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Short communication

ACTION OF PINAVERIUM BROMIDE ON CALMODULIN-REGULATED FUNCTIONS

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Pinaverium bromide at concentrations below 10^{-5} M did not inhibit calmodulin-dependent enzymes such as phosphodiesterase and the Ca transport ATPase of the plasma membrane. At higher concentrations the compound interacted with the stimulation of those enzymes by calmodulin and also inhibited the calmodulin-independent activity. A similar inhibitory action was observed for the NaK ATPase. It is concluded that the inhibitory action of pinaverium bromide on smooth muscle concentration at concentrations below 10^{-5} M was due to its interaction with the voltage-dependent Ca channels and not to its interference with the calmodulin-dependent activation of the contractile proteins.

Smooth muscle Ca antagonist Calmodulin Pinaverium bromide

1. Introduction

Pinaverium bromide (N-(bromo-2-dimethoxy-4,5-benzyl)-N([(dimethyl-6,6-norpinanyl-2)-2-ethoxy]-2-ethyl) morpholinium bromide) inhibits the contractions of intestinal smooth muscle induced by $BaCl_2$ (Bretaudeau and Foussard-Blanpin, 1980). Experimental evidence suggests that the compound acts mainly by blocking voltage-dependent Ca^{2+} channels (Droogmans et al., 1983) but an additional effect of this antagonist on intracellular systems controlling the contraction of smooth muscle has not been ruled out. Several examples of an interaction of Ca^{2+} entry blockers with calmodulin are known (Boström et al., 1981; Movsesian and Adelstein, 1984).

Calmodulin modulates a multitude of Ca^{2+}-dependent enzymes and cell functions. The calmodulin-dependent fraction of these enzyme activities can be inhibited by a large number of chemically unrelated substances that share one common characteristic in all being cationic amphiphiles (Gietzen et al., 1983). However all the inhibitors described are more or less unspecific in that they also inhibit the basal activity of the calmodulin-dependent enzymes, more in particular membrane enzymes and even the activity of some calmodulin-independent enzymes. These actions may be due to a perturbation of the lipid environment of the enzymes by the lipophilic antagonists.

Pinaverium bromide fulfils the general requirements for a putative calmodulin inhibitor in having cationic amphiphilic properties, but has a lower hydrophobicity than most calmodulin inhibitors and hence distributes less in favour of the lipid phase of biological membranes. We have therefore investigated the possibility that this substance might be a selective calmodulin antagonist. In particular we have compared the action of pinaverium bromide with that of compound 48/80, which by virtue of its low hydrophobicity is one of the most specific inhibitors of the calmodulin-induced activation of the Ca^{2+}-transport ATPase in erythrocytes (Gietzen et al., 1983).

We have examined the action of pinaverium bromide as a calmodulin antagonist in two calmodulin-dependent enzymes: the Ca^{2+}-trans-

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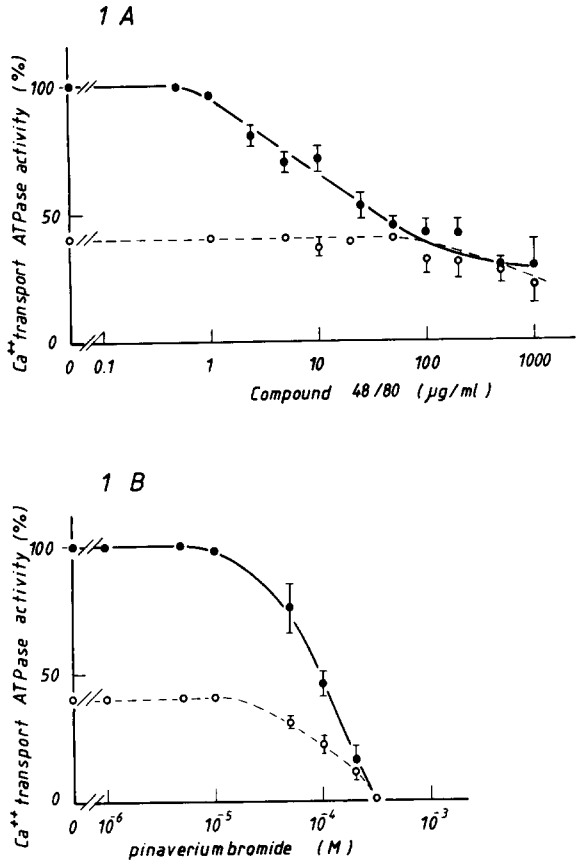

