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ACTION OF PROPRANOLOL ON MITOCHONDRIAL FUNCTIONS—EFFECTS ON ENERGIZED ION FLUXES IN THE PRESENCE OF VALINOMYCIN

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Abstract—The effects of propranolol on mitochondrial ion fluxes have been studied in oscillatory conditions in the presence of potassium plus valinomycin. The drug was able to decrease slightly the rate of swelling and that of passive contraction a little more. When the concentration of external salt was below 10 mM, propranolol decreased the extent of swelling; above 15 mM it was increased by the drug. The inhibition of contraction was abolished by nigericin or raised pH and was less evident with ATP rather than succinate as the source of energy. Propranolol only slightly affected the oxidative phosphorylation and latent ATPase activity of mitochondria. At low concentrations of salts the rate of valinomycin-stimulated respiration and ATPase activity were both inhibited. At higher concentrations of the salts, both were stimulated. It is suggested that the stimulation of respiration and ATPase activity result from increased ion uptake. Propranolol seems to stabilize an "energized" state, possibly by inhibiting the entry of protons, thereby retarding the equilibration of ionic gradients across the inner membrane.

Propranolol (1-propylamino-3(1-naphtholoxy)-propan-2-ol hydrochloride) is a β-adrenergic blocking drug. It also has local anaesthetic properties, suggested to be due to its lipophilic nature [1, 2]. Local anaesthetics, structurally related to propranolol, have been shown to decrease the permeability of liposomes [3] and energized mitochondria [4, 5] to monovalent cations and similar effects have been reported for propranolol [6]. The action of local anaesthetics on mitochondrial membranes has recently been suggested to be due to the stabilization of the inner membrane [7]. Papa *et al.* [8, 9, 10] concluded that local anaesthetics stimulate the oxido-reduction linked proton translocation in beef heart submitochondrial particles.

Non-respiring, non-energized mitochondria have been used to study the penetration of different solutes through the inner membrane of mitochondria [11, 12, 13]. Under these conditions we found that propranolol considerably increased the permeability of the inner membrane to potassium and chloride [14].

Energization changes the properties of the inner membrane considerably; in mitochondria and submitochondrial particles the binding of non-penetrating ions is changed, and the penetrating ions are extruded or taken up. This has been interpreted as being due to an alteration of charge distribution in the membrane by activation of an electrogenic proton pump [15–18] which creates a pH gradient and an electrogenic field across the membrane.

One way to study the changes of mitochondria from energized to non-energized states (and vice versa) is to follow the volume oscillations of their inner membrane [19–22]. Volume oscillations can be obtained by energizing the mitochondria in the presence of permeant cations and anions. Under these conditions the energization first induces an uptake of salts. Then this uptake ceases due to changes in structure and/or ion concentrations, and an uncoupled contraction follows. Several successive swelling–contraction cycles may occur.

We have now studied the interactions of propranolol with energized mitochondria to obtain information about the relationship of the changes which the drug causes in oscillatory conditions.

MATERIAL AND METHODS

Rat liver mitochondria were prepared and the measurements of protein concentration and changes of volume were performed as described earlier [14]. The respiration of mitochondria was recorded by means of a Clark-type electrode (Yellow Springs Co., Ohio) connected through an Aminco (Yellow Springs, Maryland) accessory to a recorder.

The ATPase activity was measured by stopping the reaction with perchloric acid, the final concentration being 1·2 M. The liberated inorganic phosphate was estimated with the Lowry procedure [23].

RESULTS

The effect of propranolol on respiration, oxidative phosphorylation and ATPase activity. Propranolol had only slight effects on respiration when succinate was the substrate. The rate of state 4 respiration was increased to some extent (*ca.* 30% at 500 nmoles/mg protein propranolol in sucrose medium containing

Abbreviations: FCCP, carbonyl-cyanide-*p*-trifluoromethoxy phenylhydrazone; Hepes, *N*-2-hydroxymethylpiperazine-*N'*-2-ethanesulphonic acid.

20 mM KCl). State 3 respiration, uncoupled respiration and ADP/O-ratio were almost unaffected even at this high concentration of propranolol.

