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ACTION OF PROPRANOLOL ON MITOCHONDRIAL FUNCTIONS—EFFECTS ON NON-ENERGIZED ION FLUXES

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Abstract—Propranolol, a β-blocking agent, increased the permeability of the inner mitochondrial membrane to the monovalent cations potassium and sodium. It also increased the net uptake of chloride and nitrate anions, but hardly affected the uptake of acetate, phosphate or sulphate. When bound to the membranes the drug released hydrogen ions. In solutions containing potassium chloride, valinomycin plus FCCP propranolol increased the apparent buffering capacity of mitochondria. These changes are interpreted to be the result of a change in the membrane charge distribution and of perturbation of the membrane structure.

Propranolol (1-isopropylamino-3-(1-naphtoloxy)-propran-2-ol hydrochloride) is widely used as a β-blocking antiarrhythmic drug. It is a lipophilic base (p*K* 9·45) with a potent local anaesthetic action [1, 2]. Under certain conditions local anaesthetics, structurally related to propranolol, decrease the permeability of phospholipid bilayers and liposomes to monovalent cations and compete with Ca^{2+} ions for binding sites [3]. Propranolol increases the exchange and efflux of potassium ions through the red-cell membrane when calcium ions are present [1].

Local anaesthetics are thought to decrease the flux of potassium through mitochondrial membranes [4]. They competitively inhibit the binding of cations to the membranes [5], but it has also been suggested that they stimulate the binding and transport of cations [6–8]. In oscillatory conditions propranolol and some local anaesthetics have been reported to affect mitochondrial ion fluxes, dampening them so that the mitochondria stay in an energy-dependent, swollen state [11].

The aim of this study was to obtain more detailed information on the interaction of propranolol with mitochondria and to study the alterations in mitochondrial permeability in non-energized conditions.

MATERIALS AND METHODS

Rat-liver mitochondria were isolated by the Schneider procedure in 0·25 M sucrose [12]. Mitochondrial protein was determined by the Lowry method [13], with bovine serum albumin as a standard. This method gives values about 20 per cent lower than the biuret procedure. The control ratios of the mitochondria exceeded 4 when succinate was the substrate. Potassium was determined with a Perkin–Elmer Model 300 atomic absorption spectrophotometer.

The changes of pH were followed with a Beckman microtip electrode connected to an EIL Vibron Model 33 B potentiometer and to a Goertz model R E 541 recorder. The changes in mitochondrial volume were monitored optically (as a change in transmission) with an Aminco Chance dual wavelength spectrophotometer, one wavelength at 630 nm, the other excluded, or with an Aminco DW 2 spectrophotometer using a split-beam against air at 630 nm.

FCCP was a gift from Dr. Heytler. X464 (Hoffman–La Roche, Basel, Switzerland), which is though to be identical to nigericin, was a gift from Dr. Harned of the Eli Lilly Co., Indianapolis, Indiana, U.S.A. Propranolol (Inderal®) was given by I.C.I., Macclesfield, England. Valinomycin was purchased from Calbiochem, Luzern, Switzerland.

To determine the potassium content of the mitochondria, samples were withdrawn at the times indicated and centrifuged rapidly through a silicone layer (Wacker ÖL A R 100) into 8 per cent perchloric acid with a Beckman 152 microfuge. The supernatant was used for the determination of potassium.

RESULTS

Effect of propranolol on mitochondrial swelling. The net movements of solutes across the inner mitochondrial membrane can be conveniently followed by measuring the volume changes optically [10].

The effect of propranolol on swelling was studied mainly in media containing potassium phosphate, potassium acetate or potassium chloride (Fig. 1). In all these solutions propranolol increased both the rate and the extent of the swelling. In media containing chloride or phosphate the swelling seemed to be similar, although the permeability of the inner membrane of mitochondria to chloride is usually thought to be small in non-energized conditions. The magnitude of the changes were roughly proportional to the concentrations of propranolol. In potassium acetate propranolol had less effect than in phosphate or chloride. In this case there was an initial phase of rapid swelling, the extent of which depended on the con-

Abbreviations used: FCCP, Carbonyl-cyanide-*p*-trifluoromethoxy phenylhydrazone; Hepes, *N*-2-hydroxymethylpiperazine-*N*-2-ethane-sulphonic acid.

