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Diltiazem inactivates acetylcholine-induced membrane channels in skeletal muscle fibres

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ABSTRACT

Diltiazem, a 'calcium antagonist', closes endplate channels irrespective of whether it is applied extracellularly or intracellularly. It also causes a marked depression of ACh-currents induced by repetitive pulses of ACh. The amplitude of miniature endplate currents is only slightly reduced. It is suggested that similar effects of diltiazem, and related drugs, may be relevant to their beneficial action in the treatment of some cardiovascular diseases.

KEY WORDS diltiazem / acetylcholine / muscle / membrane channels / angina

Some human heart ailments, such as the variant form of Angina, are being treated with a group of substances generally known as calcium antagonists (9, 8, 12, 13). The mode of action of these drugs is not yet well understood; but it is frequently assumed that their beneficial action is related to their ability to block calcium ion fluxes across membranes (3).

We have previously found (1, 2, 7) that muscle membrane channels opened by acetylcholine (ACh), are rapidly inactivated by methoxyverapamil (D 600), one of the so-called calcium antagonists. To determine if this characteristic is shared by other drugs in the group, we have studied the action of diltiazem, a benzothiazepine derivative (10), which is becoming increasingly important in clinical use (8, 13).

The experiments, briefly reported here, were made on voltage-clamped endplates in the sartorius muscle of the frog, during September–October 1980. The methods used have already been described (2, 5, 7). ACh was applied ionophoretically from a micropipette; and diltiazem was either added to the fluid bathing the muscle, or applied ionophoretically.

When a pulse of ACh is applied to the synaptic muscle membrane, an ionic current flows through the channels opened by the interaction of ACh with the ACh-receptors. With pulses such as those illustrated in Fig. 1, the ACh-induced current increased progressively throughout the pulse, and began to subside soon after its end. If a similar pulse of ACh was repeated a second or more later, the responses to both pulses were practically identical (Fig. 1A).

In contrast, when diltiazem was added the ACh-sensitivity of the membrane (6) was reduced, and the ACh-currents behaved quite differently. For instance, instead of increasing progressively, the current rose and fell during the pulse. Moreover, the response to a second pulse of ACh was markedly depressed (Fig. 1B) and took many seconds to recover. This activity-dependent inhibition of ACh action could be observed with as little as 10^{-7} M diltiazem, and its magnitude increased with the dose. The time interval that had to elapse for the second response to recover, was lengthened by lowering the temperature.

As illustrated in Fig. 2, an ionophoretic pulse of diltiazem applied extracellularly to the ACh-activated muscle membrane, produced a rapid and prolonged decrease in the current induced by ACh. The amount of inhibition depended on the intensity of the pulse of diltiazem; but the rate of recovery of the ACh-current was relatively independent of it. A pulse of diltiazem, applied alone to the resting muscle membrane, had no effect on the clamp current; but it inhibited the response to a pulse of ACh applied

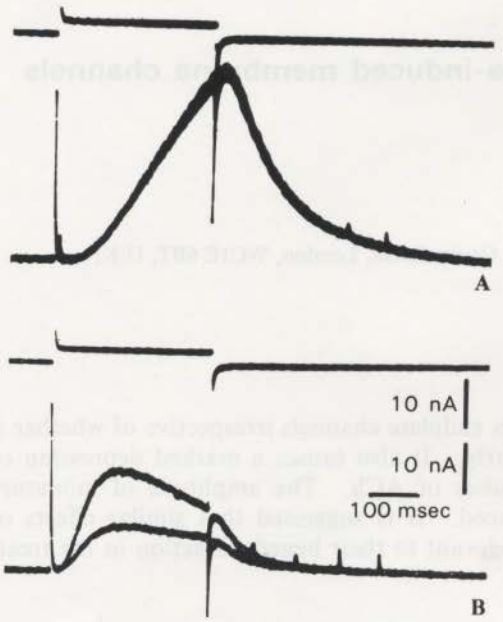

Fig. 1 Currents induced by ionophoretic application of ACh to a voltage-clamped endplate. A: control in normal Ringer. B: in diltiazem 2.2×10^{-5} M. Each frame shows the responses to two pulses of ACh separated by 3 sec interval. Amplitude of miniature endplate currents was 4.14 nA and 3.66 nA in A and B respectively. Top traces monitor ionophoretic current. Lower traces show membrane current. This and subsequent figures are from frog sartorius muscle fibres with the membrane potential clamped at −90 mV. Temperature, 20°C

many seconds later. Another interesting feature arising from these experiments, is that the depressing effect of diltiazem on ACh-currents was seen in the absence of calcium ions in the external medium (Fig. 2).

