Effect of quinidine on microsomal ATPase from rabbit taenia coli

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Quinidine inhibits the relaxation of intestinal smooth muscle in response to β-stimulants, α-stimulants, antispasmodics or non-adrenergic inhibitory stimulation (Burnstock, Campbell & others, 1970; Tomiyama, Takayanagi & Takagi, 1975). Its inhibitory action implies that it interferes with basic mechanisms of relaxation of the smooth muscle and Tomiyama & others (1975) demonstrated that it inhibits ATP-dependent uptake of Ca^{2+} ions into intestinal microsomes. Thus quinidine depresses intracellular Ca^{2+}-sequestration and reduces the effects of relaxants that lower the intracellular concentration of Ca^{2+} ions. As this sequestration is supported by ATP, we have examined the effect of quinidine on microsomal ATPase to obtain information on Ca^{2+} ion pumping mechanisms in smooth muscle. Inhibitors of Ca^{2+}-pumps, such as ruthenium red do not affect relaxation of intact muscle (Tomiyama & others, 1975). Therefore quinidine is useful for studies on the underlying mechanisms of the Ca^{2+} ion pump in smooth muscle.

Male rabbits, 2–3 kg, were killed by exsanguination after a blow on the head. Then the taeniae coli were rapidly removed, washed with ice-cold 0·25 M sucrose solution, cut into small pieces and homogenized in 9 volumes of ice-cold 0·25 M sucrose solution in a Polytron (PT 10, Brinkman) at rheostat setting 6 for three 5 s periods. The homogenate was centrifuged at 15 000 *g* for 30 min and the supernatant was further centrifuged at 40 000 *g* for 60 min. The resulting microsomal fraction is the fraction reported by Takayanagi, Hongo & Kasuya (1977) to consist mainly of plasma membrane vesicles. Contaminating actomyosin-ATPase was removed by washing the microsomal fraction twice with 0·6 M KCl.

ATPase activity was measured in 20 mM imidazole buffer (pH 7·2) containing 0·1 M KCl, 3 mM Na_2ATP, 0·1 mM ouabain and 5 mM sodium azide. Ouabain (0·1 mM) was added to block the activity of Na^+-, K^+-, Mg^{2+}-ATPase, and sodium azide (5 mM) was used to block the contaminating mitochondrial Ca^{2+}-, Mg^{2+}-ATPase.

To the reaction mixture a specified amount of Mg^{2+} and/or Ca^{2+} ions were added. ATPase activity in the presence of Mg^{2+} ions alone was denoted as Mg^{2+}-ATPase, that in the presence of Ca^{2+} ions alone as Ca^{2+}-ATPase, and the activation of Mg^{2+}-ATPase by further addition of Ca^{2+} ion, as Ca^{2+}-, Mg^{2+}-ATPase. The free concentration of Ca^{2+} ions was adjusted with EGTA and calculated as described by Ogawa (1968) assuming that $K_{app} = 3{\cdot}16 \times 10^6\ M^{-1}$ (at pH 7·20). The reaction was started by adding a suspension of the microsomal fraction to give a protein concentration of about 100 μg ml^{-1}, as determined by the microbiuret method (Itzahki & Gill, 1964). The reaction was stopped by adding a final concentration of 5% trichloroacetic acid; inorganic phosphate that split from ATP during incubation at 37° for 10 min was determined by Martin-Doty method (1949). Non-enzymatic hydrolysis of ATP was subtracted.

We tested the effect of quinidine at 0·1 mM at which concentration it shifted the dose-response curve for papaverine 10-fold to the right, reversibly, as reported by Tomiyama & others (1975) and depressed ATP-dependent uptake of Ca^{2+} ions into the microsomes of rabbit taenia coli to 80% of the control value [i.e. control = 20 ± 0·6 n mol Ca^{2+} ions mg^{-1} protein; control + quinidine (0·1 mM) = 16·2 ± 0·5 n mol; incubation for 8 min, triplicate determination, 6 rabbits], as determined according to Takayanagi, Yamashita & others (1977). This concentration of quinidine (0·1 mM) did not interfere with inorganic phosphate determination but higher values did. Furthermore, in the inhibition of relaxation of intact muscle strips, higher concentrations of quinidine were more effective but a

