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European Journal of Pharmacology 304 (1996) 237–241

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Amiodarone affects membrane water permeability properties of human erythrocytes and rat mitochondria

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Received 31 July 1995; revised 13 February 1996; accepted 16 February 1996

Abstract

Dose-dependent water exchange times and intracellular water contents were measured by NMR (nuclear magnetic resonance) in erythrocytes and mitochondria interacted with the anti-anginal and anti-arrhytmic agent, amiodarone. Addition of the drug up to 26 μM yielded 80% enhancement of the water exchange rate in erythrocytes at 37°C and 41% enhancement at 22°C with 40% and 9%, respectively, increases in the intracellular water content. Similar enhancements were obtained in mitochondria at 22°C. The data suggests a somewhat higher affinity of amiodarone to mitochondrial than to erythrocyte membranes.

Keywords: Amiodarone; Water exchange; NMR (nuclear magnetic resonance); Membrane

1. Introduction

Amiodarone hydrochloride (2-butyl-3-benzofuranyl 4-[2-(diethylamino)-ethoxy]-3,5-diiodophenylketone hydrochloride is a potent anti-anginal and anti-arrhythmic drug (Fig. 1). Most cardiovascular drugs are cationic amphiphilic molecules with a lipophilic aromatic ring system and a hydrophilic amino-substituted side-chain, usually protonated at neutral pH. The cardioactive group of drugs includes such molecules as β-adenoreceptor agonists and antagonists.

Several effects have been described in the literature dealing with the interaction of the amphiphilic amiodarone molecule with the lipid core of membranes (Chatelain, 1991). These include inhibition of ATPase activity in rat brain synaptic membranes (Chatelain et al., 1985), inhibition of lysosomal lipid catabolism (Hoestetler et al., 1986; Palmieri et al., 1991), modification of potassium permeability and activation of passive swelling of mitochondria (Da Chuna Pimental de Meira Guerreiro et al., 1986a,b), effects on mitochondrial metabolism (Fromenty et al., 1990; Manneschi and Federico, 1992), inhibition of multi-conductance channel in mitochondrial membranes (Antonenko et al., 1991a; Antonenko et al., 1991b; Zorov et al., 1992). High affinity was also demonstrated for the erythrocyte membrane, where preferential intercalation in the inner hemileaflet of the lipid bilayer was suggested (Reinhart and Rohner, 1990), yielding a stomatocytic transformation on acute exposition or an increase in the cholesterol contents on chronic treatment.

Conformational analysis of amiodarone in a simulated membrane water environment gave evidence of an inverted-cone shape as compared with the cone-shaped structure of lyso-phosphatidylcholine (Chatelain and Brasseur, 1991), such that a stable cylindrical structure was obtained by the association of the two cones.

Alteration of the lipid trimethylammonium groups by amiodarone was shown by NMR (nuclear magnetic resonance) experiments (Jendrasiak et al., 1990) and confirmed by determination of liposome size (Sautereau et al., 1992). In this last case, incorporation of the drug within the lipid core was suggested as explanation for the favoured drug-photoinduced peroxidation of lipids.

Since the NMR properties of intracellular water have long been known to be affected by changes induced by effectors at the membrane surface, we measured cell water NMR proton relaxation in either erythrocytes or mitochondria upon treatment with different doses of amio-

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PII S0014-2999(96)00148-3

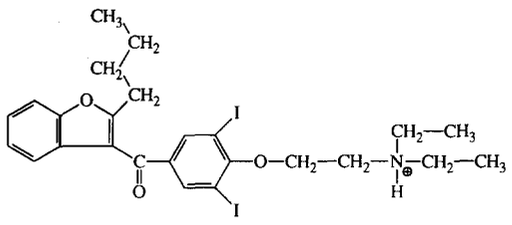

Fig. 1. Amiodarone hydrochloride (2-butyl-3-benzofuranyl 4-[2-(diethylamino) ethoxy]-3-,5-diiodophenylketone hydrochloride.

darone in order to ascertain the effects on membrane permeability.

2. Materials and methods

2.1. Materials

Amiodarone, glucose, sodium chloride and *N*-2-hydroxyethyl-piperazine-N′-2-ethane sulfonic acid (HEPES) were obtained from Sigma and used without further purification. Manganese chloride tetrahydrate was obtained from Carlo Erba (Italia).

