ACTA CHEMICA SCANDINAVICA 17 (1963) 2113–2119

Aldehydes as Inhibitors of Mitochondrial Respiration

1. The Effect of Biological Aliphatic Aldehydes, especially Methylglyoxal, on Brain and Liver Mitochondria *in vitro*

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Six aldehydes which might occur *in vivo* have been studied for their inhibition of pyruvate oxidation in liver and brain mitochondria. Of these, methylglyoxal has been more extensively studied.

Methylglyoxal inhibited pyruvate oxidation more in mitochondria from cerebellum than from cerebrum or liver, and equalled in this respect the previously found effect of acetaldehyde. Also the glutamate oxidation was obviously decreased in both brain and liver mitochondria. Neither the oxidation of succinate nor of α-glycerophosphate was influenced by methylglyoxal.

The inhibition of pyruvate oxidation is more or less abolished by addition of NAD, but only if the mitochondrial membranes have been previously disorganized by aging of the mitochondria.

Methylglyoxal does not affect the oxidation rate of NADH, which indicates that the area or inhibition lies between the substrate and NADH dehydrogenase.

It has been shown previously [1,2] that acetaldehyde *in vitro* strongly inhibits pyruvate oxidation in rat mitochondria especially from the cerebellum, but does not notably affect mitochondrial oxidation of NADH.* Under certain conditions, the inhibition could be completely abolished by the addition of TDP and NAD.

Of mitochondria from other rat tissues, only those from skeletal muscle showed the same lasting sensitivity to the aldehyde as those from cerebellum. Liver and kidney mitochondria rapidly escaped from the inhibition.

In the present work other biologically occurring aldehydes, methylglyoxal, lactaldehyde, glyceraldehyde, glyceraldehyde-3-phosphate, glyoxylic acid and succinic semialdehyde, have been studied for their effect on mitochondrial oxidation.

* Abbreviations: NAD nicotinamide adenine dinucleotide; NADH reduced nicotinamide adenine dinucleotide; TDP thiamine diphosphate; CoA coenzyme A.

EXPERIMENTAL

Male albino Wistar rats from this institute's stock were used. The tissues were homogenized, the mitochondria separated, the Warburg vessels filled and the protein determined as described previously.[2] The substrates were either 30 μmole pyruvate together with 2.5 μmole malate to each vessel, or 30 μmole succinate, glutamate, α-glycerophosphate or NADH as stated in the tables.

The aldehydes were placed in the main compartment of the Warburg vessel immediately before the start of the experiment.

The glyceraldehyde, glyceraldehyde-3-phosphate and Na-glyoxylate were from Sigma Chemical Company. Succinic semialdehyde was synthesized according to a modified method by Albers[3] referred by Jacoby, as were lactaldehyde after Huff and Rudney,[4] and methylglyoxal by the method of Shroeder and Woodward.[5]

The figures and tables present results from single typical experiments.

RESULTS

The effect of various aldehydes on the oxidation of pyruvate by liver and brain mitochondria

Of the aldehydes tested, Na-glyoxylate, succinic semialdehyde and methylglyoxal inhibited pyruvate oxidation in liver mitochondria, whereas the others did not (Table 1). Increasing membrane permeability by aging the mitochondria for 3 min in water at 30°C before incubation, did not potentiate the activity of the non-active aldehydes.

With brain mitochondria only methylglyoxal was an inhibitor when present in moderate concentrations. High concentrations of Na-glyoxylate and succinic semialdehyde were required to afford any effect. Additions of smaller amounts of succinic semialdehyde resulted in an increase of the respiration.

Mainly on account of this preliminary orientation (Table 1), five of the compounds have been excluded from further studies as inhibitors of mitochondrial respiration. Only methylglyoxal has been more extensively investigated.

Effect of methylglyoxal on oxidation of various substrates by liver and brain mitochondria. Table 2 shows that pyruvate and glutamate oxidation is strongly inhibited by methylglyoxal in both brain and liver mitochondria. The oxidation of succinate and α-glycerophosphate, however, is only slightly inhibited in liver mitochondria and not at all in brain. Even a slight increase in the oxygen consumption is sometimes observed. This increase may depend on methylglyoxal itself serving as a substrate either directly or by transformation to pyruvate.

Pyruvate oxidation in mitochondria with various concentrations of methylglyoxal. Like acetaldehyde,[2] methylglyoxal inhibits pyruvate oxidation much more in cerebellar than in cerebral or liver mitochondria (Fig. 1). The oxygen consumptions given in the figure are calculated from 20-min incubations and listed as oxygen consumption per hour. However, brain mitochondria as distinguished from liver mitochondria, gradually escape from the inhibition (Fig. 2). This means that the initial inhibition of brain mitochondria, at least with low concentrations of methylglyoxal, is more pronounced than is apparent from the slope of the curves.

