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EFFECTS OF VERAPAMIL AND MANGANESE ON THE VASOCONSTRICTOR RESPONSES TO NORADRENALINE, SEROTONIN AND POTASSIUM IN HUMAN AND GOAT CEREBRAL ARTERIES

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Abstract—The effects of verapamil and manganese (Mn^{2+}) on the noradrenaline (NA), serotonin (5-HT) and potassium (K^+)-induced contractions were studied in human and goat cerebral arteries. Verapamil and Mn^{2+} relaxed both kinds of cerebral vessels previously contracted with 10^{-5} M NA, 10^{-5} M 5-HT and 75 mM K^+. The ID_{50} (50% inhibition of maximum contraction) was around 10^{-7} M for the organic antagonist and 10^{-3} M for the inorganic one. The ID_{50} for the Ca^{2+} antagonists in K^+-induced contractions was smaller than that for NA and 5-HT-evoked contractions. Preincubation of segments with verapamil (10^{-6} M) or Mn^{2+} (2×10^{-3} or 5×10^{-3} M) caused inhibition of the contractions evoked by the three agents that was greater in the case of K^+. The inhibitory effects of verapamil were reversed by adding Ca^{2+} to the bath. The removal of Ca^{2+} from the extracellular medium reduced the contractions elicited by the three vasoconstrictor agents in both cerebral blood vessels. This reduction was greater for K^+ than for the other two. These results indicate that both cerebral vessels are very susceptible to Ca^{2+} omission and to Ca^{2+} entry blockers such as verapamil and Mn^{2+}, which could be of interest to treat cerebral vasospasm.

Calcium ions play an important role in the control of contraction of smooth muscle [1]. This contraction is initiated by several stimuli that increase the intracellular concentration of free Ca^{2+} which activates the contractile proteins [1–3]. It has been reported that two essential sources of activator Ca^{2+} exist: the extracellular pool of Ca^{2+} and that sequestered mainly in the sarcoplasmic reticulum and mitochondria [1,4,5]. Some vasoconstrictor agents used both sources of Ca^{2+} differently [1,2].

Calcium antagonists, such as verapamil, are used to treat a variety of cardiovascular disorders, which include arrhythmias, angina pectoris, hypertension, etc. [6]. These agents induced relaxation of vascular muscle due to transmembrane blockade of Ca^{2+} influx [1,7]. Recently it has been reported that cerebral vessels from different animals are sensitive to the effects of some verapamil-type vasodilators [8,9]. Furthermore, these agents induce considerable inhibition of cerebral [10] and coronary vasospasm [11]. In spite of these interesting results, to our knowledge little is known about effects of Ca^{2+} antagonists in human cerebral arteries. Therefore, the present study was undertaken to investigate the effect of two calcium antagonists, verapamil and manganese (Mn^{2+}), on the noradrenaline (NA), serotonin (5-HT) and potassium (K^+)-induced contractions in human cerebral arteries. Also the effects of suppression of Ca^{2+} from the medium on the contractile responses evoked by these three agents were studied. For comparative purposes the same experiments were designed for goat cerebral arteries.

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MATERIALS AND METHODS

Cerebral arteries and recording systems. The goat brain arteries were obtained from female goats (25–40 kg) which had been killed by injecting 30 ml of a saturated solution of potassium chloride i.v. The brain was removed and the middle cerebral arteries were dissected out.

The human cerebral arteries were obtained from adult individuals 4–6 hr after death. The cause of decease was acute myocardial infarctions in four cases and infection of the small bowel in one case. The dissected arteries were small branches of middle cerebral arteries of a similar size to goat middle cerebral arteries.

The vessels were cut into cylindrical segments 4 mm in length. Each cylinder was set up in an organ bath containing 6 ml of Krebs–Henseleit solution (KHS) at 37° through which a 95% O_2–5% CO_2 mixture provided a pH of 7.4–7.5. Two stainless-steel pins, 150 μm diameter, were introduced through the lumen of each segment. One pin was fixed to the bath wall, the other, connected to a strain gauge for isometric recording, was in a parallel position but movable, thus permitting the application of resting tension in a perpendicular plane to the long axis of the vascular cylinder.

