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Inhibition of oxidative phosphorylation by an antipyretic drug

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IN THE course of a study on the mechanism of the pharmacological action of antipyretics, an inhibitory effect "in vivo" on rat oxygen uptake and "in vitro" on isolated rat liver mitochondria was observed using 2-allyl-oxybenzamide, a salicylamide (2-hydroxybenzamide) derivative.[1]

The main results, obtained with rat liver mitochondrial preparations, are summarized in this report. Table 1 shows the effect of 2-allyl-oxybenzamide (AOB)* on the oxidative phosphorylation

TABLE 1. OXIDATIVE PHOSPHORYLATION IN PRESENCE AND IN ABSENCE OF 2-ALLYLOXYBENZAMIDE (AOB)

	substrate	AOB	O_2 uptake (μatoms/mg N)	P esterified (μmoles/mg N)	P/O
(a)					
	α-ketoglutarate	—	12·59	31·44	2·50
	α-ketoglutarate	0·002 M	3·90	10·51	2·70
	β-hydroxybutyrate	—	4·70	13·20	2·80
	β-hydroxybutyrate	0·002 M	1·67	4·30	2·57
	succinate	—	16·30	29·80	1·83
	succinate	0·002 M	16·50	23·60	1·43
(b)					
	DPNH	—	10·10	10·67	1·06
	DPNH	0·002 M	9·10	8·07	0·89
(c)					
	ferrocytochrome C	—	5·76	2·70	0·47
	ferrocytochrome C	0 002 M	6·10	2·74	0·45

The oxygen uptake was measured manometrically at 26 °C in the Warburg apparatus. The vessels contained 0·005 M $MgSO_4$, 0·03 M glucose, 0·0014 M ATP, 0·8 mg yeast hexokinase (Sigma, type II), 0·00001 M cytochrome C, 0·09 M sucrose, and:

(a) α-ketoglutarate (0·01 M), succinate (0·01 M) and β-hydroxybutyrate (0·02 M) as substrates, 0·03 M potassium phosphate buffer, pH 7.4, 0·00036 M $MnCl_2$, mitochondria (isolated in 0·25 M sucrose) 1 mg of N; time of incubation 20 min.;

(b) DPNH (0·002 M) as substrate, 0·009 M potassium phosphate buffer, pH 7·4, 0·04 M *tris* buffer, pH 7·4, 0·001 M ethylendiaminotetracetate, mitochondria (isolated in 0·25 M sucrose and pretreated for 15 min. at 0 °C with 0·075 M sucrose) 0·5 mg of N; time of incubation 15 min.;

(c) reduced cytochrome C as substrate, 0·009 M potassium phosphate buffer, pH 7·4, 0·02 M *tris* buffer, pH 7·4, 0·01 M ascorbate, 0·00036 M $MnCl_2$, 0·01 M KF, mitochondria (isolated in 0·25 M sucrose and pretreated for 15 min. at 0 °C with 0·075 M sucrose) 1 mg of N; time of incubation 30 min.;

* 2-allyl-oxybenzamide was kindly supplied by Cassella-Curta (Frankfurt/M).

final volume 2·8 ml; gas phase air; KOH and filter paper in the centre well.

supported by several substrates, included added reduced diphosphopyridine nucleotide (DPNH) and ferrocytochrome C. It is evident that AOB (0·002 M) produces a marked inhibition of respiration with α-ketoglutarate and β-hydroxybutyrate as substrates; the same concentration of AOB is without effect on the oxidation of succinate, added DPNH and cytochrome C reduced by ascorbate.

The possibility of two alternative pathways for oxidation of added DPNH may explain the failure of AOB to produce inhibition on oxygen uptake with this substrate, but the partial depression of the coupled phosphorylation, observed in hypotonically-treated mitochondria, may indicate that the internal pathway is AOB-sensitive; in this case the phosphorylation observed in the presence of AOB could originate in a great degree from the oxidation of cytochrome C, reduced by *external* pathway (P/O = 0·89).

With α-ketoglutarate and β-hydroxybutyrate as substrates, AOB inhibits electron transport, without affecting the efficiency of the coupled phosphorylation, while with succinate the P/O ratio is slightly reduced (this effect might be due to inhibition of oxidation of fumarate derived from succinate). AOB was found to have no uncoupling effect on the phosphorylation coupled to the ferrocytochrome C oxidation if the P/O ratios are corrected for endogenous phosphorus uptake; the endogenous phosphorus uptake, observed in the absence of ascorbate and cytochrome C, is partially inhibited by AOB.

