Ca^{2+}-Antagonistic Properties of Phospholipase A_2 Inhibitors, Mepacrine and Chloroquine

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Abstract. The effects of putative phospholipase A_2 inhibitors mepacrine and chloroquine on membrane ionic currents were studied in intact frog atrial trabeculae. Both agents decreased slow calcium channel current I_{si} and fast sodium channel current I_f. I_{si} was affected twice at least in comparison to I_f. Half-block of I_{si} was observed at $\sim 10^{-6}$ mol/l mepacrine and at $\sim 10^{-5}$ mol/l chloroquine. These effects on transmembrane ionic transport should be considered when using the above agents as phospholipase inhibitors or antiarrhythmic drugs.

Key words: Mepacrine — Chloroquine — Frog atrial membrane — Ca^{2+}-current — Sodium current — Phospholipase A_2 inhibitors

Introduction

A number of agents have been described so far, which inhibit phospholipase A_2, including synthetic antimalarial drugs mepacrine and chloroquine, widely used to investigate the role of the enzyme in metabolic and pathological processes (Mallorga et al. 1980; Billah et al. 1981; Chen et al. 1982; Kunze et al. 1982; Severson et al. 1980). In addition to the inhibitory effect on phospholipase A_2 in various tissues the same concentrations of mepacrine (0.2—1 mmol/l) have been reported to directly interact with membrane phospholipids and to alter the membrane structure (Dise et al. 1982). It can be expected that alteration of the phospholipid structure of the membrane is associated with changes in the functioning of the protein ionic channels in the membrane. This problem was investigated in the present work by studying mepacrine and chloroquine action on transmembrane ionic currents in frog atrial fibres.

Materials and Methods

Voltage-clamp experiments were performed on frog (*Rana ridibunda*) atrial trabeculae, 0.1—0.15 mm in diameter, using the double sucrose-gap technique as described previously (Filippov and

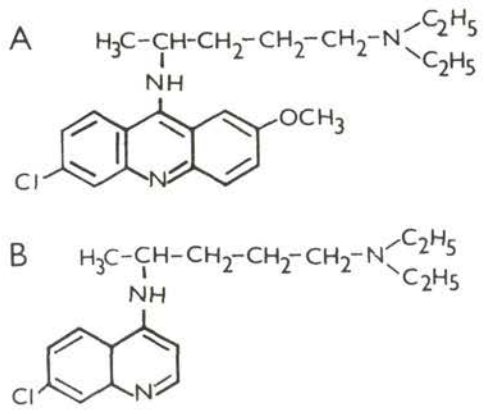

Fig. 1. Chemical structure of mepacrine (*a*) and chloroquine (*b*).