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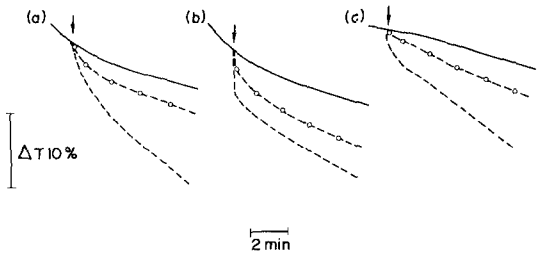

Fig. 1. Effect of propranolol on mitochondrial swelling in various potassium solutions. (a) 0·15 M KH_2PO_4, (b) 0·15 M potassium acetate, (c) 0·15 M KCl, in the presence of 2 μM rotenone and 1 mM KCN. The arrow indicates when propranolol or ethanol (control) was added: ——, control; O—O—O, 160 μM propranolol; – – –, 480 μM propranolol. Mitochondrial protein was 1·2 mg/ml, the solutions were buffered with 0·02 M Hepes, and pH was adjusted to 7·2 with KOH.

centration of the drug. This was followed by a slow swelling, the rate of which did not differ significantly from that of the control (Fig. 1b). In potassium malate, propranolol did not cause any swelling in the absence of phosphate. Addition of ammonium phosphate initiated swelling, the rate being higher in the presence of propranolol.

In the absence of electrolytes (0·25 M sucrose) propranolol was not able to cause a change in the transmission of the mitochondrial suspension.

The effect of propranolol in sodium chloride or sodium malate was essentially the same as in solutions of the potassium salts. In sodium phosphate and sodium acetate the extent of the swelling was greater in the controls [14], although with these salts, too, propranolol further increased the swelling.

Mitochondria swollen in 0·15 M potassium phosphate, pH 7·2, were analyzed for their potassium content (Table 1). The results were in agreement with the data of the swelling experiments. Propranolol increased the mitochondrial potassium content considerably.

Propranolol-induced production of hydrogen ions. The addition of propranolol induced a rapid release of hydrogen ions into the solution (Fig. 2a). This was followed by a rather rapid consumption of the hydrogen ions, so that about one-third of the ions released disappeared from the medium in 2 min. Addition of 400 nmoles of propranolol caused the release of 60 nmoles of hydrogen ions. With further additions

Table 1. Effect of propranolol on mitochondrial potassium content

Time (min)	Mitochondrial potassium (nmol/mg protein)	
	Control	Propranolol
0	870	870
5	920	1120
15	1080	1380

Mitochondria were incubated at 30° in 0·15 M KH_2PO_4, pH adjusted to 7·2 with KOH, 2 μM rotenone, 1 mM KCN, 640 μM propranolol, when present. Mitochondrial protein was 3·5 mg/ml (for the samples, see Methods). The values are arithmetical means of three separate determinations.

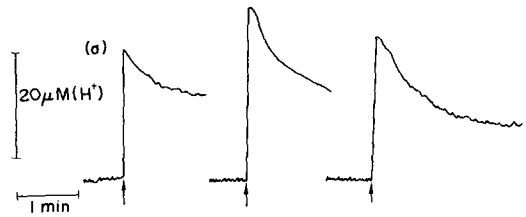

Fig. 2 (a). Production of hydrogen ions by addition of propranolol. The solution contained 0·075 M KCl, 0·05 M sucrose, and 10 μM rotenone and 2 mM KCN. Mitochondrial protein was 6·2 mg/ml. In the first curve the amount of propranolol added was 400 nmoles, in the next 800 nmoles, and in the last 1600 nmoles. The amounts were added successively; after each addition the pH was re-adjusted to 6·9 with KOH.