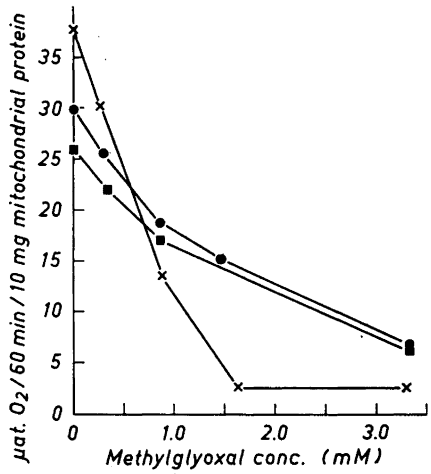

Fig. 1. Methylglyoxal inhibition of pyruvate oxidation in mitochondria from brain and liver. The results, expressed as µat. O_2/60 min, are based on 20 min incubations. Methylglyoxal was present in the main compartment of the manometer vessels from the beginning. For further details see "Experimental". ● cerebrum, × cerebellum, ■ liver.

Attempts to abolish the inhibition. Acetaldehyde inhibition of pyruvate oxidation is rapidly abolished in liver but not in brain mitochondria.[2] As can be seen in Fig. 2, no tendency to escape from the inhibition is noted in liver mitochondria when methylglyoxal is the inhibitor. However, with brain mitochondria, either from cerebrum or cerebellum, there is a tendency to abolish the inhibition of low concentrations of methylglyoxal.

Addition of various cofactors also neutralizes the inhibition of pyruvate oxidation in both liver and brain mitochondria (Table 3). Of these only NAD has a remarkable neutralizing effect. At the concentration used, however, even NAD only completely restores respiration in aged liver mitochondria oxidizing glutamate.

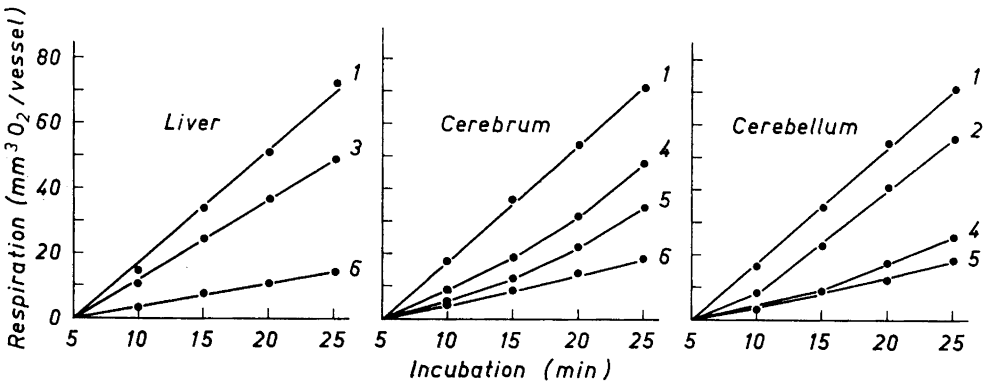

Fig. 2. Time lapse of the methylglyoxal inhibited pyruvate oxidation in mitochondria from brain and liver. Methylglyoxal was added as in Fig. 1. The first manometer reading was after five min incubation. The figures 1—6 refer to the following methylglyoxal concentrations: 1: no methylglyoxal, 2: 0.3 mM, 3: 0.7 mM, 4: 0.9 mM, 5: 1.5 mM, 6: 3.0 mM.

The effect of methylglyoxal on the mitochondrial oxidation of NADH. Mitochondria can be made accessible to NADH, by treating or "aging" them with water before incubation. Such water-aged mitochondria ozidize NADH rapidly without any signs of inhibition by methylglyoxal in concentrations causing a 50 % inhibition of the pyruvate and glutamate oxidation (Table 2). The results with acetaldehyde [2] were the same, indicating that both aldehydes achieve their inhibition between the substrate and the NADH dehydrogenase.

DISCUSSION

The present results together with previous investigations [1,2,6,7] show that certain aliphatic aldehydes are inhibitors of mitochondrial respiration while others are not. The reason for these differences is not clearly understood. It is

Table 1. The effect of various aldehydes on pyruvate oxidation by liver and brain mitochondria. Liver and brain mitochondria were incubated in Warburg vessels with 30 μmole pyruvate together with 2.5 μmole malate and with remaining additions as described earlier.[2] Incubation time 20 min at 30°C.

Aldehyde	Final conc. (mM)	μat. O_2/60 min/10 mg protein liver	brain
Glyoxylate	—	25.8	26.0
	0.5	20.4	26.4
	2.5	14.0	26.4
	12.5	7.4	7.7
Succinic semialdehyde	—	25.0	26.3
	0.2	24.4	39.4
	1.0	14.0	41.6
	2.0	4.5	26.8
	4.0	0.8	0.0
Glyceraldehyde-3-phosphate	—	19.9	31.5
	0.5	23.3	32.5
	1.0	19.5	27.9
	2.5	21.8	32.8
Glyceraldehyde	—	18.6	29.6
	0.5	17.0	31.0
	1.0	16.6	30.6
	5.0	17.4	26.2
Lactaldehyde	—	20.1	33.0
	0.5	20.6	34.8
	1.5	24.2	38.7
	7.5	22.8	36.3
	15.0	23.0	36.8
Methylglyoxal	—	21.1	26.0
	0.5	15.5	19.2
	2.5	6.3	6.5
	12.5	1.5	0.0

not due to differing mitochondrial permeability, as the inactive aldehydes still fail to inhibit respiration even in aged mitochondria.

