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Change of Mitochondrial Buffering Capacity Induced by Propranolol

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Abstract

Propranolol is able to increase the amount of the titratable groups of mitochondrial membranes. This effect occurs with sonicated particles and with liposomes, too. The phenomenon is only seen in the presence of salt solutions, not in sucrose. Propranolol increases the fluorescence of anilino-naphthalene sulphonate (ANS) in mitochondrial suspensions. The increase is counteracted by increasing concentrations of potassium chloride. It is suggested that the increase of the titratable groups results from a decrease of the aggregation of the phospholipids of the membranes. At the same time the environment of the bound ANS molecules is more hydrophobic in sucrose than in potassium chloride. The amount of the buffering groups and the hydrophilicity are in direct and the amount of the buffering groups and the fluorescence of ANS in inverse correlation.

Introduction

The penetration of protons through the inner membrane of mitochondria can be studied by adding small amounts of acid to an unbuffered suspension of mitochondria [1]. The mitochondria partly consume the added acid, and in a short time a new equilibrium is reached. The level of the equilibrium is determined by the buffering

Nonstandard abbreviations: ANS, 1-Anilino-8-naphthalene sulphonate; HOQNO, 2-Heptyl-4-hydroxyquinone N-oxide; FCCP, Carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone; Propranolol, 1-isopropylamino-3(1-naphtholoxy)-propran-2-ol hydrochloride; M, mol/l.

capacity of the membranes. In the presence of valinomycin and FCCP the equilibrium is reached more quickly.

With acid pulse techniques we found that in the presence of propranolol (a β-blocking agent) the titration of the added acid was faster than that of the control [2]. Furthermore, we found that the drug increased the amount of the consumed acid, suggesting an increase of the buffering capacity of the membranes.

Propranolol is a lipophilic drug [3, 4] with local anaesthetic properties. In biological membranes the molecules of ANS are situated close to those of local anaesthetics [5]. The fluorescence of ANS [6, 7, 8, 9] serves as a probe in the study of the interactions of propranolol and the membrane on which the drug is bound. In this study we have tried to correlate the changes of the fluorescence of the dye and the buffering capacity induced by propranolol.

Materials and Methods

Rat liver mitochondria were prepared and the mitochondrial protein determined as described earlier [2]. Sonicated particles of mitochondria were prepared as described by Kielley and Bronk [10]. Liposomes were prepared from mitochondrial phospholipids [11] as described by Papahadjopoulos *et al.* [12]. The organic phosphorus present in liposomes was determined with a slightly modified method of Fiske and Subbarow [13].

In the binding experiments the mitochondria were incubated with ^{3}H-propranolol for 10 min, and then separated from the supernatant by centrifuging. The radioactivity present in the supernatant was counted in a Packard Scintillation Counter using Instagel as the scintillation liquid. When the binding of ANS was determined the method of Azzi *et al.* [7] was used.

ANS fluorescence was determined with a Zeiss spectrophotometer with fluorimetric accessories. The wave length of excitation was 365 nm and that of emission 490 nm (both uncorrected). When propranolol was added to the solution of ANS and mitochondria, a fast increase of fluorescence occurred, followed by a slow quenching. The maximal initial increase was used in the figures.

In the pH titrations a fast responding glass electrode (Beckman combination electrode 39030) was used with the equipment described elsewhere [2].

ANS was obtained from K.K. Lab., Plainview, N.Y., U.S.A., and tritiated propranolol was kindly donated by the I.C.I., Macclesfield, Sussex, England. Other reagents were obtained as described earlier [2].

Results

The Effect of Propranolol on the Titratable Groups of Mitochondria

It has been shown earlier that the buffering capacity of mitochondria is higher in salt solutions [14, 15]. That has been interpreted to be the result of the changed pK values of the dissociable groups [14].

When the effect of propranolol on the buffering capacity of mitochondria is studied in the presence of valinomycin, FCCP and cyanide in potassium chloride, propranolol still increases the titratable groups of the membranes, Fig. 1A. Yet, the effect cannot be found in sucrose solutions, Fig. 1B. Propranolol increases the titratable groups of SMP (sonicated particles) (not shown) and liposomes as well, Fig. 1C. Mitchell and Moyle [1] found a similar effect with Triton X-100. However, the concentrations of propranolol used do not cause any lysis of mitochondria, as checked with electron microscopic controls (not shown). Our effect may be related to the optical changes that we found earlier [2].

