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The effect of adenine nucleotides and pH on the inhibition of oxidative phosphorylation by bongkrekic acid

The effect of bongkrekic acid on oxidative phosphorylation was first studied by WELLING *et al.*[1]. LARDY[2] and HENDERSON AND LARDY[3] demonstrated that its effect is due to inhibition of the adenine nucleotide translocator. This has been confirmed by our observation[4] that the hydrolysis of endogenous ATP is not inhibited by bongkrekic acid, and by direct measurements by KLINGENBERG *et al.*[5] of the activity of the adenine nucleotide translocator. WEIDEMANN *et al.*[6] reported that bongkrekic acid binds adenine nucleotides to the mitochondrial inner membrane.

In contrast to atractyloside, bongkrekic acid acts with a time-lag, even after 3-min preincubation as is demonstrated in Fig. 1, where the time course of the succinate oxidation is given in the presence of different concentrations of bongkrekic acid.

It was found that the duration of the time-lag was very dependent on the preincubation conditions. The presence of either ATP or ADP during the preincubation (in the latter case State-3 respiration was started by adding succinate) greatly shortened the time-lag, as is demonstrated with ATP in Fig. 2. AMP and GDP had no effect.

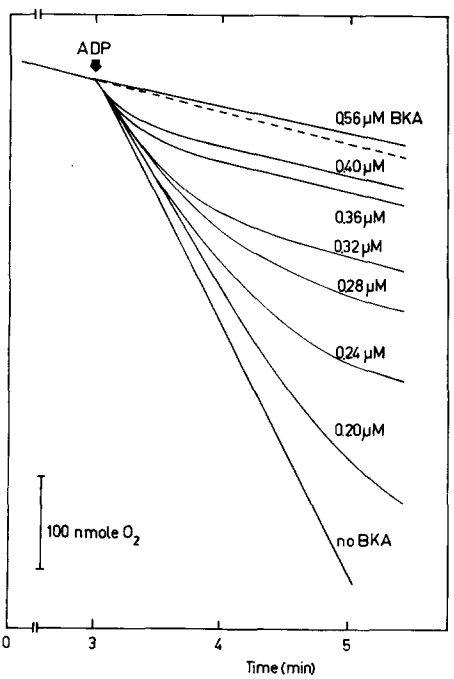

Fig. 1. The effect of bongkrekic acid on succinate oxidation by rat-liver mitochondria. The oxidation was measured polarographically using a Clark-type electrode in 1.4 ml of a reaction medium containing 15 mM KCl, 25 mM Tris–HCl (pH 7.4), 2 mM EDTA, 5 mM $MgCl_2$, 70 mM sucrose, 25 mM potassium phosphate buffer (pH 7.4), 12 mM succinate, rotenone (0.2 μg/mg protein) and 0.78 mg mitochondrial protein. Temp. 25°. After 3-min preincubation State-3 respiration was started by adding 1.29 mM ADP. – – – –, no bongkrekic acid or ADP. BKA, bongkrekic acid.

In contrast to atractyloside the rate with which bongkrekic acid acts is strongly dependent on the pH of the medium as can be seen from the results given in Fig. 3, in which the rate of succinate oxidation in the absence and in the presence of bongkrekic acid (1 min after adding ADP after preincubation for 3 min) is plotted as a function of pH. The pH dependence suggests that an acidic group, either in the inhibitor or the translocator, is involved in the mechanism of action of bongkrekic acid. It is less likely that it is the translocator, since neither the activity of the translocator[7] nor inhibition by atractyloside is pH-sensitive. Inhibition at pH 6.9 cannot be reversed by raising the pH to 7.3, nor by addition of adenine nucleotides.

Hydration of the six double bonds in bongkrekic acid[8] resulted in a loss of its

