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THE EFFECT OF CHLORPROMAZINE ON RESPIRATION OF BRAIN MITOCHONDRIA AS A FUNCTION OF METABOLIC STATE

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Abstract—The effect of chlorpromazine on brain mitochondrial respiration is studied during various metabolic states and certain transitions between them. It is found that for a given low concentration of the drug, there may be no effect, a stimulation, or a prevention of stimulation of respiration, depending on metabolic state at time of exposure to the drug. These findings are discussed in light of the "charge transfer" properties of chlorpromazine as well as possible permeability factors.

ALTHOUGH it has been demonstrated that chlorpromazine (CPZ) inhibits mitochondrial respiration and uncouples oxidative phosphorylation, these effects have not seemed pertinent to its action *in vivo* because of the high concentrations of drug usually required *in vitro* (>100 to 200 μM).[1–5] Moreover, one study[3] reports that brain mitochondria are even less sensitive than liver to these effects of CPZ, although this claim has been disputed.[4] In any case mitochondrial respiration appears inhibited only at much higher concentrations than would be found *in vivo* with pharmacologic doses. Respiration in certain other systems *in vitro*, however, is inhibited by CPZ in the pharmacologic range of concentration. For example, quite low quantities of the drug (10 μM) can prevent electrical stimulation of brain slice respiration, suggesting therefore a nonmitochondrial site of action.[6]

It may be noted, however, that these studies of mitochondrial respiration were carried out during only one metabolic state—that of maximum activity supported by the presence of excess substrate and adenosine diphosphate (ADP). This contrasts with the situation of stimulated brain slices and presumably of brain *in vivo* where there are fluctuations in activity and consequently in metabolic state.

The present study concerns the effect of CPZ on respiration of brain mitochondria during various metabolic states of mitochondria and certain transitions between them, as described and classified by Chance and Williams[7] (Table 1). In view of the hypothesis that CPZ may act by virtue of its "charge transfer" properties,[8] the possibility may be considered that the effect of CPZ, at least on mitochondrial respiration, varies according to the steady state of the respiratory carriers, rate of electron transport, and associated circumstances characteristic of each metabolic state. In addition, since it has been reported that CPZ decreases mitochondrial "permeability",[9] the possibility of indirect effects on respiration must also be considered. This is especially so in certain cases studied here in which ADP or substrate is added after CPZ, since

their subsequent access to active sites may be hindered by changes in permeability induced by the drug.

METHODS

Oxygen uptake was followed polarographically with a 120-cycle/sec vibrating platinum electrode (Oxygraph). This method was chosen because it permits measurement of instantaneous rates and rapid transitions in rate within a single reaction mixture.

TABLE 1. METABOLIC STATES OF MITOCHONDRIA (AFTER CHANCE AND WILLIAMS[7])

State	O_2	ADP	Substrate	Respiration	Rate-limiting substance
1	excess	low	low	slow	ADP
2	excess	high	0	slow	substrate
3	excess	high	high	fast	respiratory chain
4	excess	low	high	slow	ADP

Mitochondria were prepared from a single rat brain (Wistar strain, male, 175 to 200 g) according to Dahl *et al.*,[10] with the following modifications. Homogenization was carried out at low speed in 10 ml of 0·25 M sucrose by only three passes of a selected loosely-fitting Teflon pestle, since it was found that this relatively gentle handling yielded mitochondria of high stability. The supernatant of an initial spin of 1,000 × *g* for 10 min was recentrifuged for 12,000 × *g* for 10 min. The resulting sediment was suspended in 10 ml of 0·25 M sucrose and centrifuged again for 12,000 × *g* for 10 min. The second high-speed sediment revealed two clear-cut layers: a lower, tightly packed, dark one and an upper, loosely packed, light one (as described by Dahl *et al.*). The upper layer has little or no respiratory activity and is presumably composed mainly of amine-containing particles.[11] This was easily removed by gentle agitation with several 2-ml washes of 0·25 M sucrose. The remaining dark sediment was suspended to 150% of wet brain weight and used in 0·1-ml aliquots. The removal of the fluffy layer was performed for two reasons. Substances in it might have an effect on respiration and its larger particles would contribute to electrode "noise". Mitochondria were used within an hour of preparation.