Fig. 1. Inhibition of smooth muscle Ca^{2+}-transport ATPase by compound 48/80 in the presence (●) or absence (○) of 0.6 µM calmodulin. ATPase activity was measured at 37°C in 1 ml of a medium containing: 100 mM KCl, 30 mM imidazole-HCl (pH 6.8), 5.7 mM $MgCl_2$, 5 mM Na_2 ATP, 5 mM NaN_3, 1 mM EGTA, 1.5 mM Phosphoenolpyruvate, 0.26 mM NADH, 40 IU pyruvate kinase, 36 IU lactate dehydrogenase (both enzymes from rabbit muscle, Boehringer). The decrease in absorbance at 340 nm was recorded. One hundred µl of the purified calmodulin-dependent Ca^{2+}-transport ATPase (5 µg protein) in 0.05% Triton X-100, 0.05% phosphatidylcholine were added to 900 µl of the medium after addition of compound 48/80 and calmodulin. The enzyme was preincubated for 15 min at 37°C with the drug and calmodulin before addition of 0.87 mM $CaCl_2$ (resulting in a final [Ca^{2+}] of 10^{-5} M). ATPase activity is expressed as the percentage of the activity obtained in the presence of 0.6 µM calmodulin and in the absence of the inhibitor. Each point represents the mean of at least 3 determinations. (B) Inhibition of smooth muscle Ca^{2+}-transport ATPase by pinaverium bromide in the presence (●) or absence (○) of 0.6 µM calmodulin. One hundred µl of the purified Ca^{2+}-transport ATPase (5 µg protein) in 0.05% Triton X-100, 0.05 phosphatidylcholine were added to 900 µl of the medium after addition of pinaverium and calmodulin. The enzyme was preincubated with the drug for 15 min at 37°C (in the absence or presence of calmodulin) before Ca^{2+} was added up to a final concentration of 10^{-5} M. The stock solution of pinaverium bromide contained 10^{-2} M in ethanol. Each point is the mean of at least 2 determinations.

port ATPase from pig antral smooth muscle which is a typical example of an intrinsic membrane enzyme, and the calmodulin-dependent brain phosphodiesterase which can be considered a soluble enzyme. We have also analyzed the effect of pinaverium bromide on the NaK ATPase from pig kidney, as an example of its action on a calmodulin-independent membrane enzyme.

2. Materials and methods

Bovine brain calmodulin and calmodulin sensitive Ca^{2+}-transport ATPase from pig antral smooth muscle were prepared by standard procedures (De Schutter et al., 1984). NaK ATPase was purified from pig kidney by the method described by De Smedt et al. (1979). Calmodulin-sensitive phosphodiesterase from bovine heart was obtained from Boehringer. Activities of ATPase and phosphodiesterase were assayed as described by Wuytack and Casteels (1980) and Van Belle (1981) respectively. Pinaverium bromide was a gift from Dr. M.O. Christen of the Société Berri-Balzac, Suresnes, France.

3. Results

Fig. 1A demonstrates the stimulation of the Ca^{2+}-transport ATPase of smooth muscle by 0.6 µM calmodulin and the inhibition of this activation by compound 48/80. Fig. 1B shows the results of similar experiments in the presence of pinaverium bromide.

In the absence of the agents, as indicated by the points marked on the ordinate, this purified Ca^{2+}-transport ATPase could be stimulated by 0.6 µM calmodulin to a maximum of 2.5 times the basal activity of Ca^{2+}-transport ATPase, as described previously (De Schutter et al., 1984).

Compound 48/80 specifically antagonized the calmodulin-induced activation of smooth muscle

