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EFFECTS OF QUINIDINE AND PROPRANOLOL ON ENERGY TRANSDUCTION IN BEEF HEART MITOCHONDRIA

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Abstract—The effects of quinidine and propranolol on mitochondrial respiration and on three respiration-dependent functions (oxidative phosphorylation, calcium uptake, and uncoupler-stimulated respiration) were studied. Both quinidine and propranolol inhibited respiration and three respiration-dependent functions in the presence of pyruvate plus malate or succinate plus rotenone as the substrates. Furthermore, both quinidine and propranolol inhibited ATP–P_i exchange, a mitochondrial energy-coupling reaction that does not involve respiration. Inhibition by propranolol but not by quinidine was strongly depended on the KCl concentration of the medium, the inhibition being maximal at low concentrations. The effect of KCl appears to lower the affinity of propranolol to the mitochondrial membrane.

The effects of quinidine and propranolol on mitochondrial functions have been the subject of study by a number of investigators. Noack and Greeff [1] showed that quinidine and propranolol inhibited respiration-dependent ADP- and Ca^{2+} uptake, whereas Sobel *et al.* [2] reported that propranolol at a concentration of 1 mM did not inhibit oxidative phosphorylation. Järvisalo and Saris [3] reported propranolol inhibition of energized swelling and nonenergized contraction in K^+ media, and suggested, from the effect of nigericin which relieved the propranolol inhibition of the contraction, that propranolol stabilized the "energized" state, possibly by inhibiting the entry of H^+. These authors also reported that propranolol inhibited respiration and ATPase activity at low KCl concentrations and stimulated these activities at high KCl concentrations. In an *in vivo* study, Conrad and Baxter [4] showed that quinidine but not propranolol inhibited ^{45}Ca uptake by rat heart mitochondria, and Harrow and Dhalla [5] showed quinidine inhibition of ATP-dependent calcium uptake by isolated mitochondria *in vitro*.

In the present paper we report the effects of quinidine and propranolol on energy transduction in isolated beef heart mitochondria. The aim of the study was to see whether quinidine and propranolol are specific inhibitors of particular coupled functions, or alternatively, if they are more general inhibitors of various coupled functions of mitochondria.

MATERIALS AND METHODS

Heavy beef heart mitochondria were prepared according to the method of Hatefi and Lester [6]. Under the conditions of assay used in the present study (see below), 26 different batches of heavy beef heart mitochondria preparations had the following characteristics (measured with pyruvate plus malate as the substrates): ADP/O ratio, 2.2 ± 0.3 (range 1.8 to 2.8), and respiratory control ratio, 3.6 ± 0.6 (range 2.8 to 4.6). Protein concentration was determined by the biuret method [7]. Oxidative phosphorylation, calcium-stimulated respiration, and uncoupler-stimulated respiration were measured polarographically at 30° using a Beckman oxygen analyzer. The basal medium (4.2 ml) for the assay of oxidative phosphorylation (ADP-stimulated respiration) contained 0.25 M sucrose, 10 mM Tris–HCl (pH 7.4), 10 mM potassium phosphate (pH 7.4), 2.5 mM pyruvate, 2.5 mM malate, and 0.6 mg mitochondrial protein/ml. The rate of State 3 respiration was determined by the addition of 1 μmole ADP. The ADP-stimulated respiration was obtained by subtracting the State 4 rate from the State 3 rate, and the uncoupler-stimulated respiration by subtracting the State 4 rate from the rate in the presence of 1 μM carbonylcyanide *m*-chlorophenylhydrazone (m-ClCCP). The basal medium (4.2 ml) for the determination of calcium-stimulated respiration contained 0.25 M sucrose, 10 mM Tris–HCl (pH 7.4), 10 mM potassium phosphate (pH 7.4), 12.5 mM potassium succinate, 2.5 μM rotenone, and 0.6 mg mitochondrial protein/ml. The calcium-stimulated respiration and the uncoupler-stimulated respiration were determined by subtracting the basal rate from the rate in the presence of 0.25 mM calcium chloride or the rate in the presence of 1 μM m-ClCCP. The medium used for the measurements of the effect of KCl on propranolol inhibition of uncoupler-stimulated respiration was the same as that used for the assay of oxidative phosphorylation except that potassium phosphate was omitted. ATP–P_i exchange activity was measured as described earlier [8]. Rotenone, D, L-propranolol, and quinidine sulfate were obtained from the Sigma Chemical Co. (St. Louis, MO).

RESULTS AND DISCUSSION

Uncoupler stimulation of respiration is a reliable index of the respiration and energy-transducing capabilities of mitochondria. An uncoupler relieves inhibition of respiration due to a specific inhibition of an energy-requiring (endergonic) reaction (e.g. inhibition of ATP synthesis by oligomycin), but not inhibition by such respiratory chain inhibitors as KCN or antimycin A.