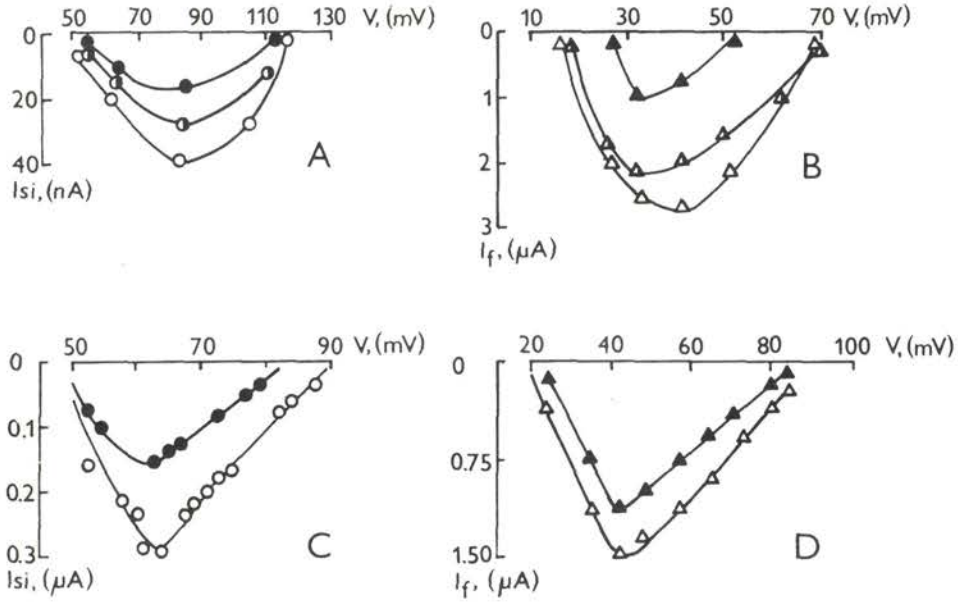

Fig. 2. Effects of mepacrine and chloroquine on the inward currents in frog atrial preparations. The peak slow inward current (I_{si}) and the fast inward sodium current (I_f) plotted against the test potential. *A*: Effect of mepacrine on peak I_{si}: 10^{-7} mol/l (half closed circles); 10^{-6} mol/l (closed circles); control (open circles). *B*: Effect of mepacrine on peak I_f: 10^{-6} mol/l (half closed triangles); 10^{-4} mol/l (closed tirangles); control (open triangles). *C*: Effect of chloroquine, 10^{-5} mol/l (closed symbols) on I_{si} and on I_f (*D*). Open symbols indicate control values.

Table 1. Effects of mepacrine and chloroquine on ionic currents

Compound	I_{si} (%)	I_f (%)	E_{si} (%)	E_f (%)
MEPACRINE (mol/l)				
10^{-7} ($n = 3$)	75 ± 5	94 ± 1	104 ± 0.5	96 ± 4
10^{-6} ($n = 3$)	46 ± 10)	81 ± 3	95 ± 1	102 ± 2
10^{-5} ($n = 2$)	0	—	—	—
10^{-4} ($n = 3$)	0	38.4 ± 3	—	81 ± 6
CHLOROQUINE (mol/l)				
2.10^{-6} ($n = 3$)	100 ± 2	84 ± 6	100 ± 2	91 ± 3
10^{-5} ($n = 9$)	52 ± 6	75 ± 2	88 ± 3	82 ± 4

Control peak slow (I_{si}) and fast (I_f) currents and reversal potentials E_{si} and E_f were compared with those measured 10 min after the addition of the respective drug to the bathing solution. Values are mean percentages ± SEM of the respective controls.

Porotikov 1983). The resting potential in control fibres was taken as zero, the membrane potential was measured as the deviation from the resting potential. Slow inward calcium currents (I_{si}) were recorded during the test clamp pulses immediately after a 100 ms conditioning prepulse 40 mV positive to the resting potential. The amplitude and the duration of the conditioning prepulse were sufficient to inactivate fast sodium current I_f (Kohlhardt et al. 1972; Reuter 1973 1979; Filippov and Porotikov 1983). I_{si} and I_f were measured as the difference between the peak and the steady current 70—100 ms after switching on the test clamp pulse (Horackova and Vassort 1976). Stimulation of the preparation, data recording and processing were performed automatically using an SM-3 computer system and CAMAC-modules (Polon, Poland).

The solutions used contained (in mmol/l): Ringer solution (pH = 7.5) — NaCl 110, KCl 2.5, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 2.4, glucose 5.5; isotonic sucrose solution 230. The solutions were not gassed. All experiments were carried out at room temperature (18—20 °C). In these conditions the activity of the preparations maintained stable during 1—1.5 hours.

Mepacrine was from Serva, chloroquine was kindly provided by Prof. L. Szekeres (Szeged, Hungary).

Results

The chemical structure of drugs is shown in Fig. 1. In 16 experiments mepacrine (10^{-7}—10^{-6} mol/l) superfusion for 10—15 min produced a significant dose-dependent decrease of action potential duration measured at 50% and 90% repolarization without affecting the resting potential (data not shown). This suggested that the agent could have influenced some ionic currents responsible for the generation of action potential.

