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The Influence of Respiratory State on Monoamine Oxidase Activity in Rat Liver Mitochondria

By G. S. SMITH and R. A. REID
Department of Biology, University of York, York YO1 5DD, U.K.

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Changes in the respiratory state of rat liver mitochondria caused significant changes (up to 10-fold) in the rates of oxidative deamination of tyramine, indicating interactions between the inner coupling membrane and the monoamine oxidase sites in the outer membrane, and suggesting the possibility that monoamine oxidase is regulated by the thermodynamic state of the mitochondria.

Monoamine oxidase (EC 1.4.3.4) is important in the oxidative deamination of dietary amines and the regulation of catecholamines (McGeer, 1971). It is located in the outer mitochondrial membrane (Schnaitman *et al.*, 1967; Tipton, 1967), and there is evidence for at least two enzyme species with different substrate and inhibitor specificities (Johnson, 1968; Houslay & Tipton, 1974). Investigations into the kinetics and reaction mechanisms of the enzyme have been made primarily on partly purified preparations (Tipton, 1968; Oi *et al.*, 1970), mitochondrial outer membranes (Houslay & Tipton, 1974) and whole mitochondria incubated, however, under conditions disruptive to the membranes and energy-coupling systems (Ekstedt, 1976). Relatively little work has been done on the enzyme in bioenergetically competent mitochondria under defined conditions, and the question of the biological significance of its cellular location has received little attention. The present paper reports findings that demonstrate a relationship between monoamine oxidase activity and mitochondrial respiratory state, which may be relevant to the latter issue.

Materials and Methods

Chemicals

Hexokinase/glucose 6-phosphate dehydrogenase, ADP and inhibitors were obtained from Boehringer Corp., Lewes, Sussex, U.K. [*side-chain*-2-^{14}C]-Tyramine was from The Radiochemical Centre, Amersham, Bucks., U.K. Tyramine and butyl-PBD [5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole were obtained from Koch–Light Laboratories, Colnbrook, Bucks., U.K. All chemicals were analytical grade.

Mitochondria

Mitochondria were isolated from the livers of male Wistar or Alderley Park strain rats (about 250g) by the method of Schneider (1948), as modified by Kielley & Kielley (1951). The final pellet was resuspended in 250mM-sucrose to a concentration of 100mg of protein/ml and stored on ice. Protein was determined by a biuret method (Layne, 1957).

Outer membranes

Outer-membrane fractions were prepared by the Jones & Jones (1968) modification of the swell–shrink method of Sottocasa *et al.* (1967).

Incubations

Mitochondria (5mg of protein) were incubated in 3ml of medium (120mM-sucrose, 6mM-$MgCl_2$, 12mM-potassium phosphate buffer, pH7.0) at 30°C. The vessel was open to the atmosphere, and the contents were vigorously stirred, a high oxygen concentration being maintained throughout the experiments, as measured by a Rank oxygen electrode. Succinate, ADP and inhibitors were added as described in the Figure legends. Samples were removed at intervals for monoamine oxidase assay.

Assay of monoamine oxidase

Monoamine oxidase activity was measured as described by Wurtman & Axelrod (1963) modified as follows. A sample of the mitochondrial incubation medium (0.4ml) was added to 0.1ml of the incubation medium described above containing [^{14}C]tyramine (400μM; 1.562Ci/mol). After 2min incubation in a shaker bath (30°C), 0.2ml of 2M-HCl was added to stop the reaction. The ^{14}C-labelled products (aldehyde and acid) were extracted into 3ml of toluene and 2ml of this was added to 1ml of scintillation fluid (giving a final concentration of butyl-PBD of 0.6%, w/v), and counted for radioactivity with an efficiency of 90%. Reaction rates were calculated after correction for blank values and extraction and counting efficiencies. Under these conditions monoamine oxidase activity was linear for 15min of assay time

and directly proportional to mitochondrial protein up to four times the amount usually added.

Respiration rates

These were determined by using a Rank oxygen electrode, the incubation mixture being sealed to the atmosphere and stirred continuously.

