SELECTIVE INHIBITION OF THE TONIC COMPONENT OF POTASSIUM CONTRACTURE OF SMOOTH-MUSCLE CELLS IN THE PORTAL VEIN BY VERAPAMIL

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Experiments were carried out on smooth-muscle cells of the rat portal vein by the double sucrose gap method. Verapamil in concentrations of $1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$M was shown to block the tonic component of the potassium contracture selectively. Since under these circumstances potassium depolarization of the membrane was preserved, this is evidence that verapamil has a specific effect on the coupling of excitation with contraction, by blocking the passive inflow of calcium into the muscle cells. In higher concentrations ($1 \cdot 10^{-6}$-$1 \cdot 10^{-5}$M) verapamil inhibits the phasic component of potassium contracture, by blocking potential-dependent calcium channels of the membrane of the portal vein smooth-muscle cells responsible for action potential generation.

KEY WORDS: smooth muscles; phasic and tonic contraction; calcium channels; verapamil.

Although verapamil is widely used as a nonionic blocker of calcium channels, according to evidence obtained by several workers it acts differently on different smooth muscles. In the coronary arteries, for instance, verapamil considerably weakens contraction induced by potassium chloride [3]. In the smooth muscles of the stomach it selectively depresses phasic contraction [2]. Meanwhile, in the smooth muscles of the ureter verapamil, during the first minutes of its action, increases the amplitude of the action potential (AP), but reduces the supernumerary spikes and the duration of the plateau, while at the same time gradually reducing the amplitude of contraction also [4].

The object of the present investigation was to study the action of various concentrations of verapamil on electrical and contractile activity of the smooth-muscle cells (SMC) of the portal vein during depolarization of the membrane by potassium chloride.

EXPERIMENTAL METHOD

SMC of the rat portal vein were used as the test object. Experiments were carried out on isolated segments 5-6 mm long by the double sucrose gap method [1], with simultaneous recording of electrical and contractile activity of the SMC. Potentials were derived by Ag—AgCl electrodes. Contact between electrodes and muscle was ensured by means of agar bridges. The initial Krebs' solution had the following composition (in millimoles/liter bidistilled water): NaCl 134.0, $NaHCO_3$ 16.3, NaH_2PO_4 1.38, KCl 5.0, $CaCl_2$ 2.8, $MgCl_2$ 0.1, glucose 7.8. The Krebs' solution with an increased potassium ion concentration (80 mmoles/liter) was made up by adding dry KCl salt to the Krebs' solution. Verapamil was used in concentrations of $1 \cdot 10^{-9}$-$1 \cdot 10^{-5}$M. The temperature of the testing solutions was maintained between 34 and 35°C.

Changes in amplitude of the phasic and tonic components of potassium contracture following administration of verapamil were expressed as percentages; the amplitude of phasic and tonic contraction of SMC during potassium depolarization under normal conditions was taken as 100%; each experiment was accompanied by a control.

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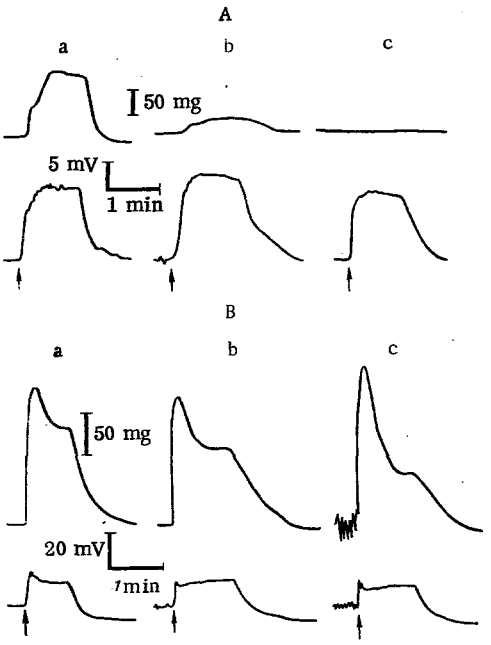

Fig. 1. Action of 80 mM KCl on electrical and contractile activity of portal vein SMC in calcium-free Krebs' solution + 2 mM EDTA (A) and in presence of $1 \cdot 10^{-9}$M verapamil in normal Krebs' solution (B). A: a) Normal, b, c) at 5th and 10th minutes respectively of action of calcium-free Krebs' solution + 2 mM EDTA. B: a) Normal, b, c) at 15th and 25th minutes respectively of action of verapamil ($1 \cdot 10^{-9}$M). Here and in Fig. 2, arrows indicate beginning of action of KCl. Top curves denote contractile, bottom curves electrical response of SMC.