Voltage-clamp experiments were carried out to check this possibility. The

results are summarized in Table 1. In these experiments mepacrine decreased the calcium current I_{si} already at 10^{-7} mol/l, and the effect was dose-dependent. An absolute block of I_{si} was observed at 10^{-5} mol/l. The effect on the fast sodium current I_f was less pronounced: a small decrease was observed at 10^{-6} mol/l, and no absolute block was obtained even at 10^{-4} mol/l. Fig. 2*a*, *b* show an example of the mepacrine effects on I_{si} and I_f. The reversal potentials of the slow and the fast inward current were determined by extrapolating the current-voltage characteristics to the intercept with the potential axis. Obviously, 10^{-7}—10^{-6} mol/l mepacrine did not in fact change the reversal potential of either current; at 10^{-4} mol/l however, it decreased the reversal potential of the fast inward current. The threshold voltage of I_f increased at 10^{-4} mol/l (Fig. 2*b*). The effect on this parameter was further not studied.

All the above effects were partially reversed upon washing the fibres with drug-free Ringer solution for 15 min.

Qualitatively, the effect of chloroquine resembled that of mepacrine as far as the inward currents are concerned; however, higher concentrations were required to obtain the same effects. The drug had practically no effects even at $2 \cdot 10^{-6}$ mol/l. Significant activity was observed only at 10^{-5} mol/l. Similarly as mepacrine decreased I_{si} more than it did I_f. A nearly complete restoration of I_f was observed upon a washout for 20 min whereas I_{si} restored only partially. Fig. 2*c*, *d* show typical effects of the agent on the inward currents.

Discussion

In our experiments mepacrine and chloroquine were shown to inhibit I_{si} in frog atrial membrane at concentrations much lower than have been reported for the inhibition of phospholipase A_2 in various tissues by both compounds, or for the interaction of mepacrine with membrane phospholipids. If membrane phospholipids are not affected by the drug, the effects observed can be suggested to result from some direct action of mepacrine on membrane protein components, i.e. ionic channels or regulating enzymes. Special experiments with radioligands are required to answer this question directly.

It should be stressed that at 10^{-7}—10^{-6} mol/l the primary effect of mepacrine is inhibition of the calcium current. This inhibition is not due to a decrease of the transmembrane electrochemical gradient since the reversal potential for calcium current remained practically unchanged. The drugs seem to affect conductance properties of Ca channels, and this can be investigated by patch-clamp experiments. The Ca antagonist potencies of mepacrine and chloroquine can be compared with those of the conventional Ca^{2+} channel blockers: at similar experimental conditions dihydropyridine Ca channel antagonists nimodipine

and nitrendipine produce half-block of Ca current in cardiac cells at $\sim 10^{-6}$ mol/l (Bean 1984; Sanguinetti and Kass 1984). This value is approximately equal to effective mepacrine concentration, and 10-fold less than the respective value for chloroquine. It is, in some way, the pyridine ring in the molecule of all these compounds that may be responsible for Ca channel block (Mannhol et al. 1982). If we compare the effects and the chemical structure of mepacrine and chloroquine the first impression is that the more voluminous hydrophobic substituent in the pyridine ring of the molecule increases the activity. This can be the consequence of an easier penetration of the molecule through the phospholipid membrane to the active binding sites. However other analogs must be studied to provide basis for speculations on this possibility.

In addition to the inhibition of the calcium current both agents produced a weaker decrease of the fast sodium current, I_f. The partial inhibition of I_f can be explained by a decrease of the reversal potential E_f that may indicate an elevation of external Na concentration. The decrease of the fast sodium current is typical for class I antiarrhythmic drugs (Vaughan and Williams 1981). To some extent, the clinically well known antiarrhythmic properties of chloroquine may be related to drug-induced decrease of the fast sodium current. However, effective concentrations of the compounds for mammalian tissues can differ from those for frog heart.

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