Conditions of experiments are noted with the appropriate figures. Usually a pair of substrates was used since this seemed to provide good respiratory control ratios even in the absence of fluoride (perhaps because of inhibition of endogenous ATPase by increased diphosphopyridine nucleotide reduction[12]).

RESULTS

The results are grouped according to various metabolic states of mitochondria, since it was found that the effect of CPZ on respiration differed from state to state. For convenience the numerical classification of Chance and Williams, as outlined in Table 1, was followed. It may be seen from the table that for each combination of levels of ADP and substrate there is a characteristic respiratory rate and rate-limiting substance.

Metabolic states 3 *and* 4

During state 4 there is a relatively high steady-state reduction of the respiratory carriers, due to an excess of substrate, and a slow rate of respiration, ADP being limiting in a "tightly coupled" system. In a typical experiment, illustrated in Fig. 1A, when ADP is added to a medium containing α-ketoglutarate and malate, making the

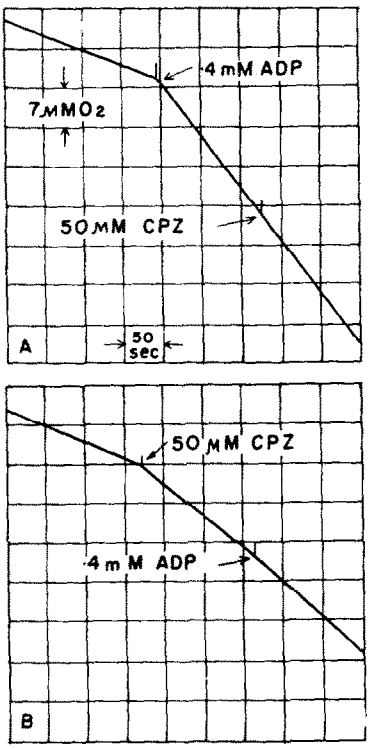

FIG. 1. Rate of O_2 consumption, metabolic states 3 and 4. In A, CPZ is added after ADP, in B prior to ADP. Reaction is initiated by adding mitochondria to a polarograph vessel containing the following medium: 2 mM α-ketoglutarate, 2 mM malate, 0·1 M sucrose, 0·04 M KCl, 0·01 M phosphate buffer (pH 7·4), 0·008 M $MgCl_2$. A and B represent consecutive experiments using equal aliquots of the same mitochondrial preparation. Values are expressed as final concentrations. Temperature, 25·5°.

transition from state 4 to state 3, a 3·6–fold increase in oxygen consumption occurs. Under these circumstances, 50 μM CPZ has no inhibitory effect. This is in accord with most reports in which considerably higher concentrations were necessary for a significant inhibition during state 3. Matters are quite different when 50 μM CPZ is applied during state 4 (excess substrate, low ADP). Here, as shown in Fig. 1B, there is a 100 per cent stimulation of respiration immediately upon addition of CPZ. Although a stimulation seems paradoxical it corresponds to the reported uncoupling effect of the drug in state 3. Now, however, ADP itself no longer induces a stimulation of respiration, the final rate falling considerably short of that in Fig. 1A.

Distinct effects of lesser magnitude are obtainable with as little as 10 μM CPZ. For example, in an experiment in which 10 μM CPZ was added to a medium containing 2 mM glutamate and 2 mM malate (other conditions as in Fig. 1), a 24 per eent stimulation of respiration occurred. The subsequent addition of 0·4 mM ADP resulted in only a 2·2-fold increase in respiration as compared with a 5-fold increase in the absence of CPZ. In the latter instance 10 μM CPZ had no inhibitory effect when added after the ADP (*i.e.* during state 3).

A variety of diphosphopyridine nucleotide-linked substrates as well as succinate gives the same qualitative picture—namely that low concentrations of CPZ, while having no effect on respiration during state 3 (high ADP, substrate), induce a partial stimulation during state 4 (low ADP, high substrate), any further effect by ADP being to some extent prevented.