EXPERIMENTAL RESULTS

During the first moment of the action of 80 mM KCl membrane depolarization of SMC developed and an AP appeared, accompanied by phasic contraction. During the subsequent action of KCl the AP died away and lasting membrane depolarization was observed; correspondingly, the phasic contraction was converted into tonic (Fig. 1A, B, a).

Removal of calcium ions from the Krebs' solution by means of 2 mM EDTA was followed by slight depolarization of the SMC membrane by 2-3 mV. Under the influence of KCl, 5 min after removal of calcium from the external solution a sharp decrease was observed in both the phasic and the tonic components of potassium contracture (Fig. 1A, b). During depolarization of the membrane by potassium chloride 10 min after removal of external calcium AP disappeared and both the phasic and the tonic components of potassium contracture were completely suppressed (Fig. 1A, c).

The study of the action of verapamil on portal vein SMC showed that in a concentration of $1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$M verapamil leads to slight depolarization of the SMC membrane (by 1-2 mV) and to an increase in the spontaneous discharge frequency; during the action of verapamil in concentrations of $1 \cdot 10^{-6}$-$1 \cdot 10^{-5}$M, however, the membrane was depolarized by 3-4 mV and spontaneous activity was inhibited. As Fig. 1B, curve b shows, a very slight decrease in phasic contraction (by 8%) and a more marked decrease in tonic contraction (by 20%) were observed at the 15th minute. At the 25th minute (Fig. 1B, c) the phasic response was actually a little increased (by 15%), whereas the tonic continued to decline (by 45%). During further repetition of potassium depolarization of the membrane at the 30th-40th minutes of action of verapamil it was found that the reaction remained essentially the same as at the 20th-25th minutes of action of verapamil, i.e., the response

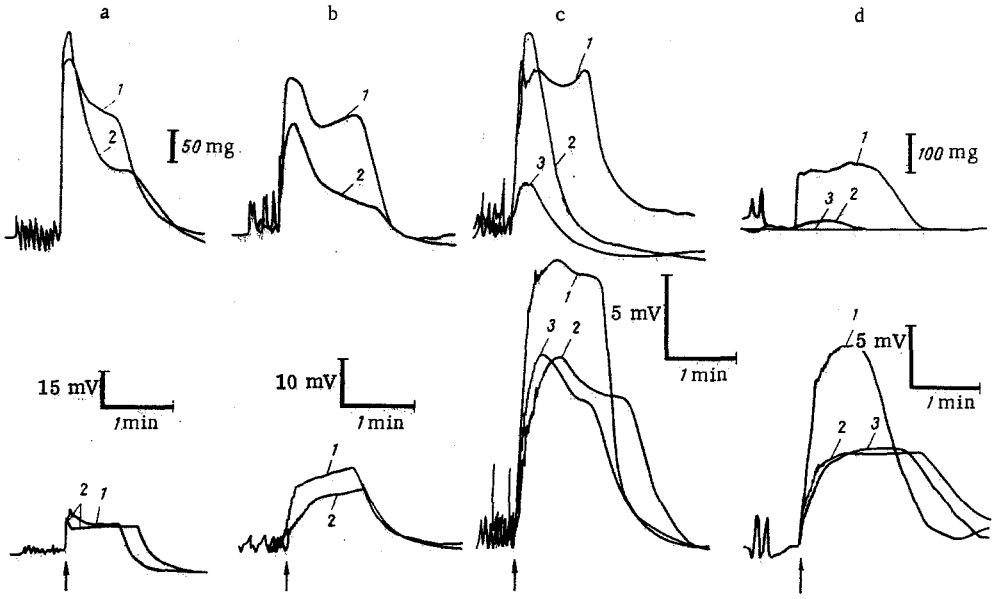

Fig. 2. Action of 80 mM KCl on electrical and contractile activity of portal vein SMC in presence of different concentrations of verapamil in Krebs' solution. a) 1) Normal, 2) verapamil $1 \cdot 10^{-9}$M; b: 1) normal, 2) verapamil $1 \cdot 10^{-8}$M; c: 1) normal, 2, 3) verapamil $1 \cdot 10^{-7}$ and $5 \cdot 10^{-7}$M respectively; d: 1) normal, 2, 3) verapamil $1 \cdot 10^{-6}$ and $1 \cdot 10^{-5}$M respectively.