The recording system included a force–displacement transducer (Grass FTO3c) connected to a poligraph (Grass Model 7D). The cylindrical segments were submitted to different resting tensions, from 0.3 to 1.5 g, and 1 g was selected because it produced the maximal contraction with the vasoconstrictor agents used. This tension was readjusted every 15 min during a 90–120 min equilibration

period during which the basal tension became stable. This tension was readjusted throughout the experiment. Contractile responses were then evoked by NA, 5-HT or K^+.

Analysis of the effects of the drugs. NA (10^{-5} M), 5-HT (10^{-5} M) and K^+ (75 mM) induced maximal contractions in goat and human cerebral arteries. When the vasoconstriction elicited by these drugs reached a stable plateau either verapamil (2×10^{-8} to 10^{-6} M) or Mn^{2+} (10^{-4} to 5×10^{-3} M) was added in a cumulative manner to the bath; i.e. once a dose of verapamil or Mn^{2+} had elicited its maximal relaxing effect the next dose was added. At the end of the dose–response curve the bath medium was changed several times during 20 min until the base line was recovered. Again, NA, 5-HT or $K^+$was administered to test the endurance of the verapamil and Mn^{2+} effects. Since the relaxing response to Mn^{2+} had disappeared after this washing period, the same arterial segments were used to analyze the effects of Mn^{2+} on the contraction induced by NA, 5-HT and K^+. To achieve this, Mn^{2+} (2×10^{-3} or 5×10^{-3} M) was added 10 min before the contractile agents. As the effect of verapamil did not disappear after washing, the action of 10^{-6}M of this drug on the vasoconstriction induced by NA, 5-HT and K^+ was studied in different arterial segments following an identical procedure as indicated for Mn^{2+}.

Solutions, drug and statistical evaluation. The composition of the KHS was (mM): NaCl, 115; KCl, 4.6; $CaCl_2$, 2.5; KH_2PO_4,1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$, 25; glucose, 11.1; disodium salt of ethylenediamine tetraacetic acid (Na_2EDTA), 0.03. The solution for K^+-depolarization contained 75 mM KCl and only 45 mM of NaCl in order to maintain the osmolarity. Ca^{2+}-free KHS was prepared by omitting $CaCl_2$ and 1 mM ethyleneglycol-bis(beta-aminoethyl ether)*N,N*-tetraacetic acid (EGTA) was added to reduce contaminating Ca^{2+}. These solutions were prepared on the day of use and the chemicals were of analytical grade.

NA, 5-HT and verapamil hydrochloride were prepared as stock solutions in physiological saline containing 0.01% (m/v) ascorbic acid, and were kept frozen.

The drugs used were: noradrenaline bitartrate (Sigma), 5-hydroxytryptamine creatinine sulphate (Sigma), potassium chloride (Merck), manganous chloride (Merck) and verapamil hydrochloride (Knoll AG).

Statistical significance was evaluated by Student's *t*-test for paired or independent experiments and P values of 0.05 or less were considered to be significant. The inhibitory effects of verapamil and Mn^{2+} on the contraction caused by the drugs were expressed as a percentage of the response caused by the agents in the absence of verapamil or Mn^{2+}. Fifty per cent inhibitory doses (ID_{50}) were estimated according to Fleming *et al.* [12].