Figure 1 suggests that the pK value of the membrane buffer is not changed but rather the amount of the buffering groups. As shown in Fig. 1C the effect seems to be the result of changes of the phospholipid part of the membranes. The effect of propranolol on the buffer of the membranes is maximal at high pH values. Yet, propranolol itself cannot be the buffer increased, because the concentration of the drug is approximately one order lower than the extra consumption of the added acid.

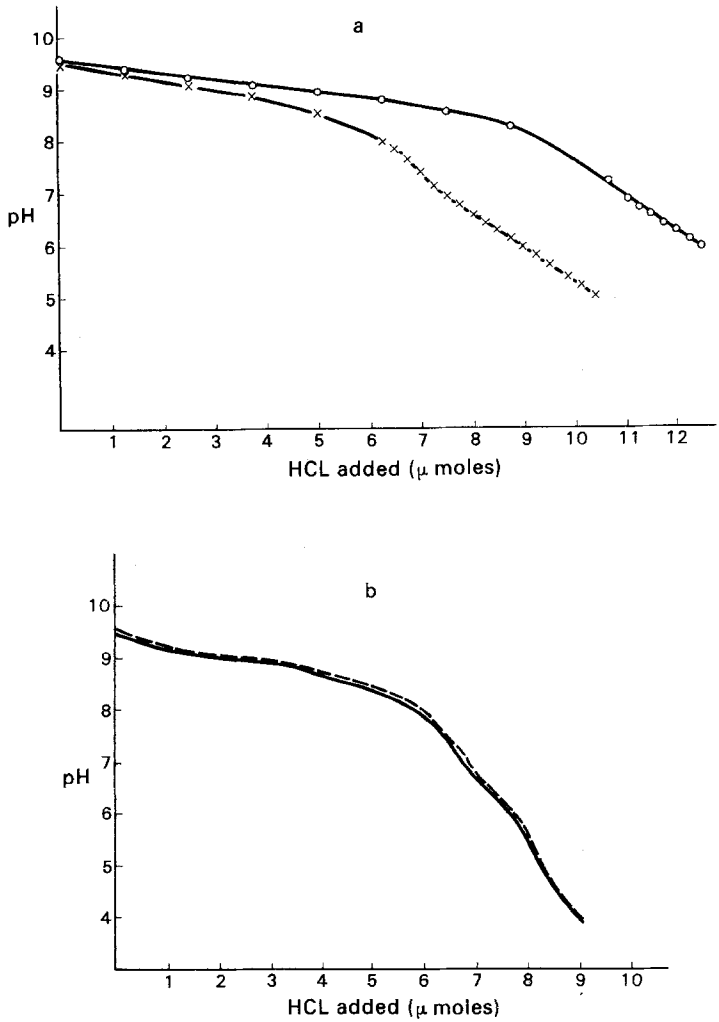
The Effect on the Equilibration of Acid Pulses

As mentioned above, propranolol seemed to increase the rate of the titration of the acid pulses [2]. Because we could not see any effect of propranolol on the titratable groups in sucrose (Fig. 1B) we studied the rate of equilibration of added acid pulses in sucrose and potassium chloride, Fig. 2. Even in sucrose the rate of the titration is faster in the presence of propranolol. In potassium chloride propranolol increases the rate even more, and there is an increase of the amount of the buffer, as found above. So, we can conclude that the increased rate of the titration we found even in sucrose is not related to the amount of the buffering groups but rather results from the changed permeability characteristics of the membranes [2].

The Change of the Fluorescence of ANS

Propranolol like local anaesthetics increases the fluorescence of ANS in mitochondrial membranes, Fig. 3A. This increase, however, greatly depends on the concentration of the external salt, as does the buffering

capacity in the presence of propranolol. Even the ionophores used in the experiments of Fig. 1 decrease the fluorescence that propranolol causes, Fig. 3B. It is obvious that the increase of the fluorescence and the change of the buffering capacity are in inverse relation. It is evident that the decrease of the fluorescence cannot be related to the membrane potential only, because it can be seen in the presence of valinomycin and FCCP, Fig. 3C.