Results

Monoamine oxidase activity towards tyramine in respiratory States 1–4 (Chance & Williams, 1955) is shown in Fig. 1. Over an incubation period of 20min mitochondria in States 2 and 3 showed low and constant monoamine oxidase activity. In contrast, State-4 mitochondria showed high and constant activity, and State-1 mitochondria underwent a transition from an initial low activity to a final high activity comparable with that of State 4.

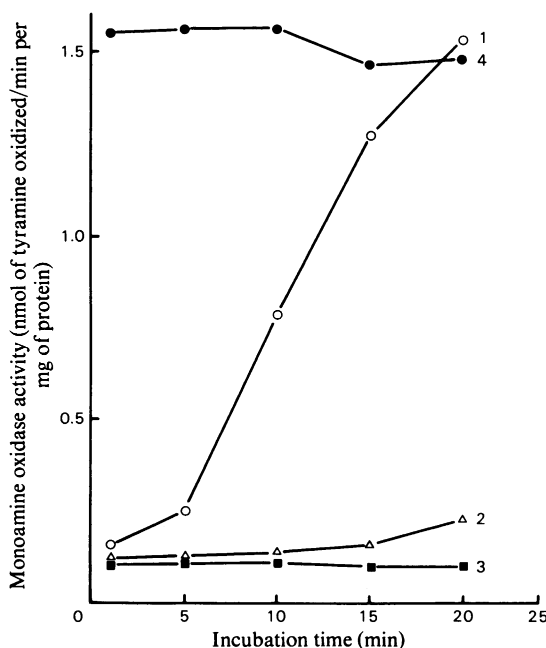

Fig. 1. *Effects of respiratory state on monoamine oxidase activity*

At zero time 0.05ml of mitochondrial suspension was added to 2.95ml of medium as specified in the text (State 1; ○), 2.95ml of medium containing 30μg of hexokinase/glucose 6-phosphate dehydrogenase, 6.7mM-glucose and 1μmol of ADP (State 2; △), 2.90ml of medium containing hexokinase trap components and ADP as above plus 0.05ml of 200mM-sodium succinate (State 3; ■) or 2.90ml of medium plus 0.05ml of 200mM-succinate (State 4; ●). Samples were taken at the times indicated.

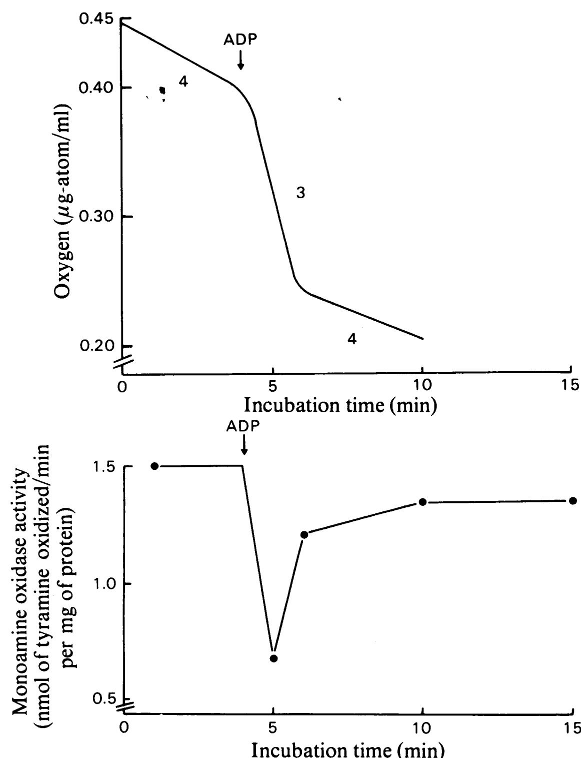

Fig. 2. *Correlation of low monoamine oxidase activity with State-3 respiration*

At zero time 0.05ml of mitochondrial suspension was added to 2.90ml of medium plus 0.05ml of 200mM-sodium succinate. After 4min 1μmol of ADP (0.02ml of 50mM) was added.