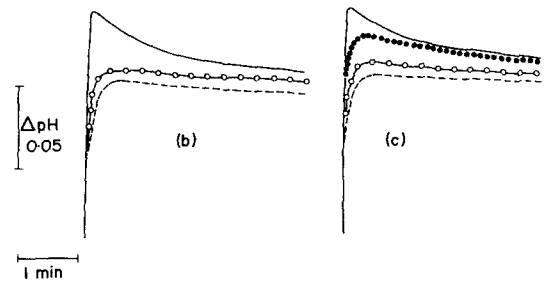

Fig. 2 (b) and (c). Effect of propranolol on the equilibration of added HCl pulses. The solution was as in Fig. 2a. Mitochondrial protein was 5·0 mg/ml. The amount of HCl added was 300 nmoles. (b) ——, Control; O—O—O, 2·5 μM FCCP and 0·8 μg valinomycin were added; – – –, FCCP; valinomycin and 1·8 mM propranolol were added. (c) ——, control; · · · · · ·, 460 μM propranolol; O—O—O, 920 μM propranolol; ——, 1·8 mM propranolol. The pH in these experiments was adjusted to 6·9 with KOH before each addition of the acid.

of 1·2 μmoles and 2·4 μmoles, respectively, 140 and 200 nmoles of hydrogen ions were liberated into the medium (cf. Ref. 15). This indicates a gradual saturation of the binding sites.

Effect of propranolol on the consumption of an added acid. The permeability of the inner mitochondrial membrane was studied in non-energized conditions by measuring the pH changes after additions of small amounts of hydrochloric acid [16, 17]. As shown in the control experiment in Fig. 2b, part of the added acid pulse was consumed by the mitochondria. The rate of this reaction is thought to be limited by the exchange of cations against hydrogen ions [17]. The hydrogen ion consumption indicates the inside buffering capacity [17]. In the presence of valinomycin plus FCCP, the protons penetrate rapidly. The recorded pH reaches the level determined by the buffering capacity immediately [17]. This level was lowered by propranolol (Fig. 2b), indicating an apparently increased buffering capacity. The same kind of effect is also seen in Fig. 2c, where different concentrations of propranolol were used (no valinomycin or FCCP added). The extent of the initial pH rise was reduced, too, indicating a more rapid equilibration.

Figure 3 shows that propranolol increased the swelling in the presence of FCCP or FCCP plus valinomycin in this solution (buffered with 0·02 M Hepes, pH 6·9). In the presence of valinomycin the

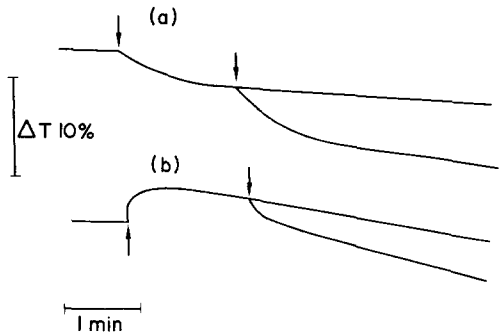

Fig. 3. Effect of propranolol on mitochondrial volume changes in uncoupled conditions. The medium consisted of 0·075 M KCl, 0·05 M sucrose, 1 μM rotenone, and 1 mM KCN. In the upper part the first arrow indicates the addition of 1·6 μM FCCP, in the lower part the addition of 1·6 μM FCCP and 0·4 μg valinomycin. The second arrow shows the addition of 320 μM propranolol. Mitochondrial protein was 1·4 mg/ml. pH was adjusted to 6·9 with HEPES buffer.

stimulation caused by propranolol was almost the same as in the presence of FCCP (not shown).

Effect of propranolol on anion permeabilities. When potassium permeation is promoted by addition of sufficient amounts of the ionophore nigericin the rate of swelling is limited by anion entry. Under these conditions propranolol did not cause further swelling in potassium acetate or potassium phosphate, but in potassium chloride swelling was considerably stimulated (Fig. 4a).

The permeability of mitochondria to anions was studied in the presence of solutions of various ammonium salts, after the addition FCCP to increase hydrogen ion permeation. Stimulation by propranolol was maximal in ammonium chloride (Fig. 4b) and ammonium nitrate (not shown). In the presence of sulphate swelling was stimulated slightly. The rate of the swelling was rapid even in the absence of propranolol [14].