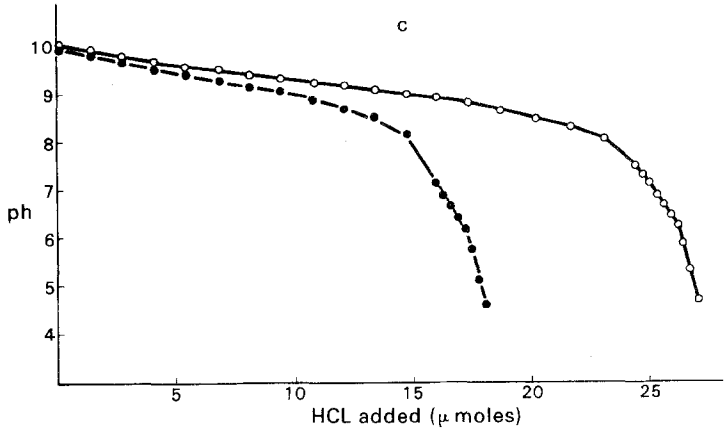


Figure 1. Effect of Propranolol on the Titratable Buffer. (A) 2.4 mg/ml protein, medium 0.07 M KCl and 0.06 M sucrose; (B) 2.7 mg/ml protein, medium 0.2 M sucrose; (C) liposomes, organic phosphorous 21 μg/ml. In all the figures, the upper curve, 530 μM propranolol present. Other additions were 7 μM rotenone, 2.7 μM FCCP, 0.4 μg/ml valinomycin, pH adjusted to 9.4 with KCN.

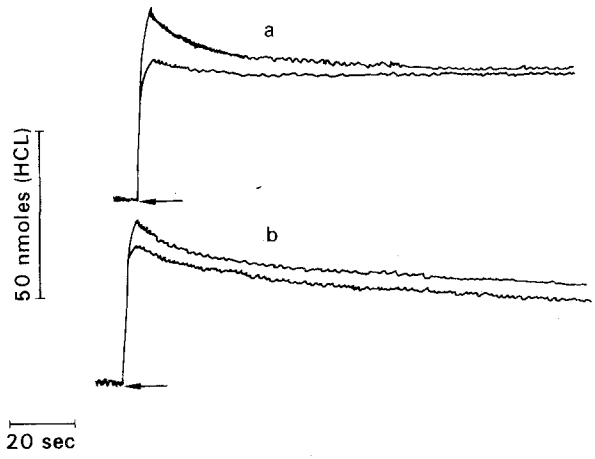


Figure 2. Effect of Propranolol on Acid Pulses. (A) 0.2 M sucrose, (B) 0.07 M KCl and 0.06 M sucrose. The upper curve in A and B, the control, the lower 320 μM propranolol. 12 μM rotenone, 2.4 μM HOQNO and 0.4 μg/ml valinomycin present, mitochondrial protein 2.8 mg/ml, pH adjusted to 7.2 before each acid pulse with choline.