RESULTS

Relaxant effects of verapamil and Mn^{2+} on NA, 5-HT and K^+ evoked contractions

Human and goat cerebral arteries previously contracted with 10^{-5} M NA, 10^{-5} M 5-HT and 75 mM

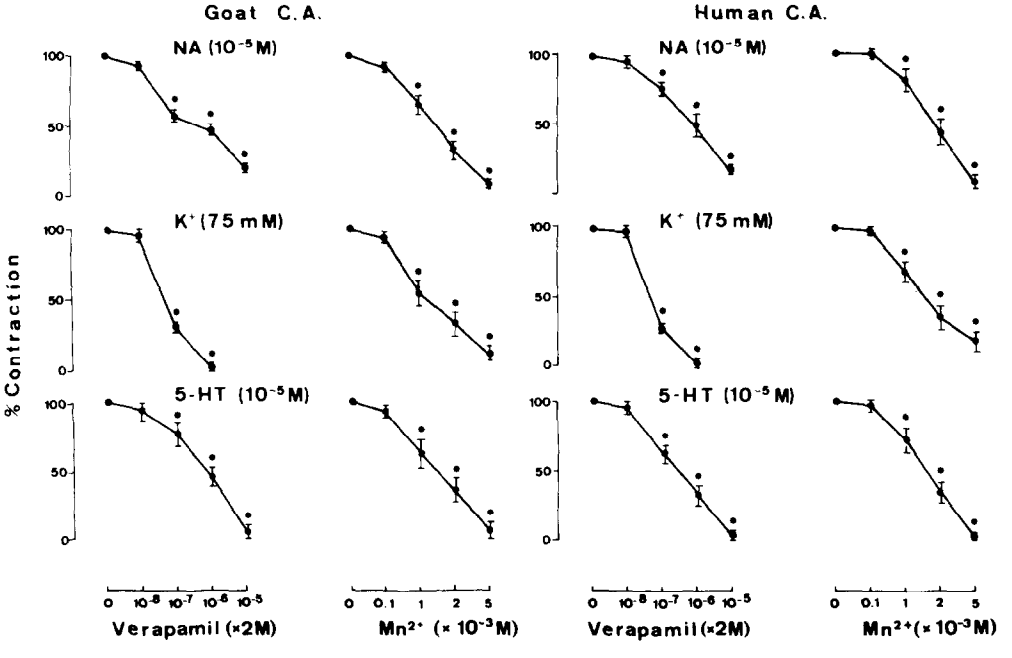

Fig. 1. Vasodilator effects of verapamil and Mn^{2+} on NA-, 5-HT- and K^+-induced contractions in segments of human and goat cerebral arteries. After the height of the contraction evoked by these drugs was obtained, verapamil and Mn^{2+} were added cumulatively. Values are mean ± S.E.M.; five to seven different arterial segments were used. *P < 0.05 or less.

Table 1. Concentrations of verapamil and Mn^{2+} producing 50% inhibition of contraction of 10^{-5} M NA, 10^{-5} M 5-HT and 75 mM K^+ in human and goat cerebral arteries

	ID_{50} (M)*		
	Mn^{2+}	Verapamil	ID_{50} Mn^{2+}/ID_{50} verap.†
		Human C.A.	
NA	1.7×10^{-3} (4) (1.28–2.45)	7.2×10^{-7} (4) (2.5–19)	2.3×10^{-3}
5-HT	1.51×10^{-3} (4) (0.57–3.9)	3.1×10^{-7} (3) (2.05–4.48)	4.8×10^{-3}
K^+	1.21×10^{-3} (4) (0.7–20.3)	1.24×10^{-7} (3) (0.62–2.3)	9.7×10^{-3}
		Goat C.A.	
NA	1.42×10^{-3} (4) (1.23–1.69)	1.78×10^{-6} (6) (1.34–2.3)	0.79×10^{-3}
5-HT	1.6×10^{-3} (4) (0.7–3.6)	9.5×10^{-7} (7) (3.12–28)	1.6×10^{-3}
K^+	9.2×10^{-4} (14) (6.9–12.5)	1.03×10^{-7} (3) (0.78–1.33)	8.9×10^{-3}

* Values are geometric mean ID_{50} with 95% confidence interval. The number of experiments is in parentheses.
† Ratio of potencies.