Propranolol inhibited the rate of respiration in the presence of valinomycin and potassium phosphate at less than 10 mM. This inhibition was abolished, when the concentration of the salt was raised to 15 mM or more.

Propranolol did not stimulate latent ATPase activity below 500 nmoles/mg mitochondrial protein. However, in the presence of valinomycin and 30 mM potassium acetate ATPase was stimulated even at low concentrations of the drug (Fig. 1). As in the respiration experiments, an inhibition was seen at low concentrations of the salts.

Effects of propranolol on volume oscillations driven by respiration. Seppälä *et al.* [22] have shown that propranolol damps down mitochondrial volume oscillations. We studied this effect in greater detail using potassium plus valinomycin as the cation and phosphate or acetate as the anion. It can be seen in Fig. 2A that the rate of the swelling was decreased in the presence of propranolol. The mitochondria were damped down in a swollen state. After the respiration was blocked, the rate of the contraction was decreased by propranolol. It can be noted that at low concentrations of the electrolytes (Fig. 2B) propranolol inhibited the extent of swelling. This finding might be related to the inhibition of respiration and ATPase activity that occurs in similar conditions.

In the presence of phosphate the oscillatory behaviour of mitochondria was essentially similar to that in the presence of acetate. Fig. 3 shows the effect of propranolol in the presence of varying concentrations of potassium phosphate. The rates of energized swelling rose with increasing concentrations both in the presence and absence of propranolol (Fig. 3A) though

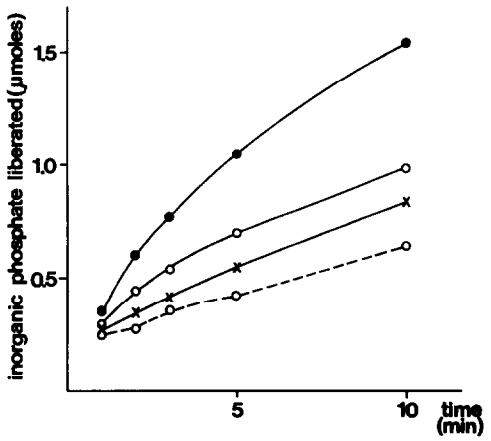

Fig. 1. The effect of propranolol on the ATPase activity. The solutions in the upper two curves were 0·12 M sucrose, 30 mM potassium acetate, 2 mM EDTA, 20 mM Hepes buffer, pH 7·2 with Tris. In the lower two curves 4 mM potassium acetate was present, and the osmolarity maintained with sucrose. Other additions: 10 μM rotenone, 1 mM KCN, 40 ng/ml valinomycin; mitochondrial protein 3·5 mg/ml. The reaction was started with 5 mM Tris–ATP. At the times indicated, double samples were drawn and mixed with perchloric acid. O–O–O and ×–×–×, controls; .–.–. and O–O–O, 500 μM propranolol.

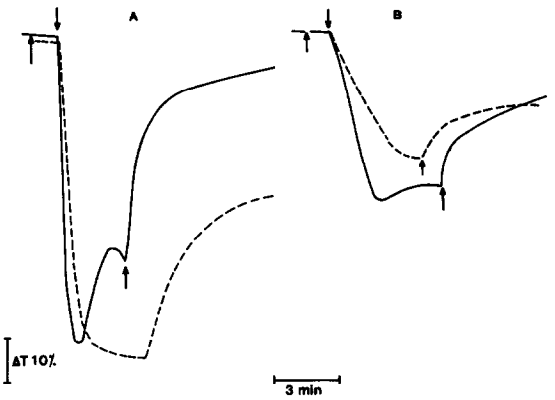

Fig. 2. The damping effect of propranolol. In (A) the concentration of potassium acetate was 30 mM and in (B) 4 mM. The osmolarity was maintained with sucrose. The other additions were 7·5 μM rotenone, 16 ng/ml valinomycin, 2 mM EDTA, 20 mM Hepes buffer, pH adjusted to 7·2 with Tris; mitochondrial protein 1·3 mg/ml. —— the control; – – – – – 160 μM propranolol. The first arrow indicates the addition of ethanol or propranolol, the second that of Tris–succinate (8 mM). The third arrow shows where potassium cyanide (1·2 mM) was added.

they were clearly lowered by the drug. The rate of contraction increased up to almost 15 mM potassium phosphate (Fig. 3C). At higher concentrations the rate was lowered in the control experiments but not in the presence of propranolol. The drug strongly inhibited the overall rate of the contraction. As in the presence of acetate, the extent of swelling cannot be explained by the changes in the relative rates of swelling and contraction.