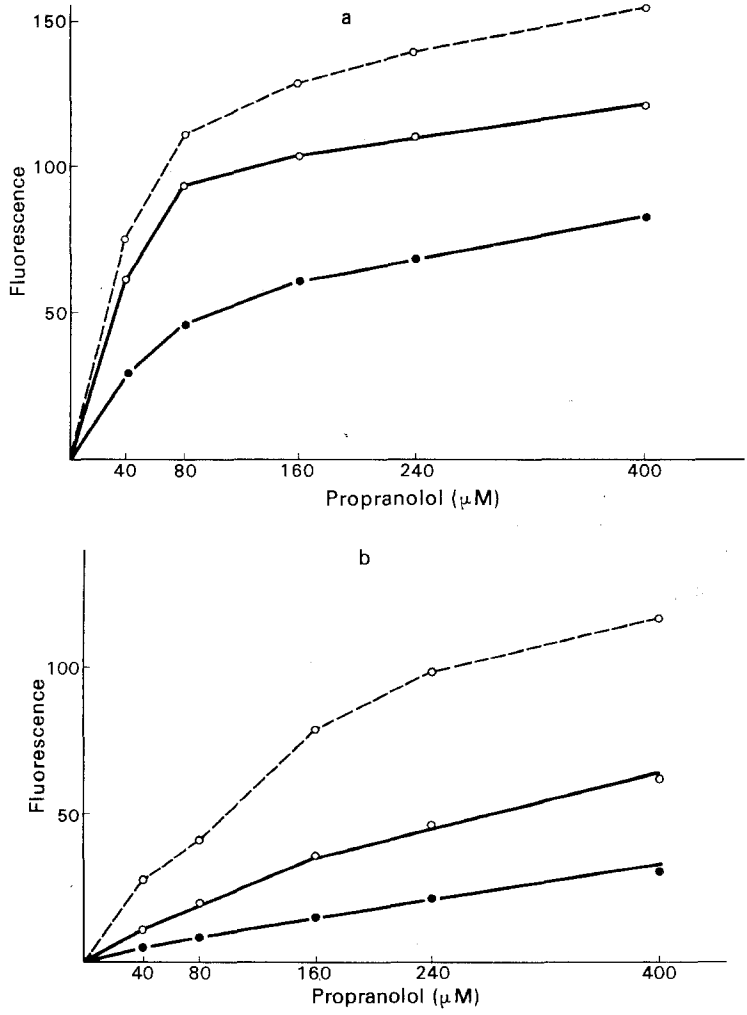


Figure 3. Increase of Fluorescence of ANS. (A) 1.2 μM HOQNO and 12 μM rotenone, (B) HOQNO and rotenone, and 1.0 μM FCCP and 0.2 μg/ml valinomycin. ○-----○; KCl, ○——○——○ 27 mM KCl, ●——————● 90 mM KCl. Mitochondrial protein 1.4 mg/ml, 0.02 M HEPES buffer, pH to 7.2 with Tris, 50 μM ANS.

The Effect of Potassium Chloride on the Binding of Propranolol and ANS

Propranolol has been shown to increase the binding of ANS to erythrocytes [16] and ANS the binding of propranolol to rat liver

mitochondria [17]. It has been suggested to result from the changed charge of the membranes in the presence of the drugs.

Using small concentrations of potassium chloride Huunan-Seppälä [17] could not find any competition between the binding of propranolol and potassium. Still, an obvious competition was found between propranolol and protons [18].

The binding of propranolol is almost unaffected by potassium chloride when the concentration of propranolol is rather small (100 μM), Fig. 4. When the concentration is higher (400 μM), the drug may be bound to more unspecific sites, because potassium chloride can now decrease the binding. When a small concentration of ANS (50 μM) is present, more propranolol is bound, and the binding is not affected by potassium chloride. This suggests that the new binding sites are rather similar to the sites to which the drug is bound when its concentration is low.

The decrease of the binding of ANS is not the reason for the decrease of the fluorescence of ANS that is seen in potassium chloride, because the salt even increases the binding of ANS to the membranes. This decrease is rather a result from an increase of the hydrophilicity of the binding sites of the drugs when the concentration of potassium chloride is increased.

Discussion

This study shows that propranolol is able to increase the buffering capacity of rat liver mitochondria. The change is related to the lipid part of the membrane, and it depends on the presence of external salts. The increase of the fluorescence of ANS that propranolol causes depends on external salts, too, and it diminishes when the concentration of potassium chloride is increased.

Potassium chloride does not decrease the binding of propranolol when the concentration of propranolol is rather low. In these conditions we found that propranolol causes the swelling of mitochondria [2]. In the swelling phenomenon the inner membrane is expanding, and it is likely that the lipid groups on the surfaces of the membrane are increased, too. Butler *et al.* [18] have reported that local anaesthetics promote the generation of multilamellar arrays in lipid membranes and the membranes become more stabilized. Then more of the phospholipids might become accessible to protons on the surfaces of the arrays than if the lipids were in aggregated form. Ohnishi and Ito [19] have recently reported that tetracaine disaggregates acidic phospholipids in liposome membranes

ANS and the molecules of local anaesthetics are suggested to situate close to each other in lipid membranes [5]. If the fluorescence of ANS is the measure of the hydrophobicity of the binding sites of the dye and propranolol, external potassium chloride is likely to increase the