The action of AOB is also present in a ~ P acceptors-deficient system activated by 2, 4 dinitrophenol and in water pre-incubated mitochondria oxidizing β-hydroxybutyrate in the presence of diphosphopyridine nucleotide (DPN) but in the absence of cytochrome C. In this case (water pre-incubated mitochondria) the addition of cytochrome C partially removes the effect of AOB; the antagonism is more evident if at the same time the level of added DPN is increased. Data obtained with these experiments are plotted in Fig. 1.

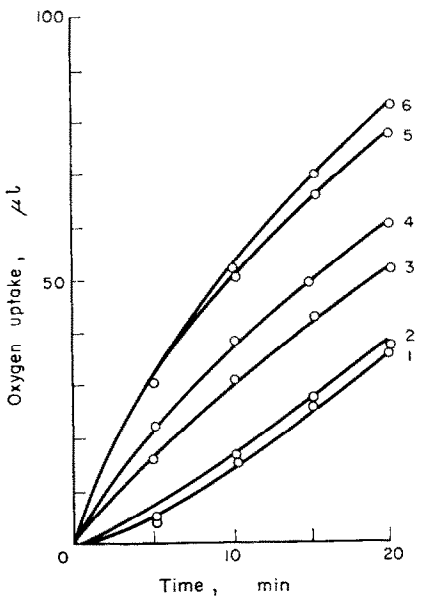

FIG. 1. The effect of AOB on oxygen uptake of water-pretreated mitochondria. The vessels contained 0·03 M potassium phosphate buffer, pH 7·4, 0·005 M $MgSO_4$, 0·001 M ethylendiaminotetracetate, 0·02 M β-hydroxybutyrate, mitochondria (isolated in 0·25 M sucrose and pre-incubated in water for 30 min. at 0 °C) 1 mg of N; other conditions as indicated in Table 1. Additions: (1) 0·002 M AOB + 0·00036 M DPN; (2) 0·002 M AOB + 0·0014 M DPN; (3) 0·002 M AOB + 0·00036 M DPN + 0·00002 M cytochrome C; (4) 0·002 M AOB + 0·0014 M DPN + 0·00002 M cytochrome C; (5) 0·00036 M DPN or 0·00036 M DPN + 0·00002 M cytochrome C; (6) 0·0014 M DPN or 0·0014 M DPN + 0·00002 M cytochrome C.

It may also be pointed out that cytochrome C is unable to remove the inhibition of respiration in phosphorylating mitochondria and that in water pre-treated mitochondria oxidizing succinate the drug is ineffective both in the presence and in the absence of cytochrome C.

The reversal of AOB inhibition by added cytochrome C may be explained if one considers cytochrome C as an electron acceptor bypassing the point of attachment of the inhibitor (AOB) to the electron transport chain. With water pre-treated mitochondria cytochrome C may be thought to accept electrons directly from flavoproteins as it has been observed in disrupted mitochondria.[2, 3] In these conditions, with amytal (5-ethyl-5-isoamylbarbituric acid) are obtained similar results.

The present data appear to support the hypothesis that AOB inhibits electron transport in the respiratory chain, without affecting the coupled phosphorylation reactions and suggest that the effect of the drug on mitochondrial respiration might be due to the inhibition of DPNH-cytochrome C reductase. In this respect, AOB has an action similar to that of amytal.[4]

In regard to the pharmacological implications, these experiments can support the following considerations:

(a) the inhibition of respiration by AOB might play an important part in the antipyretic effect of this substance in as much as it might lead to reduced heat formation;

(b) the allyl group is important for the inhibition of oxygen uptake. In this respect salicylamide (2-hydroxybenzamide) is much less active and this substance differs from AOB in that a OH replaces the allyl group. It is of interest to note that AOB is an antipyretic more active than salicylamide;

(c) since AOB has a strong depressing action upon central nervous system, the present data confirm the probability that the inhibition of DPNH-cytochrome C reductase can be related to the depression of central nervous system induced by several drug (hypnotics, sedatives, tranquillizers).

Indeed, the action of AOB is similar to that of amytal and, in some way, it resembles the action of chlorpromazine.[5]

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