2.2. Methods

2.2.1. Erythrocytes

Blood was collected into heparinized tubes by venipuncture of healthy individuals and stored at 4°C. The samples were centrifuged at $1000 \times g$ for 5 min; the erythrocytes were washed 3 × with 150 mM NaCl, 5 mM HEPES and 10 mM glucose, resuspended to 45% haematocrit with a solution containing 40 mM $MnCl_2$ and 95 mMNaCl. NaCl was added in order to adjust the osmolarity of the solution, even though the diffusional water permeability was shown to be little affected (Chien and Macey, 1977). The cell suspension was kept in a warm bath (22 or 37°C) for 2 min, then the NMR sample was prepared by adding the desired amount of amiodarone (5.8–50 μM). The samples were incubated for 5 min before NMR measurements. The washed erythrocyte model was chosen because amiodarone may strongly interact with serum proteins (Lalloz et al., 1984). NMR experiments on amiodarone-free erythrocytes were carried out on cells washed 3 ×, resuspended in the medium and centrifuged at $1000 \times g$ for 30 min. The top buffer-saline solution was removed and packed cells were used.

2.2.2. Mitochondria

Mitochondria were isolated from adult (250 g) male Wistar rats decapitated after a rapid anaesthesia by 1 atm of CO. The liver (5–6 g) was removed and immediately placed into-ice cold homogenization medium (225 mM mannitol, 75 mM sucrose, 10 mM Tris-HCl, 100 mM EDTA potassium salt, pH 7.4), chopped and washed several times. Homogenization was carried out with a glass-Teflon homogenizer (B-Braun Homogenizer) with 6 up-and-down strokes of the pestle rotating at 500–1000 rpm. The homogenate was centrifuged at $1000 \times g$ for 5 min in a Beckman J2-21 centrifuge. The supernatant was carefully removed and centrifuged at $10\,000 \times g$ for 10 min. The pellet was resuspended and centrifuged at $1000 \times g$ for 5 min and the supernatant was again centrifuged at $10\,000 \times g$ for 10 min. The final pellet contained relatively pure mitochondria.

2.2.3. NMR measurements

^{1}H-NMR experiments were carried out with a low-resolution home-made spectrometer equipped with a 2.1 T Bruker magnet and a Stelar console that allows measurements at the three different ^{1}H-Larmor frequencies of 10, 30 and 90 MHz. Spin-spin relaxation rates were measured with the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, collecting 32 echoes ranging from 0.2 to 6 ms (erythrocytes) or from 1 to 30 ms (mitochondria) with 100 and 500 μs as the interecho times, respectively. T_2 values were calculated by biexponential regression analysis of the decay curves of the water proton transverse magnetization. The error was evaluated at ±5%.

3. Results

Cell water transport rates can be measured by the ^{1}H-T_2-NMR technique with the CPMG pulse sequence applied in the presence of a membrane-impermeable paramagnetic relaxation agent. The technique, as first described by Conlon and Outhred (1972) and Conlon and Outhred (1978), has been the basis for investigations in whole blood, washed erythrocytes (Morariu et al., 1985, 1986) and resealed erythrocyte ghosts (Benga et al., 1987). The method has been reviewed recently (Chao and Butterfield, 1990).

In the ^{1}H-T_2-NMR method, the paramagnetic agent (usually Mn^{2+}) is added to provide a magnetic label for the extracellular compartment. It is assumed that Mn^{2+} ions do not penetrate the cell so that the extra- and intracellular environments can be distinguished in the NMR relaxation experiment. The spin-spin relaxation rates of intra- (R_{2int}) and extracellular (R_{2ext}) water protons can then be measured with the CPMG pulse sequence. The decay of the echo envelope is initially dominated by the rapid decay (R_{2ext}) of the Mn^{2+}-labelled extracellular compartment which ends within 1 ms. Thereafter the decay of the remains of intracellular water (R_{2int}) is much slower and has a contribute by the water exchange rate τ_{ex}^{-1}. This latter is calculated from the following simplified equation:

$$\tau_{ex}^{-1} = R_{2f} - R_{2a} \quad (1)$$

where R_{2a} is the natural spin-spin relaxation rate of intracellular water (without contributions from exchange) in packed drug-free cells.

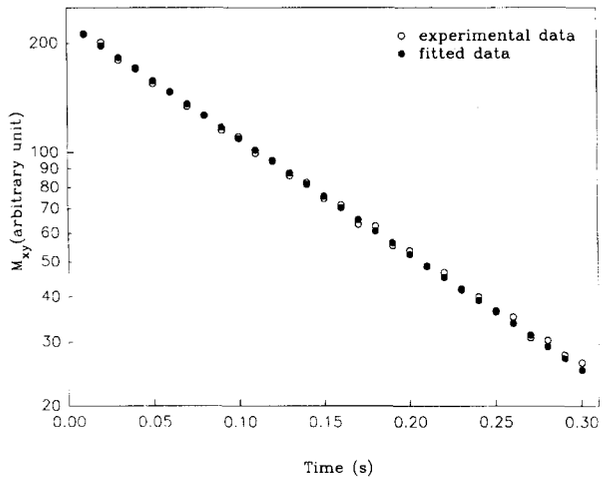

Fig. 2. Decay of transverse magnetization of water protons in packed erythrocytes. $T = 37°C$.