K^+ showed vasodilator responses when verapamil (2×10^{-8} to 2×10^{-5} M) was added to the bath (Fig. 1). Verapamil (10^{-6} M)practically abolished the contraction evoked by K^+, whereas it needed to reach a concentration of 10^{-5} M, in order to annul the vasoconstrictions elicited by NA and 5-HT.

The vasodilator responses caused by Mn^{2+} (10^{-4} to 5×10^{-3} M) under the same conditions as verapamil are depicted in Fig. 1. The relaxation became significant from 10^{-3} M Mn^{2+} in human and goat cerebral arteries contracted with the three agents.

The values of the concentrations of Mn^{2+} which

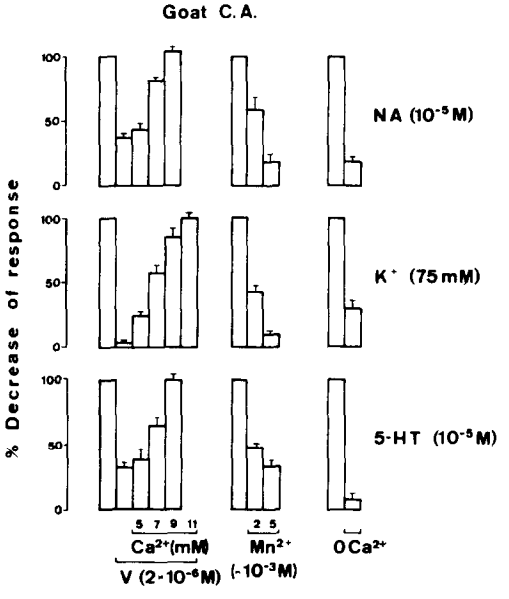

Fig. 2. Inhibition of the contractions evoked by NA, 5-HT and K^+ in goat cerebral arteries (CA) by (i) verapamil (V, 10 min preincubation); (ii) Mn^{2+} (10 min preincubation); (iii) removal of Ca^{2+} from the medium; and the effect of addition to the bath of Ca^{2+} to obtain concentrations from 5 to 11 mM. Mean 100% isometric responses for NA, 5-HT and K^+ were (in mg): 550.88 ± 75.75; 1605 ± 111; and 1450 ± 293.9, respectively. Values are mean ± S.E.M.; five to seven segments were used for each agonist and Ca^{2+} antagonist used. *$P < 0.05$ or less.

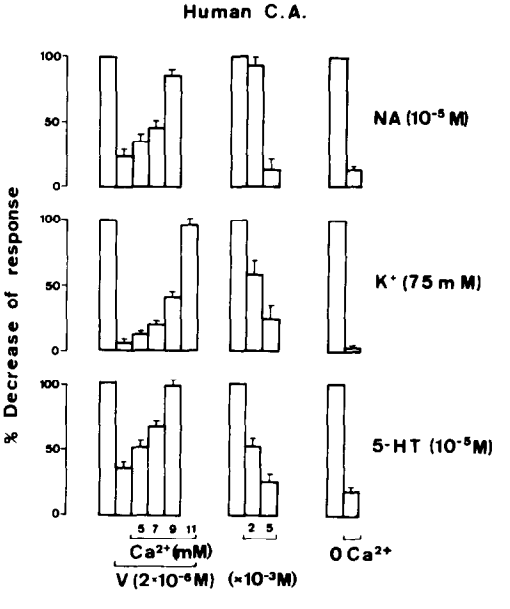

Fig. 3. Inhibition of the contractions evoked by NA, 5-HT and K^+ in human ccrebral arteries (CA) by (i) verapamil (V, 10 min preincubation); (ii) Mn^{2+} (10 min preincubation); (iii) removal of Ca^{2+} from the medium; and the effect of addition to the bath of Ca^{2+} to obtain a cation concentration from 5 to 11 mM. Mean 100% isometric responses for NA, 5-HT and K^+ were (in mg): 965 ± 103.3; 1236.83 ± 75.27; and 1390 ± 94.42, respectively. Values are mean ± S.E.M.; five to seven segments were used for each agonist and Ca^{2+} antagonist used *$P < 0.05$ or less.

gave a 50% inhibition of the contractile responses were the same in human and goat cerebral arteries (Table 1). Similar results were obtained with verapamil although the ID_{50}s were less than those of Mn^{2+}. The ratio of potencies of Mn^{2+} vs verapamil ranged between 2.3×10^{-3} and 9.7×10^{-3} when the experiments were undertaken in human cerebral blood vessels, and practically the same array was obtained with goat pial vessels (Table 1).

