoral arterial dilator action exhibited by verapamil after systemic injection into intact animals (Ono & Hashimoto, 1979).

The failure of our experiments to reveal either an arteriolar or venular dilator action for the perivascular application of verapamil in mesenteric and pial vasculatures would seem to question the concept that verapamil acts as a peripheral vasodilator solely by inhibiting Ca^{2+} influx across vascular smooth muscle cell membranes (Fleckenstein, 1977). It is of important, additional interest to note that our direct microscopic observations reveal that verapamil, contrary to Zsotér's (1980) suggestion, can cause potent and dose-dependent venodilatation, at least in skeletal muscle capacitance microvessels. In view of these new findings, it will be necessary to examine carefully the *in situ* microvascular actions of other so-called Ca antagonists.

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References

ALTURA, B.M. (1971). Chemical and humoral regulation of blood flow through the precapillary sphincter. *Microvasc. Res.* **3,** 361–384.

ALTURA, B.M. (1978). Pharmacology of venular smooth muscle. New insights. *Microvasc. Res.*, **16,** 91–117.

ALTURA, B.M. & ALTURA, B.T. (1974). Peripheral vascular actions of glucocorticoids and their relationship to protection in circulatory shock. *J. Pharmac. exp. Ther.* **190,** 300–315.

ALTURA, B.M., GEBREWOLD, A. & LASSOFF, S. (1980). Biphasic responsiveness of rat pial arterioles to dopamine: direct observations on the microcirculation. *Br. J. Pharmac.*, **69,** 543–544.

BAEZ, S. (1973). An open cremaster muscle preparation for the study of blood vessels by in vivo microscopy. *Microvasc. Res.* **5,** 384–394.

CHURCH, J. & ZSOTÉR, T.T. (1980). Calcium antagonists. Mechanism of action. *Canad. J. Physiol. Pharmac.*, **58,** 254–264.

FLECKENSTEIN, A. (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. *A. Rev. Pharmac. Tox.* **17,** 149–166.

HEUPLER, F.A. & PROUDFIT, W.C. (1979). Nifedipine therapy for refractory coronary arterial spasm. *Am. J. Cardiol.*, **44,** 798–803.

HOSADA, S. & KIMURE, E. (1976). Efficacy of nifedepine in the variant form of angina pectoris. In *Third International Adalat Sympsoium*, Rio de Janeiro. ed. Jatene, A.D. & Lichtlen, P.R. pp. 195–199. Amsterdam: Excerpta Medica.

LIVESLEY, B., CATLEY, P.F., CAMPBELL, R.C. & ORAM, S. (1973). Double-blind evaluation of verapamil, propranolol and isosorbide dinitrate against a placebo in the treatment of angina pectoris. *Br. med. J.*, **i,** 375–378.

ONO, H. & HASHIMOTO, K. (1979). Ca^{2+} antagonism in various parameters of cardiac function including coronary dilatation with the use of nifedipine, perhexiline, and verapamil. In *Ischemic Myocardium and Antianginal Drugs.* ed. Winbury, M.M. & Abiko, Y. pp. 77–88. New York: Raven Press.

SANDLER, G., CLAYTON, G.A. & THORNICROFT, S.G. (1968). Clinical evaluation of verapamil in angina pectoris. *Br. med. J.* **iii,** 224–227.

SCHAMROTH, L. KRIKLER, D.M. & GARRETT, G. (1972). Immediate effects of intravenous verapamil in cardiac arrhythmias. *Br. med. J.* **i,** 660–662.

YAMAGUSHI, I., IKEZEWA, K., TAKADA, T. & KIYOMOTO, A. (1974). Studies on a new 1,5-benzothiazepine derivative (CRD-401). Effects of renal blood flow and renal function. *Jap. J. Pharmac.*, **24,** 511–522.

ZSOTÉR, T.T. (1980). Calcium antagonists. *Am. Heart J.* **99,** 805–810.

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