It can be seen in Table 2 that an increase in valinomycin concentration was accompanied by progressively less effective propranolol inhibition of active swelling. Valinomycin distinctly increased the rate of passive contraction, yet, the amount of valinomycin had less effect on this process in the presence of propranolol.

The effect of pH on the inhibition of oscillation. Nigericin is known to equilibrate the pH and potassium ion gradients across the membrane. It abolished the inhibition of the contraction even in the absence of valinomycin (Fig. 4). The fast contraction induced by nigericin was followed by a slow one. The role of pH was also studied by changing the pH of the solution. As shown in Fig. 5, the inhibitory effects of propranolol on the rates of volume changes were diminished by increasing pH. The extent of swelling was still greater in the presence of the drug, however.

Effect of propranolol on oscillations driven by ATP. Uptake of ions can be driven by the energy of ATP instead of by the oxidation of succinate. The rates of ensueing changes in volumes are less influenced by propranolol (compare Fig. 6A with Fig. 2A). Yet, if succinate plus ATP are added in conditions where the respiration is inhibited, the main effects of propranolol are restored, i.e. retardation of contraction and damping down in a more expanded state (Fig. 6B). These data clearly refer to the role of anions, the release of which can be inhibited in the presence of propranolol.

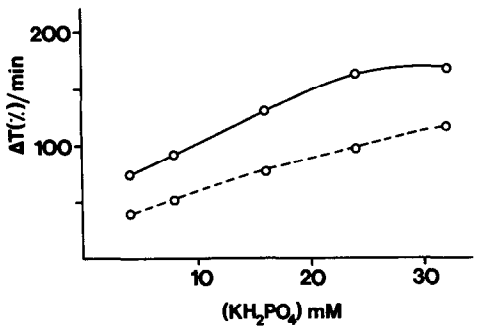

Fig. 3(A).

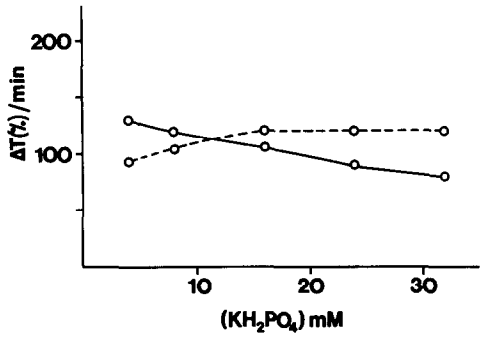

Fig. 3(B).

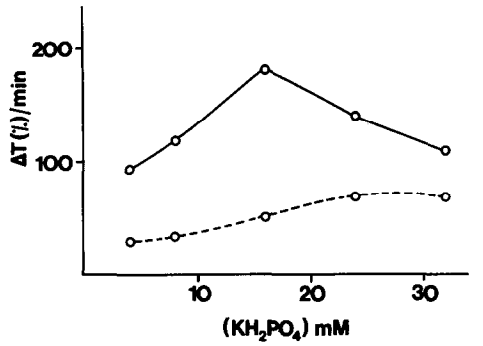

Fig. 3(C).

Fig. 3. The effect of the concentration of the salts. (A) The initial rate of swelling, (B) the extent of swelling, (C) the initial rate of contraction. The points are means of two experiments. The osmolarity was kept constant with sucrose; mitochondrial protein 1·2 mg/ml. Potassium phosphate was added as KH_2PO_4, and pH was adjusted to 7·2 with Tris. Other additions as in Fig. 2.