As illustrated in Fig. 3, an intracellular pulse of diltiazem again depressed the current induced by ACh. This shows that diltiazem, like procaine and D 600, blocks ACh-induced membrane channels irrespective of the route of administration; while other antagonists of ACh action, such as curare, gallamine and tetraethylammonium are ineffective when applied intracellularly (5, 7). Thus it appears that, like procaine and D 600, diltiazem gains access to the ACh-receptor through the lipid phase of the membrane; and blocks the active ACh-receptor-channel complex through binding at a site which differs from that which combines with ACh (see also 5, 7). This hypothesis is supported by preliminary experiments which indicate that diltiazem decreased only slightly, or not at all, the amount of α-bungarotoxin bound to the sartorius muscle.

Other effects of diltiazem will be described later. However, it should be mentioned here that, as in earlier observations on vascular smooth muscle by Ito *et al.* and Tajima *et al.* (4, 11), diltiazem did not affect the resting potential or input resistance of skeletal muscle fibres. There was not much change either, on the equilibrium potential of ACh action or on the voltage dependence of the amplitude of ACh-currents evoked by pulses of ACh. On the other hand miniature endplate currents were reduced in amplitude and decayed more rapidly; but they were still detectable in the presence of diltiazem concentrations that abolished, almost completely, the response to ionophoretic pulses of ACh.

These observations show that 'calcium antagonists' such as diltiazem, D 600, verapamil, etc., have a powerful antagonistic effect on the active ACh-receptor-channels, which is manifested more clearly during prolonged exposure to ACh. An important consequence of this effect of diltiazem is that the action of a 'tonic' release of ACh may be annulled, while the

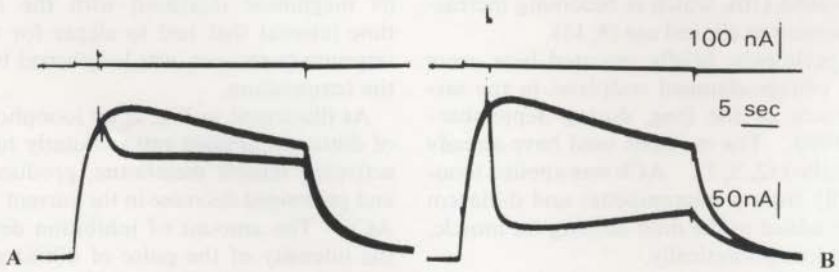

Fig. 2 Blocking of ACh-current by an extracellular pulse of diltiazem. A and B: diltiazem pulses of different intensity. Note the rapid and long lasting inhibition of ACh-current following the pulse of diltiazem. Top traces monitor ionophoretic currents through separate ACh and diltiazem micropipettes. Muscle in 0 mM Ca^{2+}, 5mM Mg^{2+}, 1 mM EGTA-Ringer. Temperature, 5°C

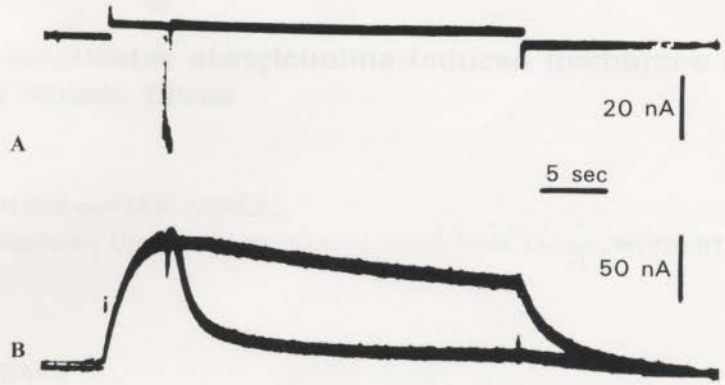

Fig. 3 Blocking of ACh-current by an intracellular pulse of diltiazem. Same endplate etc. as in Fig. 2

'phasic' action of transmitter quanta released from the nerve terminals is only slightly reduced. It is possible, that the differential effect of diltiazem on tonic and phasic transmitter action, is not restricted to the cholinergic system, but may occur also in other transmitter-receptor interactions. Ito *et al.* (4) found that diltiazem blocked the depolarization induced by noradrenaline on the pulmonary artery muscle. It would be interesting to find out if this block is analogous to the block of ACh action on frog muscle. An effect of this kind may be responsible for the therapeutic action of diltiazem, and related compounds, on some cardiovascular diseases. It is also conceivable that similar substances, which inactivate open channels, play a role at synapses in the central nervous system.

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