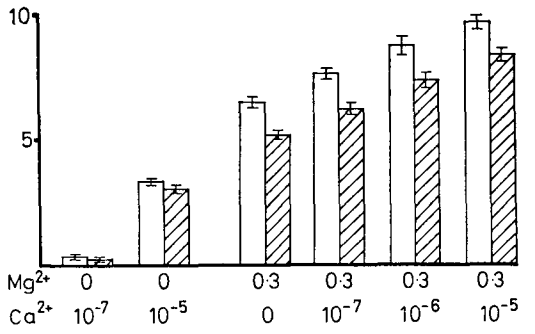

FIG. 1. Effect of quinidine on ATPase activity of microsomes from rabbit taenia coli. Microsomes from rabbit taenia coli (80–100 μg ml^{-1}) were incubated with 3 mM ATP, 100 mM KCl, 20 mM imidazole (pH 7·2), 100 μM ouabain, 5 mM sodium azide and the indicated amount of Mg^{2+} and/or Ca^{2+} ions. Free concentration of Ca^{2+} ions was regulated by Ca^{2+}-EGTA buffer system. (Ca^{2+} = 0; no added Ca^{2+} ions in the presence of 0·1 mM EGTA.) ATPase activity at 37° was expressed in terms of the amount (n mol) of inorganic phosphate liberated mg^{-1} of microsomal fraction per 10 min. Open columns show activities in the absence of quinidine and shaded columns those in the presence of quinidine (0·1 mM). Values are means ±s.e. of triplicate determinations on preparations from six rabbits. Ordinate: Pi (μ mol mg^{-1} protein per 10 min). Abscissa: concentration, Mg^{2+} ions (mM); Ca^{2+} ions (M).

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considerable time was needed to wash out its inhibitory effect.

In the presence of 2 mM Mg^{2+} ions, addition of Ca^{2+} ions ($10^{-7} - 10^{-5}$ M) did not stimulate the Mg^{2+}-ATPase further but when the concentration of Mg^{2+} ions was reduced to 0·3 mM, the activation by Ca^{2+} ions was unmasked (Fig. 1). Ca^{2+} ions ($10^{-7} - 10^{-5}$ M) caused a concentration-dependent activation of Mg^{2+}-ATPase. Quinidine inhibited Mg^{2+}-ATPase activity to the same degree (to 80%) as ATP-dependent Ca^{2+} ion uptake activity of the microsomes. However, quinidine did not inhibit the activation of Mg^{2+}-ATPase by Ca^{2+} ions. When Ca^{2+} ions were present, total ATPase (Ca^{2+}-, Mg^{2+}-ATPase) activity was increased with the increase in the amount of added Ca^{2+} ions, yet the amount of inhibition by quinidine remained unchanged. Therefore the fraction of Ca^{2+}-, Mg^{2+}-ATPase activated by Ca^{2+} ions was not influenced by quinidine. Quinidine also had only a small effect on Ca^{2+}-ATPase. Thus quinidine probably inhibits Mg^{2+}-ATPase in the microsomes of taenia coli.

The stimulation of Mg^{2+}-ATPase by Ca^{2+} ions is direct evidence of energy linked Ca^{2+} ion transport and it is observed with both skeletal and cardiac microsomes (Tonomura, 1973). Quinidine inhibited Ca^{2+}-stimulation in the microsomes of these muscles (Fuchs, Gerz & Briggs, 1968, Balzer, 1972; Harrow & Dhalla, 1976). However, our present work suggests that in microsomes of the smooth muscle examined it did not inhibit the stimulation by Ca^{2+} ions even when Ca^{2+}-activation was unmasked. This finding shows a difference between the mechanisms in Ca^{2+} ion uptake by gut smooth muscle and by other muscles. Furthermore, several workers have reported that Ca^{2+} ions did not activate Mg^{2+}-ATPase activity in smooth muscles; Verty & Bevan (1969); Boudin & Meyer (1973); and Yamashita, Aoki & others (1976) observed this with aortic microsomes and Krall, Swensen & Koreman (1976) with uterine microsomes. Yamashita & others (1976) studied the coupling of Ca^{2+} ion uptake to ATPase activity and concluded that Mg^{2+}-ATPase activity in the microsomes supplies the energy for Ca^{2+} ion uptake. Krall & others (1976) found that stimulation of rat uterine microsomal Mg^{2+}-ATPase activity by cAMP was similar to its stimulation of Ca^{2+} ion uptake. This implies a close relation of Ca^{2+} ion uptake activity to Mg^{2+}-ATPase activity.

These observations and our present results would indicate a functional importance of microsomal Mg^{2+}-ATPase in relaxation of smooth muscle.

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