Inhibitory effects of verapamil and Mn^{2+} *on the contraction evoked by NA, 5-HT and* K^+. *Influence of* Ca^{2+}*-free medium in these contractions*

Preincubation for 10 min of cylindrical segments of human and goat cerebral arteries with verapamil (10^{-6} M) or Mn^{2+} (2×10^{-3} or 5×10^{-3} M) elicited a significant reduction of the contraction evoked by 10^{-5} M NA, 10^{-5} M 5-HT and 75 mM K^+ (Figs. 2 and 3). The contractions induced by K^+ were practically annulled by verapamil and 5×10^{-3} M Mn^{2+}. The addition of Ca^{2+} in concentrations ranging from 1 to 5 mM to the bath reversed the inhibitory effects of verapamil (Figs. 2 and 3).

Ca^{2+} removal from the extracellular medium caused a reduction of the contraction evoked by the three vasoconstrictor agents in both types of cerebral vessels. This effect was greater for K^+ than for the other two agents (Figs. 2 and 3).

DISCUSSION

The results shown in the present study indicate that verapamil and Mn^{2+}, two Ca^{2+} antagonists [1,13], vasodilated the human and goat cerebral arteries previously contracted with NA, 5-HT or K^+, and inhibited the contraction induced by these agents (Figs. 1, 2 and 3). Verapamil was more potent than Mn^{2+} in these effects, which are in agreement with the results found in other vascular beds [14–16].

The removal of extracellular Ca^{2+} markedly reduced the contraction evoked by NA, 5-HT and K^+ in human and goat cerebral arteries. These experiments show that extracellular Ca^{2+} is a necessary requirement for the contraction induced by the three agents in these arteries, analogous to that occurring in other vessels [1,17,18]. Since the inhibitory effects of Ca^{2+} antagonists were mimicked by Ca^{2+} suppression, it can be assumed that the action of verapamil and Mn^{2+} is mediated by interference with Ca^{2+} entry to vascular smooth muscle as has been reported [13–16].

The contractions evoked by K^+ were more inhibited by Mn^{2+} and verapamil than those produced by NA and 5-HT. This agrees with the fact that this contraction was also more blocked by Ca^{2+} suppression and with the higher relaxation evoked by Mn^{2+} and verapamil in cerebral arteries contracted with this ion (Figs. 1, 2 and 3). This indicates that the sources of Ca^{2+} used for contraction with each agent are different as has been reported [1]. This K^+-depolarization induced influx of Ca^{2+} across potential-sensitive Ca^{2+} channels, whereas the agonists evoked Ca^{2+} entry through receptor-operated Ca^{2+} channels [1]. The first channels seem in several tissues to be more sensitive to Ca^{2+} antagonists than the second ones [1], which coincides with the present results.

The depressor effect of verapamil, to differentiate that occurring with Mn^{2+}, did not disappear after repeated washing periods, but was reversed with Ca^{2+} addition to the medium (Figs. 2 and 3). The antagonism between Ca^{2+} and these agents is probably due to an increase in extracellular Ca^{2+} which augments the Ca^{2+} influx and hence the intracellular concentration of this ion at the level of contractile proteins [3].

In conclusion, verapamil and Mn^{2+} are two Ca^{2+} blockers which interfere with vascular contraction elicited by the three agents. The high sensitivity of cerebroarterial contractions to this organic Ca^{2+} antagonist suggests the possibility of using such an agent to treat cerebral vasospasm.

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