Table 1. Quinidine inhibition of calcium-stimulated respiration and uncoupler-stimulated respiration*

	Concentration of quinidine (mM)				
	0	0.5	1.0	1.5	2.0
Calcium-stimulated respiration	209 ± 18	161 ± 17	135 ± 25	72 ± 21	53 ± 4
Uncoupler-stimulated respiration	197 ± 32	170 ± 3	139 ± 9	79 ± 12	55 ± 19

* Average values from three experiments with standard deviations are shown. Unit of activity: natoms [O]/min·mg. Succinate in the presence of rotenone was used as the substrate.

To see whether quinidine is a specific inhibitor of calcium uptake, we have compared the effects of varying concentrations of quinidine on calcium uptake (measured as calcium-stimulated respiration) and uncoupler-stimulated respiration. As can be seen in Table 1, there was no significant difference between the inhibitory effects of quinidine on calcium-stimulated respiration and uncoupler-stimulated respiration at each concentration of quinidine tested. While Noack and Greeff [1] reported that the transport of Ca^{2+} was less sensitive than that of ADP to the inhibitory action of several cardioactive drugs including quinidine and propranolol, we did not find quinidine to be a specific inhibitor of ADP-stimulated respiration. Figure 1 shows that the concentration of quinidine for half-maximal inhibition of ADP-stimulated respiration was about 1 mM, a value comparable to those found with calcium- and uncoupler-stimulated respiration.

Propranolol resembles quinidine, as both compounds inhibit respiration and respiration-dependent functions of mitochondria. To see whether propranolol selectively inhibits a certain respiration-dependent function, we compared the effect of propranolol on calcium- and uncoupler-stimulated respiration (Fig. 2) and on ADP- and uncoupler-stimulated respiration (Fig. 3). The concentration of propranolol required for half-maximal inhibition of these three functions was about 0.4 mM, indicating that propranolol is not a specific inhibitor of any particular respiration-dependent function. Noack and Greeff [1] reported the concentrations of propranolol needed for 25 per cent inhibition of Ca^{2+} and ADP uptake by rat liver mitochondria were 0.88 and 0.57 mM respectively.

A characteristic of propranolol is that its effect on mitochondrial functions is strongly dependent on the KCl concentration of the medium [3]. Table 2 shows that propranolol inhibition of ADP-stimulated respiration was substantially relieved in the present of 50 mM KCl. A similar effect of KCl concentration in the medium was found with propranolol inhibition of uncoupler-stimulated respiration, indicating that the effect was not limited to oxidative phosphorylation. The result of the experiment in which the concentration of propranolol was varied in the presence or the absence of 50 mM KCl (Fig. 4) suggested that the affinity of propranolol to the mitochondrial membrane was lowered in the presence of a high concentration of KCl. Unlike propranolol inhibition, quinidine inhibition was found to be insensitive to the KCl concentration of the medium, indicating a largely hydrophobic interaction of quinidine with the mitochondrial membrane as com-

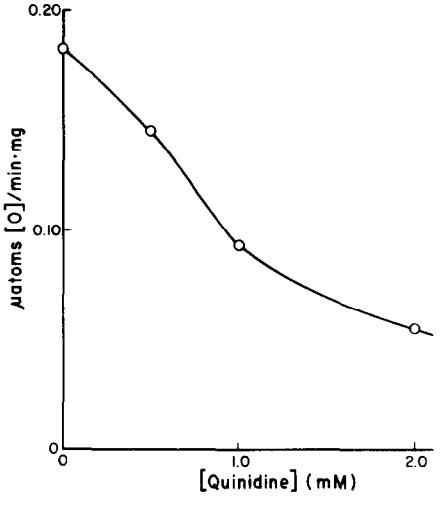

Fig. 1. Effect of quinidine on ADP stimulated respiration, determined with pyruvate plus malate as the substrates.

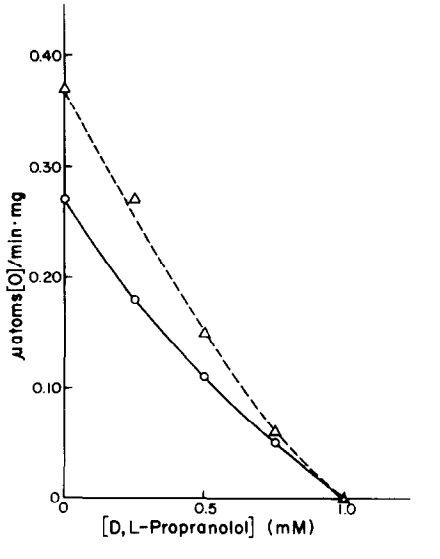

Fig. 2. Propranolol inhibition of calcium-stimulated respiration (——○——) and uncoupler-stimulated respiration (– – –△– – –), determined with succinate as the substrate in the presence of rotenone.