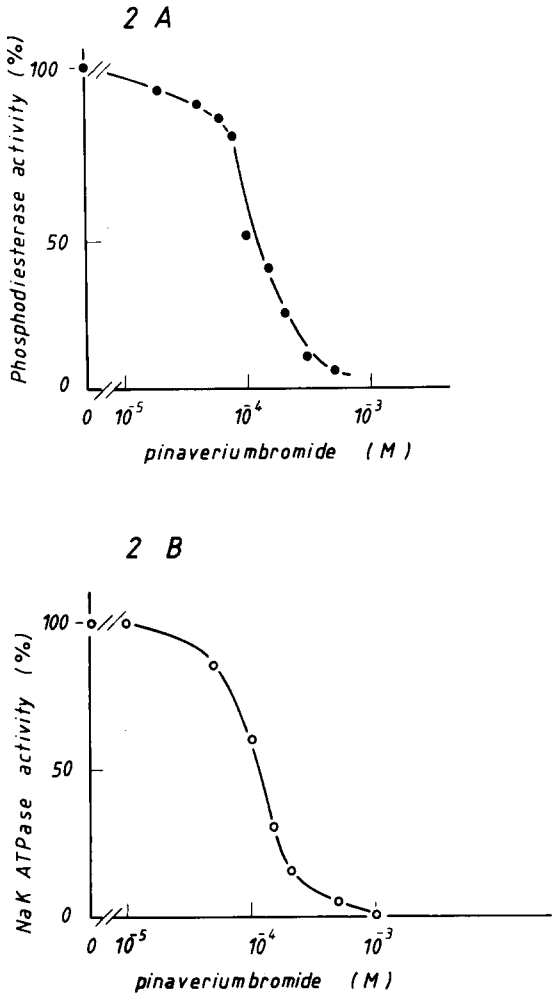

Fig. 2. Effect of pinaverium bromide on calmodulin-stimulated brain phosphodiesterase activity. The enzyme was preincubated at room temperature with the inhibitor for 30 min in the presence of 0.6 μM calmodulin before the reaction was started by adding 200 μM of cAMP to a medium containing 20 mM 4-morpholine propanesulfonic acid (MOPS), pH 7.5, 10 mM $MgCl_2$, 40 μM $CaCl_2$, 0.2 U/ml of calf intestinal alkaline phosphatase (Boehringer). The release of inorganic phosphate at 20°C was measured by an automated malachite green procedure. The enzyme activity measured in the absence of calmodulin was 0.125 times that in the presence of the activator (not shown). (B) Effect of pinaverium bromide on purified NaK ATPase activity of pig kidney medulla. ATPase activity was measured as described for Ca^{2+}-transport ATPase activity, but with 100 mM NaCl and 10 mM KCl instead of 100 mM KCl, and without addition of $CaCl_2$. The enzyme was preincubated for 15 min at 37°C with the inhibitor before the reaction was started by the addition of ATP.

Ca^{2+}-transport ATPase with an IC_{50} value of 5 μg/ml, whereas the basal activity was only slightly inhibited at 100 μg/ml (fig 1A).

Pinaverium bromide at high concentrations inhibited both the calmodulin-dependent and calmodulin-independent Ca^{2+}-transport ATPase activities. The IC_{50} values for the two activities amount to about 10^{-4} M. The same high IC_{50} values were found for the action of pinaverium bromide on calmodulin-dependent phosphodiesterase activity (fig. 2A) and calmodulin-independent NaK ATPase activity (fig. 2B).

4. Discussion

High concentrations of pinaverium bromide inhibited the calmodulin-dependent activities of both Ca^{2+}-transport ATPase and phosphodiesterase ($IC_{50} = 10^{-4}$ M). Therefore pinaverium bromide is a very weak inhibitor of the calmodulin-dependent activities compared to calmidazolium for which IC_{50} values of $0.5\text{-}1.0 \times 10^{-8}$ M or $1.5\text{-}3.5 \times 10^{-7}$ M were reported for phosphodiesterase and Ca^{2+}-transport ATPase respectively (Van Belle, 1981).

It can be assumed that an amphiphile at a concentration in the range of 10^{-4} M will disturb the lipid environment of any membranal enzyme and could thereby provoke unspecific inhibition. Moreover we have observed that pinaverium bromide had a very low selectivity for inhibiting calmodulin-dependent enzyme activity. The ratio of the IC_{50} value for the basal activity to the IC_{50} value for the calmodulin-dependent enzyme activity is a measure of the specificity of the drug to inhibit calmodulin-induced stimulation. For the Ca^{2+}-transport ATPase this ratio is larger than 200 in the case of compound 48/80 whereas it is about 1 in the case of pinaverium bromide. Gietzen et al. (1983) proposed that the high specificity of compound 48/80 could be due to the fence-like structure of this polycation, whereby the incorporation of the agent into the lipid environment of the enzyme might be hindered. The molecular structure of pinaverium bromide is more linear. This probably explains the very low specificity of this agent as a calmodulin antagonist.

After this work was completed Ronca-Testoni et al. (1985) reported on the interaction of a number of smooth muscle relaxants with calmodulin and cyclic nucleotide phosphodiesterase. These authors classify pinaverium bromide as a calmodulin antagonist. However, according to their data even 100 μM of this compound inhibits the calmodulin-dependent phosphodiesterase (at 0.3 μM calmodulin) by only 30%. This value is comparable to that observed in our study with 0.6 μM calmodulin (see fig. 2A).

It is concluded that pinaverium bromide exerts few calmodulin antagonistic effects. Therefore at concentrations of 10^{-5} M or lower its inhibitory action on the contraction of smooth muscle cannot be due to interference with the calmodulin-dependent activation of the contractile system. Hence its inhibitory action on isolated smooth muscle tissues must be largely due to its blocking action on voltage-dependent Ca channels, as suggested by the work of Droogmans et al. (1983).

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