Short communication

Effects of Diltiazem on the Electrical and Mechanical Activity of the Guinea-pig Ureter Smooth Muscle

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Diltiazem was launched as a Ca antagonist by Nakajima et al. (1975). According to the Fleckenstein original classification (Fleckenstein 1983), diltiazem belongs to group A of highly specific Ca antagonists. In ureter smooth muscle another Ca antagonist of group A, nifedipine was roughly 100 times more effective in blocking tonic tension of high-K contracture than phasic contractions associated with action potentials (Aickin et al. 1984). Na/Ca channels responsible for the generation of the plateau component of the action potential are more sensitive to inhibitory action of nifedipine than Ca-channels responsible for the fast spike component (Brading et al. 1983). The Na-free contracture which is thought to reflect the activity of the Na—Ca exchange proved to be completely insensitive to nifedipine and D-600 (Aickin et al. 1984).

The main goal of the present study was to see the effect of diltiazem on various mechanisms of Ca entry in the ureter smooth muscle of the guinea-pig.

The experiments were performed on isolated pieces of whole ureter from guinea-pig using both the double sucrose-gap method (Bülbring and Tomita 1969) for simultaneous recording of electrical and mechanical activity and the continuous superfusion technique for tension recording (Brading and Sneddon 1980).

The composition of normal Krebs solution was (in mmol/l): Na^+, 136.9; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 133.5; $H_2PO_4^-$, 1.2; HCO_3^-, 15.5; glucose, 11.5 bubbled with 97 % O_2 + 3 % CO_2 at 36—37 °C. High-K solution was prepared by replacing all external NaCl by KCl. Loading of the tissue with Na ions was obtained by inhibition of the Na—K pump by treatment of the ureter with ouabain as described earlier (Aickin et al 1984). Caffeine contractures of the ureter muscle were recorded at 20—22 °C.

Under normal conditions the action potential of the ureter smooth muscle evoked by just suprathreshold depolarization of short duration has an initial fast component consisting of repeated and gradually decaying spikes and a subsequent slow component, the so-called plateau (Fig. 1*A*,*a*). The action potential is accompanied by a brief phasic contraction.

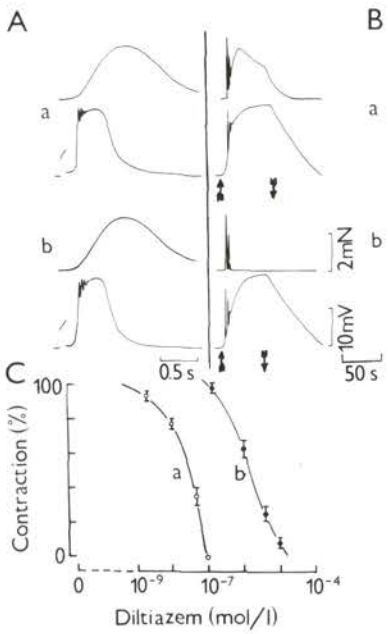

Fig. 1. Effect of diltiazem on the ureter smooth muscle. *A*, the electrical (lower trace) and mechanical (upper trace) responses of the smooth muscle to depolarizing current pulse in the absence of diltiazem (*A*, *a*) and during exposure to 10^{-7} mol/l diltiazem for 10 min (*A*, *b*). *B*, the electrical (lower trace) and mechanical (upper trace) responses of the smooth muscle to high-K solution in the absence of diltiazem (*B*, *a*) and during exposure to 10^{-7} mol/l diltiazem for 10 min (*B*, *b*). *C*, dose-response curves of the inhibition of tonic (*a*) and phasic (*b*) contractions associated with sustained depolarization induced by 126 mmol/l K^+ and evoked action potentials, respectively. (All records are taken by the double sucrose gap method; first arrow indicates the administration and the second arrow indicates the removal of high-K solution).

Fig. 1*A*, *B* shows that parameters of the action potential and phasic contraction were unaffected by diltiazem at 10^{-7} mol/l, although the tonic component of high-K contracture was completely abolished by the drug at this concentrations associated with action potentials generated in the onset of depolarization. However in higher concentration range ($10^{-6} - 10^{-5}$ mol/l) diltiazem reduced the number of spikes and the duration of the plateau. Simultaneously, the contractile response was decreased. The spike component of the action potential proved to be more resistant to diltiazem and was completely blocked by the drug 2×10^{-5} mol/l. The effect of diltiazem on the action potential and phasic contraction was reversible. Dose-response curves illustrated in Fig. 1*C* show that tonic tension (curve *a*) associated with sustained membrane depolarization is roughly 100 times more sensitive to diltiazem than phasic contractions (curve *b*) associated with the action potentials. The membrane potential and membrane resistance of the ureter muscle were practically unaffected by diltiazem. These results suggest that Ca channels normally opened by sustained depolarization do not contribute to Ca-influx during

the generation of the action potential. Fig. 2*a*, *b* shows that normal tissue completely lost its ability to respond with any contractions to high-K (Na-free) solution in the presence of 10^{-5} mol/l diltiazem. However, after 40 min treatment of the tissue with ouabain (10^{-4} mol/l) the ureter muscle responded with contracture to high-K (Na-free) solution even in the presence of diltiazem. The tissue now responded to the absence of Na rather than to the high-K (Fig. 2c). Also, Na-loaded tissue developed diltiazem-resistant Na-free (Tris) contracture, which unlike Na-free (K) contracture, was smaller in amplitude and accompanied by hyperpolarization of the membrane as was demonstrated previously (Aickin et al 1984).

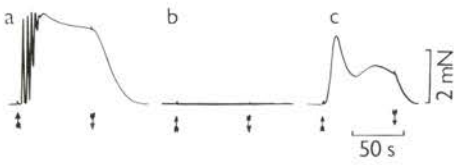

Fig. 2. Effects of diltiazem on the ureter smooth muscle. *A*, high-K (Na-free) contracture of the ureter muscle evoked in the absence of diltiazem; *B*, complete inhibiton of high-K conctracture by 10^{-5} mol/l diltiazem; *C*, the recovery of the high-K (Na-free) contracture upon 40 min treatment of the ureter muscle with ouabain at 10^{-4} mol/l in the continuous presence of 10^{-5} mol/l diltiazem (superfusion technique) (first arrow indicates the administration and the second arrow indicates the removal of KCl).

Diltiazem had no effect on the contractures of the ureter muscle stimulated by caffeine (2—20 mmol/l) normally seen in Ca-free solution at 20—22 °C.

The present results are in agreement with the idea of the existence in the ureter muscle cell membrane of several types of voltage-operated Ca channels which show different sensitivity to some Ca antagonists (Kochemasova and Shuba 1979; Brading et al 1983; Aickin et al 1984). Most effectively diltiazem blocks voltage-operated Ca channels opened during sustained depolarization. This finding is in good agreement with that of van Breemen et al. (1981) who found a close relationship between diltiazem inhibition of ^{45}Ca influx and inhibition of contractures of rabbit aortae evoked by high-K solution.

The Na-free contracture, an effect thought to reflect the activity of the Na Ca exchange mechanism (Aickin et al. 1984), and caffeine contracture which seems to reflect the release of Ca from the internal Ca store are resistant to 10^{-5} mol/l diltiazem.

Thus, we conclude that at least in ureter smooth muscle diltiazem acts as specific blocker of the voltage-operated Ca channels with high selectivity for Ca channels which do not inactivate during sustained depolarization.

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