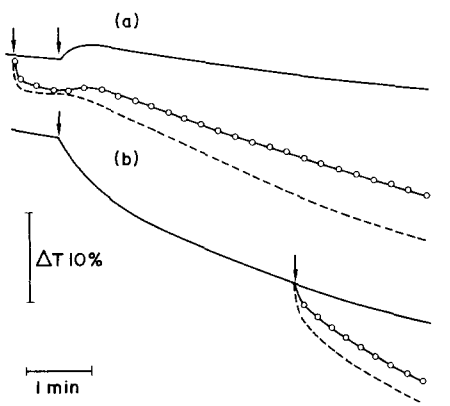

Fig. 4. Effect of propranolol on the swelling of rat liver mitochondria limited by the penetration of anions. (a) The solution consisted of 0·15 M KCl, 0·02 M Hepes, 2·0 μM rotenone and 1 mM KCN, adjusted to pH 7·2 with Tris. The first arrow indicates the addition of propranolol: O—O—O, 220 μM; – – – –, 480 μM; —— control. The second arrow indicates the addition of nigericin (0·5 μg/ml). (b) Solutions consisted of 0·15 M ammonium chloride and 0·02 M Hepes adjusted to pH 7·2 with Tris. The other additions were 2·5 μM rotenone, and 1 mM KCN. Swelling was initiated with 16 μM FCCP (first arrow); the second arrow shows where propranolol was added: O—O—O, 160 μM; – – – –, 480 μM.

DISCUSSION

In this study we found that propranolol increases the permeability of mitochondria to electrolytes. This effect was noticeable with ionic species that are comparatively poor penetrants of mitochondria.

Propranolol also had direct effects upon the membrane. When it was bound, hydrogen ions were released and the buffering capacity of the membrane was apparently increased.

Consequences of the binding of propranolol. In the physiological pH range propranolol has one positive charge. The hydrophobic part of the molecule may be assumed to penetrate the hydrophobic area of the membrane, while its charge is attracted to negative charges in and on the membrane. This will naturally cause structural alterations and reorganization of the charge distribution in the membrane. Indeed, addition of propranolol caused rapid changes in the optical properties of the mitochondria. Because such changes do not occur in sucrose, they presumably do not reflect primary structural alterations, but swelling is associated with reequilibration of ions across or in the membrane.

Rapid alterations were also seen in the hydrogen ion concentration of the solution when propranolol was added. This rapid production of hydrogen ions was followed by a partial consumption. The proton production was probably caused by the binding of the drug, which displaced the hydrogen ions. The consumption of hydrogen ions was initially more rapid than with a pure acid pulse (HCl), which suggests a component due to changes in the membrane, in addition to the diffusion of hydrogen ions.

Effects on permeation of ions. The acid pulse experiments indicated that hydrogen ion permeation increased in the presence of propranolol. This may have been due to a primary effect on hydrogen ion permeation, but also to effects on the exchange of hydrogen ions with potassium ions or to changes in chloride permeation.

The rate of swelling is stimulated by propranolol in the presence of FCCP (Fig. 3). This suggests that propranolol does not increase hydrogen ion permeation directly, but exerts its effect via an increase of membrane permeability to cations and/or anions.

When hydrated, the potassium ion has a smaller radius than the sodium ion, whereas the opposite is true of the unhydrated ions. This suggests that the penetration is determined by the unhydrated radius of the ions. Hence it is probable that these monovalent cations interact with specific components of the membrane (either some carriers or specific pores). Possibly, propranolol exerts its effect by promoting such interactions.

The net permeation of chloride and nitrate through mitochondrial membranes is usually thought to be low, although Southard *et al.* have recently suggested a ready penetration coupled to the permeation of potassium ions [20]. One possible explanation for the stimulation is that propranolol forms an ion pair with the anion (cf. Ref. 21). In that case, propranolol might

serve as an anion carrier. Another possibility is that the characteristics of the membrane are changed so that even the anions of strong acids are able to penetrate.

Buffering capacity. As shown in Fig. 4a, propranolol increased the apparent buffering capacity of the mitochondrial membranes. This indicates either a change in the pK values of the dissociable groups, or an increase in the number of groups accepting hydrogen ions. It is interesting that an anion, anilinonaphthalene sulphonate, exerts a similar effect [16].

In this paper we reported a number of changes in the properties of mitochondrial membranes brought about by propranolol. Probably, they resulted from the interaction of propranolol with phospholipids [22]. This would change the charge distribution in the membrane and cause perturbation of the water-membrane interphase and the hydrophobic core of the membrane.

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