Table 1. The effect of varying concentrations of potassium phosphate on respiration stimulated by valinomycin

Potassium phosphate (mM)	Oxygen consumed (nmoles O min^{-1} mg $protein^{-1}$)	
	Control	Propranolol
5	104 ± 3·2	71 ± 3·2
10	125 ± 6·4	95 ± 3·7
15	108 ± 3·3	109 ± 3·7
30	102 ± 0·0	108 ± 2·9

Each solution consisted of, in addition to potassium phosphate, 20 mM Hepes buffer (adjusted with Tris to pH 7·2), 5 μM rotenone, 20 μg/ml valinomycin, 8 mM Tris–succinate, 200 μM propranolol, and sucrose sufficient to maintain constant osmolarity. Mitochondrial protein concentration was 1·2 mg/ml.

Results are means ± S.E. of three determinations.

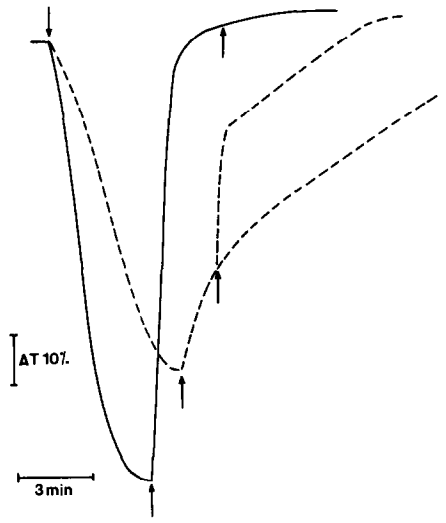

Fig. 4. The effect of nigericin on the inhibition of contraction. Conditions as in 2A, except no valinomycin was present; mitochondrial protein 1·1 mg/ml. The first arrow indicates the addition of 8 mM Tris–succinate, the second arrow the addition of KCN and the third the addition of nigericin (0·2 μg/ml).

DISCUSSION

In this study we found that propranolol had only slight effects on succinate respiration and on oxidative phosphorylation [24, 25]. It mainly affected the swelling and contraction that accompany ion fluxes. Under

Table 2. The effect of varying concentrations of valinomycin on rates of mitochondrial swelling and contraction

Valinomycin (μg/ml)	Propranolol	Rate of swelling (ΔT/min)	Rate of contraction (ΔT/min)
4	−	169 ± 6·6	239 ± 1·9
	+	112 ± 6·8	71 ± 6·6
20	−	187 ± 5·3	254 ± 4·8
	+	140 ± 1·3	121 ± 3·1
100	−	205 ± 11·0	221 ± 4·9
	+	171 ± 13·1	43 ± 1·0

The solution was as in Fig. 2. The concentration of propranolol was 160 μM, that of rotenone 10 μM. The swelling was started with 8 mM Tris–succinate and the contraction with 1·2 mM KCN. Mitochondrial protein concentration was 1·0 mg/ml, the concentration of valinomycin as indicated.

Results are means ± S.E. of three determinations.

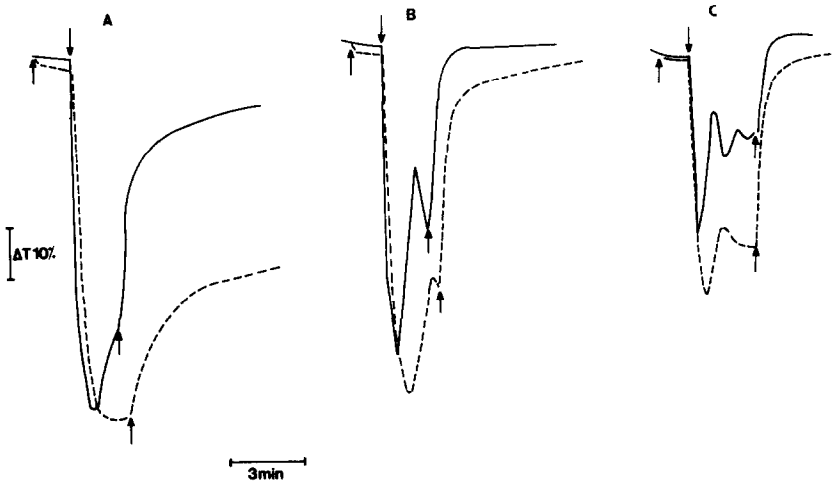

Fig. 5. The effect of pH. The solutions were as in Fig. 2A, the pH adjusted with Tris. (A) 7·2, (B) 7·7, (C) 8·2; mitochondrial protein was 1·6 mg/ml.

the conditions where the input of energy either from respiration or hydrolysis of ATP was readily directed to ion fluxes with concominant swelling, propranolol decreased the rate of the swelling to some extent. The most conspicuous effect was, however, the strong inhibition of the contraction that ensued when the input of energy ceased. This could have been due to the following interrelated factors: (1) primarily an inhibition of the efflux of ions; (2) stabilization of an 'energized' state; and (3) interaction with structural components of the inner membrane resulting in an inhibition of contraction.