Fig. 2 shows that monoamine oxidase activity is sensitive to State 4→3→4 transitions. There was a rapid decrease in activity during the ADP-induced phosphorylating respiration and a rapid recovery after the added ADP had been phosphorylated. When State-3 respiration was prevented by atractyloside inhibition of the adenine nucleotide translocase (Bruni *et al.*, 1964) or oligomycin inhibition of the ATP synthetase (Lardy *et al.*, 1964), the characteristic decrease in monoamine oxidase activity caused by added ADP did not occur (Fig. 3).

The possibility that the substrates, nucleotides and inhibitors used in the above studies were directly affecting the monoamine oxidase was tested on outer-membrane preparations at nucleotide concentrations up to 1mM and the same concentrations of substrates and inhibitors as those used in the experiments described above. The only effect noted was a small decrease in activity in the presence of

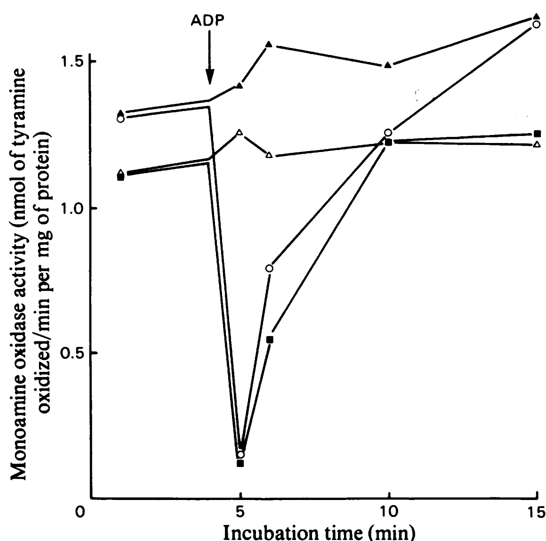

Fig. 3. *Effects of atractyloside and oligomycin*
Incubations were as for Fig. 2 in succinate-containing medium with (○) no further addition, (■) 0.02ml of ethanol, (▲) 0.02ml of atractyloside (1 mg/ml) or (△) 0.02ml of oligomycin (140 μg/ml); 0.05 ml of 50 mM-ADP (2.5 μmol) was added as indicated.

oligomycin, which is explicable in terms of inhibition by the ethanol in which the oligomycin was dissolved (Kurosawa, 1974).

Discussion

The foregoing results show that monoamine oxidase activity is influenced by the respiratory state of mitochondria. In particular, addition of succinate to coupled mitochondria increases the rate of tyramine oxidation 5–10-fold. This succinate-induced activity is diminished by addition of ADP, the lowered activity being maintained throughout phosphorylating respiration. The apparent activation and inhibition is not due to direct effects of succinate or ADP on monoamine oxidase, since these do not significantly affect the activity of outer-membrane preparations. Further, no inhibition by ADP occurs when the adenine nucleotide translocase is inhibited by atractyloside or the ATP synthetase is inhibited by oligomycin, indicating that the nucleotide must pass into the matrix and induce phosphorylating respiration before the ADP effect occurs.

It seems unlikely that the low monoamine oxidase activity caused by ADP addition is due to low oxygen tension at the site of the enzyme brought about by fast phosphorylating respiration. Low activities in State-3 mitochondria have been consistently observed even when the medium is maximally aerated. Also the initial activities of State-1 and -2 mitochondria (Fig. 1) are much lower than the activity of State-4 mitochondria, although the latter respire faster. The best evidence to date that fast State-3 respiration does not decrease monoamine oxidase activity by oxygen starvation is a recent finding that in the presence of rotenone, which inhibits electron transport through the NADH dehydrogenase, NADH-linked substrates induce high monoamine oxidase activity, which is not decreased by fast phosphorylating respiration after the addition of succinate and ADP (G. S. Smith & R. A. Reid, unpublished work).