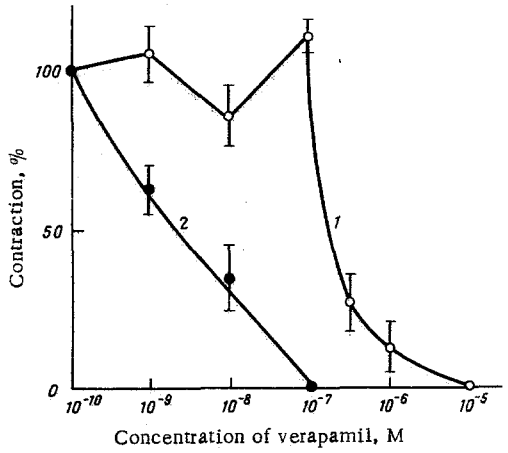

Fig. 3. Amplitude of contraction of portal vein SMC during potassium depolarization as a function of concentration of verapamil in Krebs' solution. 1) Phasic, 2) tonic component of potassium contracture of portal vein SMC.

of SMC during potassium depolarization and against the background of the action of verapamil became stabilized usually after 20-25 min. With these facts in mind, in the next experiments the effect of verapamil was usually tested during these time intervals. As the records in Fig. 1B, 2 and 3 show, verapamil in a concentration of $1 \cdot 10^{-9}$M reduced the tonic component of the potassium contracture by 30-45%, in a concentration of $1 \cdot 10^{-8}$M it reduced it by 56-78%, and in a concentration of $1 \cdot 10^{-7}$M the tonic response disappeared completely.

The phasic response of the potassium contracture during the action of verapamil depended on the presence of the AP. During the action of verapamil in concentrations of $1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$M it was either slightly reduced (by 5-25%; Figs. 2b and 3) or, if the frequency of AP rose sufficiently during potassium depolarization, it was actually increased (by 4-18%; Figs. 2a, c and 3). In concentrations of $5 \cdot 10^{-7}$-$1 \cdot 10^{-6}$M verapamil

caused inhibition of spontaneous APs and sharply inhibited (by 80-90%) the phasic response (Figs. 2c, d and 3). In a concentration of $1 \cdot 10^{-5}$M verapamil completely suppressed spontaneous and evoked APs and the phasic component of potassium contracture (Figs. 2d and 3).

The results of these investigations thus indicate that verapamil in very low concentrations ($1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$M) selectively blocks the tonic component of potassium contracture of SMC of the portal vein; as a blocker of calcium channels, verapamil acts in higher concentrations ($1 \cdot 10^{-6}$-$1 \cdot 10^{-5}$M).

Since the present experiments showed that both the phasic and the tonic components of the potassium contracture disappeared completely in calcium-free Krebs' solution in the presence of 2 mM EDTA, this could evidently indicate that both these reactions depend on the inflow of extracellular calcium into the cell.

The fact that verapamil, in concentrations of $1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$M, selectively inhibits the tonic components of potassium contracture, but that potassium membrane depolarization is preserved under these circumstances could indicate that verapamil has a specific effect on the coupling of excitation with contraction, by blocking the passive inflow of calcium into the cell. Only in higher concentrations ($1 \cdot 10^{-6}$-$1 \cdot 10^{-5}$M) does verapamil inhibit the phasic component of potassium contracture, by blocking the potential-dependent channels of the portal vein SMC membrane responsible for AP generation.

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ROLE OF VASCULAR THERMORECEPTORS IN THE MECHANISM OF COLD TREMOR INHIBITION BY OXOTREMORINE, DIAZEPAM, AND PHENTOLAMINE

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Experiments on cats showed that intravenous injection of oxotremorine, diazepam, and phentolamine not only inhibits cold tremor, but also reduces the flow of impulses from receptors of the subcutaneous veins. This decrease in the activity of the vascular thermoreceptors has been shown to be an additional component in the mechanism of the depriming action of oxotremorine and diazepam on cold tremor, whereas the decrease in thermoreceptor activity after administration of phentolamine is primary, and may perhaps be the dominant factor in the abolition of cold tremor.

KEY WORDS: cold tremor; thermoreceptors; neurotropic drugs.

Inhibition of cold tremor (CT) by certain neurotropic drugs is accompanied by an increase in the cutaneous blood flow, raising the skin temperature [3, 10, 11]. This fact suggests a definite role of the change in afferent flow from peripheral thermoreceptors in the mechanism of inhibition of CT by neurotropic drugs. The thermoreceptors of the subcutaneous and cutaneous vessels [4, 5], whose activity can be modified by central afferent influences and by the direct action of neurotropic drugs on smooth muscles, are particularly interesting from this point of view. These receptors, together with skin cold receptors, have been shown to participate in the regulation of CT [1, 2, 6].

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