In a preceding paper [14] we reported that propranolol increases the permeability of mitochondria to various ions in non-energized conditions. Therefore a primary effect on ion permeabilities seems to be unlikely here. However, the previous data on non-energized conditions may not be valid in energized conditions as many properties of the inner membrane are different. It is widely accepted that a proton gradient and a potential are generated across the membrane [15–18] which would strongly affect the behaviour of charged species in the membrane, including dipoles like phospholipids. Interaction of propranolol with the mitochondria may differ in these two conditions.

The inhibition of contraction was not so clearly seen in oscillations driven by ATP. Yet in the presence of substrate anions the inhibition was accentuated. This could be due to the function of the carrier of dicarboxylate which may have become rate-limiting in our conditions.

The influence of propranolol on the extent of swelling did not have any direct relationships with the rates of swelling and contraction which seemed to be mainly affected by the concentrations of electrolytes and hydrogen ions. At high salt concentrations the extent of swelling was greater with propranolol than in the controls, the opposite being true at low concentrations. We also found an inhibition of respiration and ATPase activity by propranolol at low salt concentrations, and a stimulation of both at high concentrations. The concentration of potassium ions (in the presence of valinomycin) where the changeover occurred is critical to anion and cation uptake of mitochondria [20, 26].

The raised pH effectively released the inhibition of contraction caused by propranolol. When the pH is

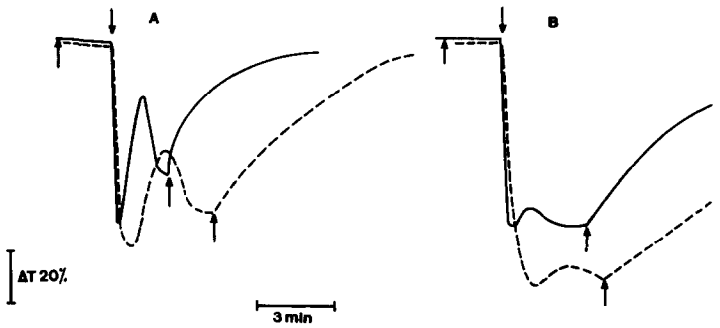

Fig. 6. The effect of succinate on the volume oscillations energized with ATP. The conditions as Fig. 2A, except in (A) 2 mM Tris–ATP was added instead of succinate and 1·5 mM KCN was present from the very beginning. (B) as in (A) but 12 mM Tris–succinate was also added with KCN. The passive contraction was induced with oligomycin, 4 μg/ml.

high the permeation of both cations and anions is increased [27, 28], and this may overcome the inhibition of propranolol.

The inhibition of contraction caused by propranolol was largely abolished by nigericin whether valinomycin was present or not. An increase in the concentration of valinomycin increased the rate of contraction only slightly in the presence of propranolol. Valinomycin increases the permeation of potassium and causes its electrogenic transport [27] whereas nigericin induces an electroneutral exchange of potassium and hydrogen ions [29]. Our findings, thus, strongly suggest that propranolol does indeed cause a stabilization of the "energized" state, probably by decreasing the influx of hydrogen ions. The inhibited efflux of cations and anions would be secondary to the decreased permeability to hydrogen ions. This effect of propranolol is of great interest both in regard to its effects on membranes, and to the special functions of mitochondria.

Addition of nigericin did not cause complete contraction of mitochondria in the presence of propranolol. The slow phase implies an interaction of propranolol with the membrane on another level, which may be called structural. The decrease of the rate of swelling may be related to this effect of propranolol on the inner mitochondrial membrane.

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