The monoexponential behaviour measured in packed erythrocytes at 37°C is shown in Fig. 2 where from the R_{2a} value of $7.35 \pm 0.60\ s^{-1}$ was calculated and was in fairly good agreement with those in previous reports (Conlon and Outhred, 1978).

The decay of the echo envelope in Mn^{2+}-labelled erythrocytes is shown in Fig. 3. Application of Eq. [1] to the spin-spin relaxation rate of intracellular water yielded an evaluation for the exchange rate of $137 \pm 11\ s^{-1}$ at 37°C. The effects brought about by the presence of increasing amounts of amiodarone are also shown in Fig. 3. R_{2int} is enhanced by the drug in a dose-dependent fashion, yielding evidence of a faster exchange rate of water protons. Such rates are plotted against the concentration of amiodarone at two temperatures in Fig. 4. It can be noticed that amiodarone causes a substantial increase in the water exchange rate, such that at the highest concentration (29 μM), 80% enhancement is observed at 37°C, and a smaller increase at 22°C.

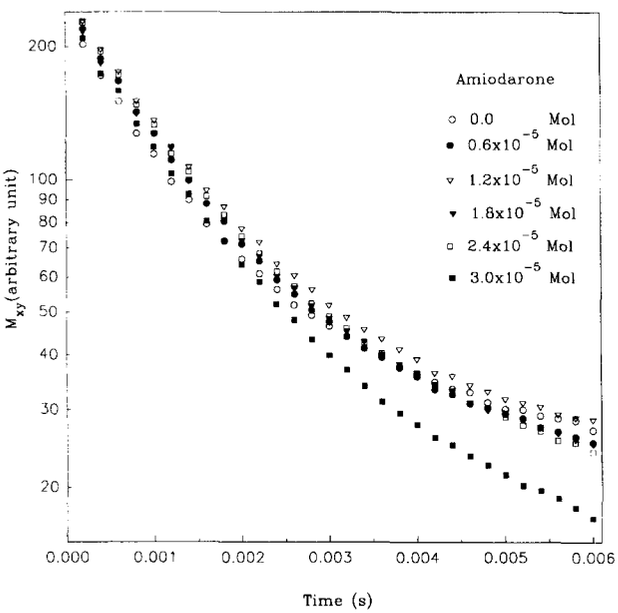

Fig. 3. Decay of transverse magnetization of water protons in suspensions of erythrocytes labelled with $MnCl_2$ 40 mM in the presence of various concentrations of amiodarone. $T = 37°C$.

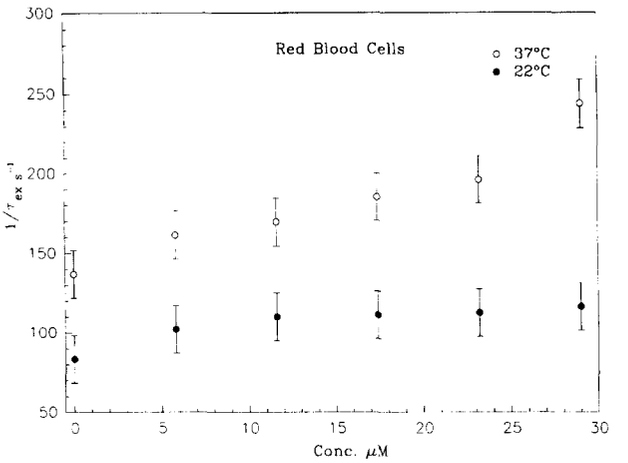

Fig. 4. Water exchange rates, τ_{ex}^{-1} (see text for details), calculated for suspensions of erythrocytes labelled with $MnCl_2$ 40 mM vs. the concentration of amiodarone at 37 and 22°C.

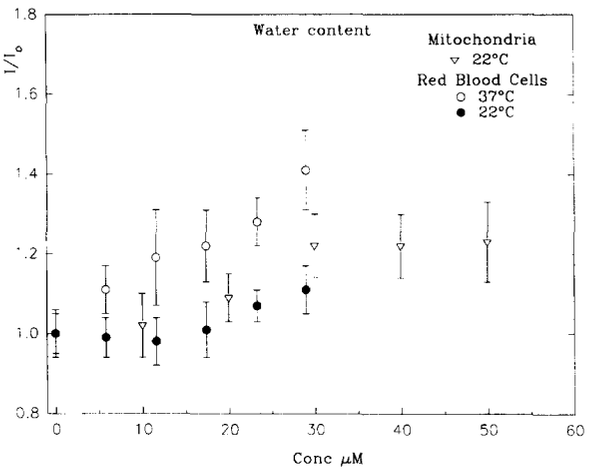

Fig. 5. Fractional increase of water content (I_o is the extrapolated value in drug-free erythrocytes and mitochondria) vs. the concentration of amiodarone at 37 and 22°C.