Metabolic state 2

During this state there is an exceedingly low rate of respiration, substrate being limiting and ADP in excess. Fig. 2 shows a typical experiment in which an 8-fold increase in oxygen uptake occurs upon addition of glutamate and malate when ADP

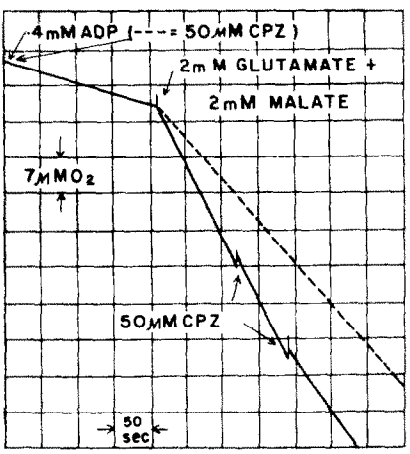

FIG. 2. Rate of O_2 consumption, metabolic states 2 and 3. CPZ is added either before or after glutamate and malate. In one case, ADP is present at the start of reaction which proceeds slowly until the addition of substrate (solid line). The broken line represents a consecutive experiment with CPZ as well as ADP initially present, the pre-substrate segments of the two reactions being identical. Other conditions as in Fig. 1.

is already present. This increased rate remains virtually unchanged by 50 μM CPZ, and is reduced only 25% by a further addition of CPZ to give a final concentration of 100 μM. Since this application is during state 3, the relative insensitivity to the drug at this time corresponds with the previous experiments. In contrast, if 50 μM CPZ is present prior to the addition of substrate there is only slightly better than a 4-fold increase in respiration (Fig. 2, broken line). This reduced rate is no greater than in a parallel experiment in the absence of ADP (*i.e.* glutamate, malate, and CPZ incubated together).

It is thus apparent that the presence of excess ADP during state 2 does not interfere with CPZ action, and that only during state 3 with both excess ADP and substrate supporting fast respiration is there a relative insensitivity to the drug.

Metabolic state 1

Brain mitochondria incubated for even a few minutes in the absence of exogenous ADP or substrate gave rather poor respiratory rates when these factors were later added, as compared to the rate when either one or both were initially present. The phenomenon is not so evident with succinate as with diphosphopyridine nucleotide-linked substrates and therefore may reflect a loss of bound nucleotide during state 1. This matter is under investigation. Although CPZ at concentrations of 50 μM or less during state 1 prevents ADP stimulation, as in the case of states 2 and 4, judgment must be reserved on these results until the basis for spontaneous loss in activity is understood.

DISCUSSION

It is interesting to find that brain mitochondrial respiration is least affected by CPZ during periods of greatest activity (metabolic state 3). In fact, at CPZ concentrations of about 50 μM or lower, no effect may be seen during state 3 whereas rather large ones are evident in other states.

During state 4, 50 μM CPZ actually induces a doubling of respiratory rate. The mechanism of this stimulation is unclear. Since the drug apparently has a reducible semiquinone form it may itself conceivably act as an electron carrier, circumventing a normally inhibited pathway.[13] There are other possibilities, of course, such as an activation or release from inhibition of endogenous ATPase.

The respiratory stimulation ordinarily induced by the addition of ADP during state 4 is particularly sensitive to CPZ. That this is not a simple competition with ADP for acceptor sites is indicated by the fact that in the transition from state 2 to 3 there is a similar degree of inhibition, although an excess of ADP is present during the initial contact with CPZ.

Just why metabolic state may be so critical a factor in the action of CPZ cannot, of course, be answered precisely by present information and theory. However, it would appear from these studies that a high rate of electron transport (as reflected by fast respiration) rather than high or low steady-state reduction of the electron carriers (states 4 and 2 respectively) is correlated with insensitivity to the drug. Conversely, during states of slow transport there is relatively great sensitivity to the drug. It must be cautioned, however, that sequences of addition, inherent in these studies, raise the issue of "permeability" factors, the effects of which may be falsely interpreted as due to metabolic conditions. For example, the presence of CPZ prior to ADP may reduce the access of ADP to mitochondrial sites. These two factors (*i.e.* metabolism and permeability) are especially difficult to separate in the case of CPZ-effects since mitochondrial swelling, used as a guide to possible "permeability" changes caused by the drug,[9] is itself dependent on electron transport.[11, 15]

On another plane these results indicate how difficult it is to interpret the effect of low concentrations of CPZ on respiration of intact cell suspensions, of tissue slices, or *in vivo*, even if the drug's ultimate site of action is assumed to be mitochondrial. Reasoning from the patterns found for isolated mitochondria, one might expect

CPZ to have no effect, to induce a stimulation, or to prevent stimulation of respiration in more intact preparations, depending on metabolic state during exposure to the drug.

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