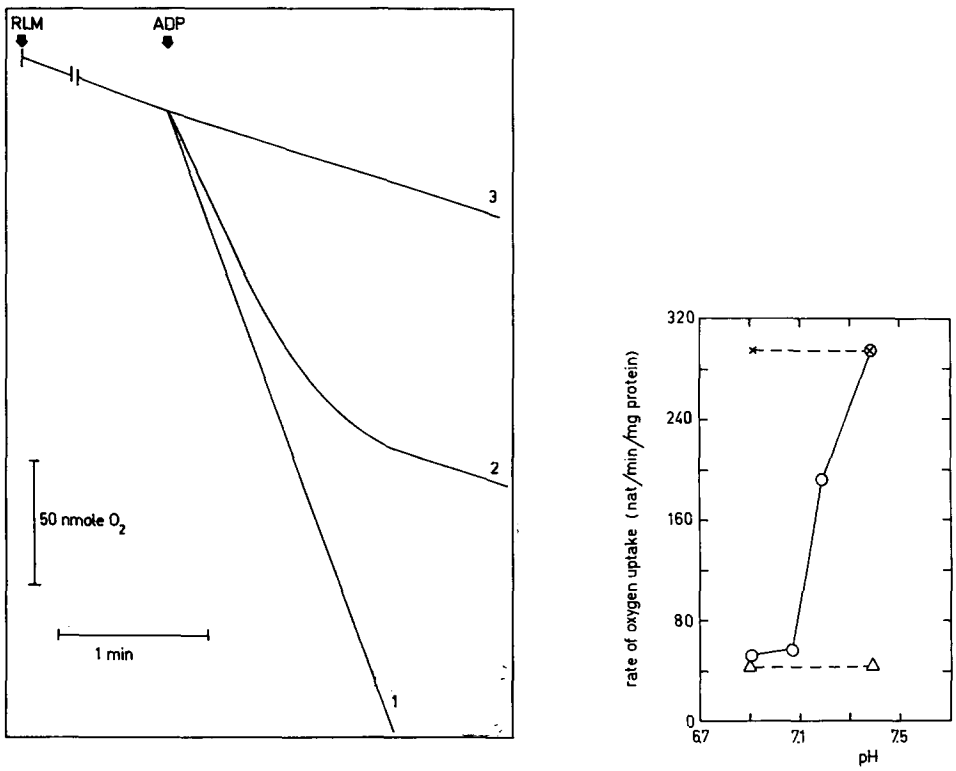

Fig. 2. Effect of ATP on the inhibition by bongkrekic acid of succinate oxidation in rat-liver mitochondria. Mitochondria (1.33 mg protein) were incubated for 3 min at 25° in a 1.6 ml of a medium containing 25 mM Tris–HCl (pH 7.4), 15 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 25 mM P_i, 12 mM succinate and rotenone (0.15 μg/mg protein). State-3 respiration was initiated by adding 0.8 mM ADP. 1, uninhibited State 3 (in absence and presence of ATP); 2, preincubated with 0.48 μM bongkrekic acid (0.60 nmole/mg protein); 3, preincubated with 0.48 μM bongkrekic acid and 0.14 mM ATP. RLM, rat-liver mitochondria.

Fig. 3. The effect of pH on the inhibition by bongkrekic acid of succinate oxidation in rat-liver mitochondria. Mitochondria (0.6 mg of protein) were incubated for 3 min at 25° in 1.0 ml of a medium containing 15 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 5 mM P_i, 12 mM succinate, 125 mM sucrose and rotenone (0.14 μg/mg protein). State-3 respiration was initiated by adding 1 mM ADP. The rates given refer to 1 min after adding the ADP. ×---×, uninhibited State-3; △---△, uninhibited State-4; ○—○, State-3 respiration in the presence of 0.6 μM bongkrekic acid (1.0 nmole/mg protein).

TABLE I

EFFECT OF INHIBITORS ON ADENINE NUCLEOTIDE TRANSLOCATION IN RAT-LIVER MITOCHONDRIA AT 0°

Rat-liver mitochondria (1.9 mg protein) were suspended in 0.7 ml of a medium containing 94 mM sucrose, 18 mM KCl, 5 mM EDTA and 18 mM Tris–HCl (pH 7.4). The incubation was started by adding 0.40 mM labeled ADP (specific activity 422 disint./min per nmole), and stopped by the rapid filtration technique. The filters were dried and counted with the use of liquid scintillation technique.

Additions	*Incorporation (disint./min) in*		
	1 min	*3 min*	*5 min*
None	607	1255	1490
Bongkrekic acid (10 μM)	117	129	137
Atractyloside (43 μM)	16	49	63

inhibitory action on the translocator. Hydrobongkrekic acid is a weak and nonspecific uncoupler.

In contrast to a report by KLINGENBERG *et al.*[5], we find that bongkrekic acid is effective at 0° in inhibiting the atractyloside-sensitive exchange (Table I).

The details of these experiments will be reported elsewhere. The mechanism of action of the inhibitor is now under further investigation.

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