The results reported in this paper are consistent with a broad correlation between monoamine oxidase activity and the oxidoreduction state of the electron carriers. Respiratory State 4 (high monoamine oxidase activity) shows a high degree of reduction of flavoprotein (Chance & Williams, 1955), which is also the case for mitochondria held in State 4 by oligomycin or atractyloside, even when ADP is present. In contrast, respiratory States 1, 2 and 3 (low monoamine oxidase activity) are characterized by respiratory carriers in more oxidized steady states. It is not clear how monoamine oxidase activity in the outer membrane could be influenced by the redox state of components of the inner membrane. However, points of attachment between the two membranes have been reported, more being apparent in State 4, where the membranes are typically close together, than in State 3, where the membranes are much further apart because of the contraction of the matrix enclosed by the inner membrane (Hackenbrock, 1966, 1968, 1972). Further, it has been shown that a large portion of the tyramine-oxidizing activity of liver mitochondria is located on the inner surface of the outer membrane (Russell *et al.*, 1978), and it may be that this activity can be influenced by the inner membrane through the points of physical contact between the membranes. The gross structural changes associated with State 4 → State 3 transitions are reversible and have been reported to take place within 20s (Lang & Bronk, 1978), which is fast enough to fit the changes in monoamine oxidase activity observed using the present experimental system. These questions and the behaviour of monoamine oxidase in State-1 mitochondria require investigation.

The results presented here raise the question of whether mitochondrial respiratory state plays a role in regulating monoamine oxidase activity *in vivo*. There are instances, outside the scope of this discussion, where some regulation of monoamine oxidase by the redox state of the mitochondria would seem sensible.

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References

Bruni, A., Luciani, S. & Contessa, A. R. (1964) *Nature (London)* **201**, 1219–1220
Chance, B. & Williams, G. R. (1955) *J. Biol. Chem.* **217**, 409–427
Ekstedt, B. (1976) *Biochem. Pharmacol.* **25**, 1133–1138
Hackenbrock, C. R. (1966) *J. Cell Biol.* **30**, 269–297
Hackenbrock, C. R. (1968) *J. Cell Biol.* **37**, 345–369
Hackenbrock, C. R. (1972) *J. Cell Biol.* **53**, 450–465
Houslay, M. D. & Tipton, K. F. (1974) *Biochem. J.* **139**, 645–652
Johnson, J. R. (1968) *Biochem. Pharmacol.* **17**, 1285–1297
Jones, M. S. & Jones, O. T. G. (1968) *Biochem. Biophys. Res. Commun.* **31**, 977–982
Kielley, W. W. & Kielley, R. K. (1951) *J. Biol. Chem.* **191**, 485–500
Kurosawa, Y. (1974) *Jpn. J. Pharmacol.* **24**, 787–795
Lang, R. D. A. & Bronk, J. R. (1978) *J. Cell Biol.* **77**, 135–147
Lardy, H. A., Connelly, J. L. & Johnson, D. (1964) *Biochemistry* **3**, 1961–1968
Layne, E. (1957) *Methods Enzymol.* **3**, 447–454
McGeer, P. L. (1971) *Am. Sci.* **59**, 221–229
Oi, S., Shimado, K., Inamasu, M. & Yasunobu, K. T. (1970) *Arch. Biochem. Biophys.* **139**, 28–37
Russell, S. M., Davey, J. & Mayer, R. J. (1978) *Biochem. J.* in the press
Schnaitman, C., Erwin, V. G. & Greenawalt, J. W. (1967) *J. Cell Biol.* **32**, 719–735
Schneider, W. C. (1948) *J. Biol. Chem.* **176**, 259–266
Sottocasa, G. L., Kuylienstema, B., Ernster, L. & Bergstrand, A. (1967) *J. Cell Biol.* **32**, 415–438
Tipton, K. F. (1967) *Biochim. Biophys. Acta* **135**, 910–920
Tipton, K. F. (1968) *Eur. J. Biochem.* **5**, 316–320
Wurtman, R. J. & Axelrod, J. (1963) *Biochem. Pharmacol.* **12**, 1439–1441