The fitting procedure of the biexponential curves also yielded the intercepts of the two decay rates which are proportional to the fractions of water protons in the two compartments. The change in fractional population of intracellular water upon addition of amiodarone could, therefore, be evaluated (Fig. 5) at the same two temperatures.

4. Discussion

It is evident that the drug causes a dose-dependent increase in cell volume, which was more pronounced at the higher temperature.

The fact that raising the temperature yields larger values of τ_{ex}^{-1} may not be exclusively due to enhanced water permeation rates, since lipid mobility and the exchange regime experienced by water protons may all contribute to the observed relaxation behaviour (Morariu et al., 1986). However, the increase in cell volume confirms what has been demonstrated by electron microscopy (Reinhart and Rohner, 1990) of erythrocytes that had been shown to change shape upon exposure to amiodarone at room temperature.

Transverse relaxation experiments were also carried out with suspensions of rat liver mitochondria. In the absence of reference data, cell suspensions were labelled with either $MnCl_2$ or $Mn(EDTA)^{2-}$ in order to ascertain whether Mn^{2+} ions penetrate the intracellular compartment. Since the paramagnetic effect is mainly due to the number of water molecules in the first coordination shell of the metal ion, the two paramagnetic probes affect the water proton relaxation rates differently. As a consequence, higher concentrations of $Mn(EDTA)^{2-}$ are expected to reproduce the relaxation behaviour obtained with $MnCl_2$ 40 mM. Since, once normalized for concentration, superimposable decay curves were obtained, it was concluded that Mn^{2+} ions are not likely to enter the mitochondrial inner space.

The same experiments as with erythrocytes were then carried out at different concentrations of amiodarone, showing the dose dependence of the change in cell volume (Fig. 5) and in the decay rate of intracellular water protons Fig. 6. Amiodarone caused a relatively greter increase in cell volume, than in erythrocytes at the same temperature. The difficulties related to obtaining packed mitochondria did not allow us to measure the intrinsic decay rate of intracellular water. However, the dose-dependent enhancement of the spin-spin relaxation rate of intracellular water protons in suspensions of mitochondria (from 34.5 ± 2.1 to 76.9 ± 4.2 s^{-1} at concentrations higher than 40 μM) is

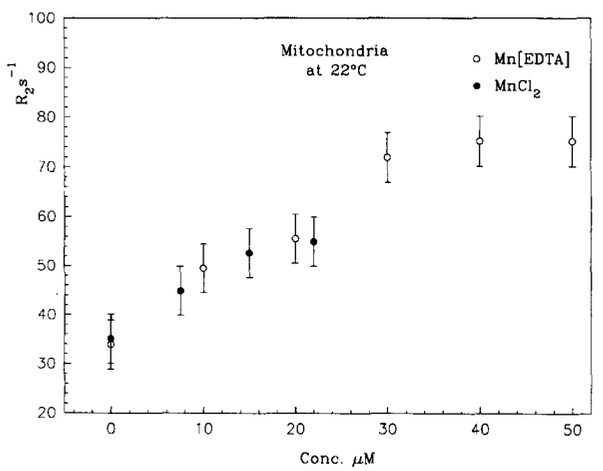

Fig. 6. Transverse relaxation rates of intracellular water protons in suspensions of rat liver mitochondria labelled with either $MnCl_2$ 40 mM or $Mn(EDTA)^{2-}$ 200 mM vs. the concentration of amiodarone. $T = 22$°C.

itself sufficient to cause a substantial increase in the water permeation rates.

All these features may be related to the interaction of the amphiphilic drug molecule with either the erythrocyte or the mitochondrion membranes. This is the key step for determining the observed changes in water permeation rates and in cell volumes, as detected by water proton transverse relaxation rates measured in the presence of paramagnetic labels of the outer compartment. The experimental findings do not allow the determination of whether amiodarone succeeds in affecting the water permeation rate by modulating the mobility of the lipidic core or by interacting directly with the protein channel. However, the fact that the drug is able to block all the channels tested in mitochondria strongly supports an indirect rather than a direct interaction. The NMR results seem to suggest that amiodarone preferentially interacts with the mitochondrial rather than with the eythrocyte membrane, at least as far as the properties of cell water are concerned.

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