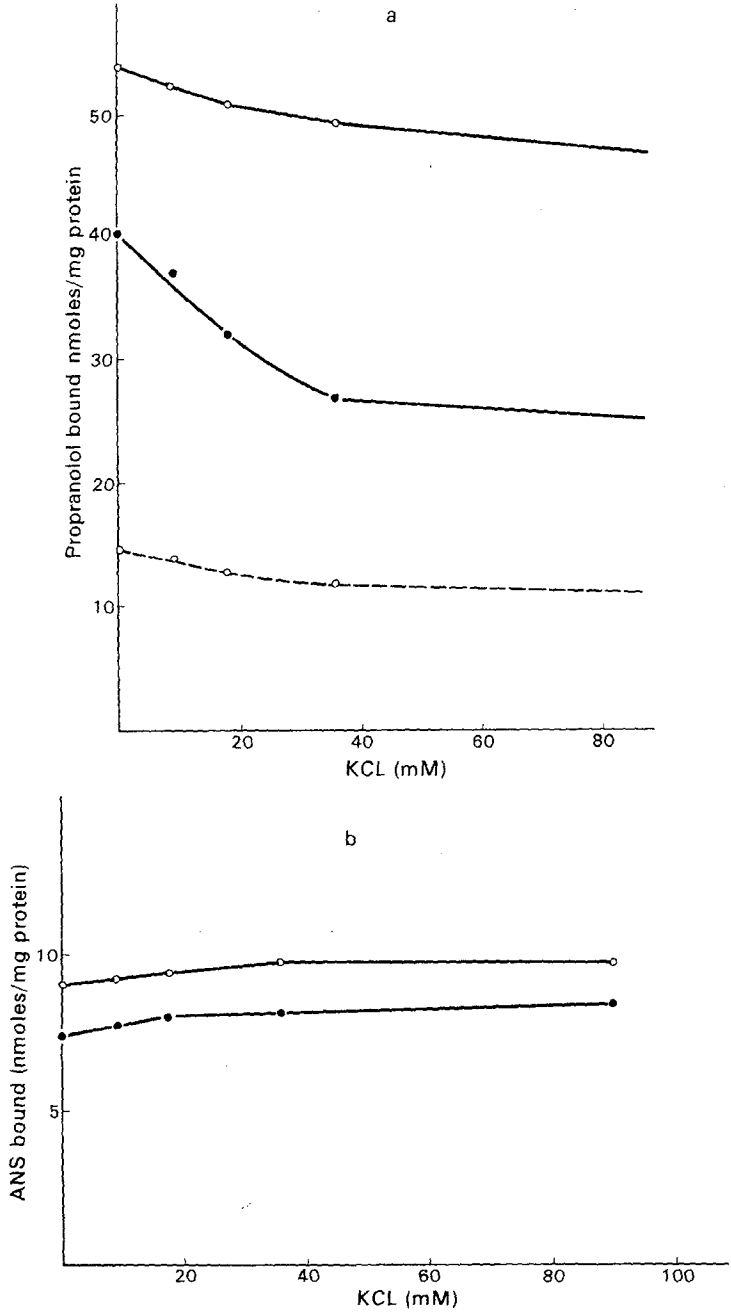


Figure 4. Effect of KCl on the Binding of Propranolol and ANS The osmolarity was maintained with sucrose. The inhibitors as in Fig. 3(A). (A) The binding of propranolol, ○——○——○ 400 μM propranolol and 50 μM ANS, ●——●——● 400 μM propranolol, ○- - -○- - -○ 100 μM propranolol, protein 2.5 mg/ml; (B) the binding of ANS, the upper curve 50 μM ANS and 400 μM propranolol, the lower 50 μM ANS, protein 2.2 mg/ml.

hydrophilicity near the sites. The decrease of the fluorescence of ANS seems to be the result of the decreased quantum yield of the fluorescence in our conditions, and the binding of the dye is even increased.

It is difficult to say how much this effect of propranolol on mitochondrial buffer operates in energized conditions. In energized mitochondria the membrane phospholipids are supposed to be arranged in an electrical field, and the effect of propranolol may differ. Yet, if the slightest change takes place in energized conditions, the distributions of protons and anions [20, 21] are changed, too, and consequently the drug could even affect the thermodynamics of oxidative phosphorylation [1, 22].

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