Table 2. Effect of KCl on propranolol inhibition of ADP-stimulated respiration measured with pyruvate plus malate as the substrates

[KCl] (mM)	D,L-Propranolol (0.5 mM)	ADP-stimulated respiration (μatoms [O]/min·mg)
0	–	0.29
	+	0.12
50	–	0.23
	+	0.20

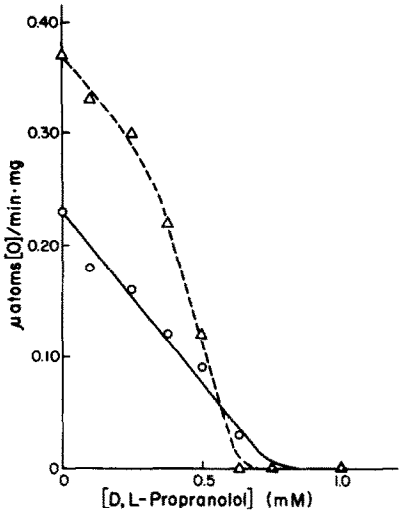

Fig. 3. Propranolol inhibition of ADP-stimulated respiration (——○——) and uncoupler-stimulated respiration (– – –△– – –), determined with pyruvate plus malate as the substrates.

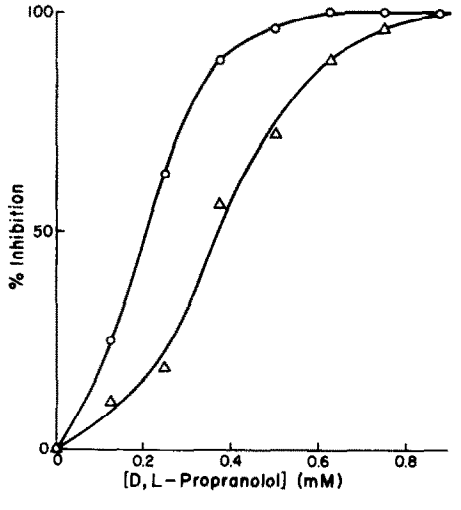

Fig. 4. Effect of KCl on the inhibition of uncoupler-stimulated respiration by varying concentrations of propranolol. The substrates were pyruvate plus malate. Key: (——○——) control; and (——△——) with KCl (50 mM). Activities in the absence of propranolol were 0.27 and 0.24 μatoms [O]/min·mg for control and with KCl (50 mM) respectively.

pared to that of propranolol in which ionic interaction is important.

While the results of the experiments described above give an impression that quinidine and propranolol are inhibitors of respiration, much like KCN and antimycin A, reports in the literature of the inhibition of ATP-dependent calcium uptake by quinidine [5] and the inhibition of ATPase activity by propranolol [3] suggested that these compounds might not be simple inhibitors of respiration. Consequently, we studied the effects of quinidine and propranolol on the ATP–P_i exchange reaction, a mitochondrial energy-coupling reaction that does not involve respiration. As can be seen in Fig. 5, quinidine and propranolol both inhibited the ATP–P_i exchange reaction. The concentration of quinidine (about 1 mM) for half-maximal inhibition of the ATP–P_i exchange reaction was essentially identical to that found with respiration-dependent reactions, whereas the concentration of propranolol (about 1.5 mM) for half-maximal inhibition of the ATP–P_i exchange reaction was considerably higher than the corresponding value (about 0.4 mM) for respiration-dependent reactions. The higher concentration of propranolol required for half-maximal inhibition of the ATP–P_i exchange reaction, as compared to that of respiration-dependent reactions, is most likely due, at least in part, to the difference in the compositions of media used for the assays.

In studying the effects of drugs on mitochondrial functions, it is convenient to consider three distinct parts of the mitochondrial energy-coupling mechanism, namely, exergonic reaction, endergonic reaction, and the coupling of these reactions. In oxidative phosphorylation, for example, the exergonic reaction is electron transport and the endergonic reaction is ATP synthesis. Compounds such as KCN and antimycin A are specific inhibitors of respiration, whereas oligomycin is a specific inhibitor of ATP synthesis and ruthenium red that of calcium uptake.

The results of the experiments described in this paper indicate that quinidine and propranolol are not specific inhibitors of any particular endergonic reaction. Furthermore, both quinidine and propranolol inhibited not

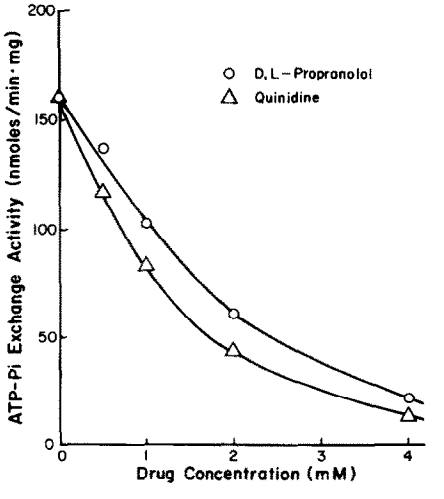

Fig. 5. Effects of quinidine and propranolol on ATP–P_i exchange activity.

only respiration-dependent reactions but also the ATP–P_i exchange reaction (this paper) and other ATP-dependent reactions [3, 5], indicating that these compounds are not simple inhibitors of respiration either. The concentrations of quinidine required for half-maximal inhibition of various coupled reactions were identical. This suggests that a single underlying effect of the drug, such as an effect on membrane structure, is responsible for the inhibition of both respiration and ATP-dependent reactions.

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