The differing capacity of the two closely related aldehydes, lactaldehyde and methylglyoxal to inhibit the oxidation of pyruvate in brain and liver mitochondria is especially interesting. The latter is a rather potent inhibitor, while the former does not inhibit at all, even in very high concentrations (Table 1).

In the present investigation, there has been no attempt to include all aliphatic aldehydes which might normally occur in tissues. Only those aldehydes which might fit into our investigations concerning the effects of ethanol on metabolism have been taken into consideration.

The central problem is to find aldehydes with the same influence on the tissue respiration, and especially the pyruvate oxidation, as previously shown for acetaldehyde.[2] The results in Table 1 in the present paper have partly been the basis for an exclusion of the inactive aldehydes, from further studies. Thus glyceraldehyde, glyceraldehyde-3-phosphate and lactaldehyde, being inactive, were excluded. Glyoxylate, being a moderate inhibitor only of liver mitochondria, and having been extensively investigated by others [8–13] was also excluded.

Table 2. Oxidation of various substrates by brain and liver mitochondria in the presence and absence of methylglyoxal. In all incubations, except when NADH (30 μmole) is the substrate, the mitochondria have been prepared in the normal way described in Experimental. With NADH added to liver mitochondria, these have been preincubated in water 3 min at 30°C to make them accessible to NADH. Besides the usual additions 0.25 mg cytochrome c was added to each vessel.

Substrate	Inhibitor Methyl-glyoxal (mM)	μat. O_2/60 min/10 mg mitochondrial protein			
		Brain			Liver
		whole brain	cerebellum	cerebrum	
Pyruvate	0	25.0	36.4	32.5	20.1
	1.5	12.5	5.0	16.2	6.1
Succinate	0	27.0	31.2	21.8	37.2
	1.5	26.2	39.7	23.7	32.3
Glutamate	0	30.0	54.7	39.2	30.9
	1.5	15.5	19.5	18.5	8.2
α-Glycero-P	0	8.6	5.7	6.6	8.2
	1.5	9.7	6.9	7.1	7.0
NADH	0				30.8
	1.5				30.0
None added	0			4.1	3.0
	1.5			3.9	5.0

Succinic semialdehyde is a comparatively strong inhibitor of pyruvate oxidation in liver mitochondria (Table 1). With brain mitochondria, however, this aldehyde strongly enhances respiration in low concentrations, probably by being transformed into succinic acid which is oxidized in the citric acid cycle. The precursor of succinic semialdehyde, γ-aminobutyric acid, has been found in large amounts in brain [14] but not in liver.[15] Spectrofluorometric assay of succinic semialdehyde in rat brain (Kiessling, unpublished results), however, showed barely detectable amounts both in the presence and absence of ethanol in the animal. Thus the lack of the precursor in liver and of the aldehyde itself in brain makes this compound unlikely to be an inhibitor of mitochondrial respiration *in vivo*.

So far methylglyoxal has been the only one of the six aldehydes deserving more extensive investigation as a possible biological inhibitor of mitochondrial respiration.

In several respects, methylglyoxal and the previously investigated acetaldehyde behave the same. They inhibit pyruvate oxidation much stronger with mitochondria from cerebellum than from cerebrum or liver (Ref.[1] and Fig. 1). With both aldehydes, the inhibition is located somewhere between the substrate and NADH dehydrogenase, as the oxidation of NADH is not at all or only slightly affected by the aldehydes (Ref.[1] and Table 2). The inhibitions are also partly abolished by NAD, especially when the mitochondria have been pretreated by aging (Ref.[2] and Table 3).

Among the differences observed between methylglyoxal and acetaldehyde, the most striking is the strong inhibition of glutamate oxidation by the former

Table 3. The effect of NAD, TDP and CoA on methylglyoxal inhibition in liver and brain mitochondria. Methylglyoxal is present in a final concentration of 1.5 mM with liver and cerebrum and 0.75 mM with cerebellum mitochondria. Final concentrations of NAD, TDP and CoA are 1×10^{-3} M, 2×10^{-4} M and 4×10^{-5} M, respectively. With aged liver mitochondria, 0.25 mg cytochrome c is present. The headings *aged* and *untreated* indicate that the mitochondria have been preincubated in glass distilled water 3 min at 30°C before addition to the Warburg vessels (aged), or were used directly after separation and washing in 0.25 M sucrose (untreated). P and G symbolize pyruvate and glutamate used as substrates.

Additions (M = methylglyoxal)	Oxygen consumed (μat. O_2/60 min/10 mg protein)								
	Liver				Cerebrum				Cerebellum
	untreated		aged		untreated		aged		untreated
	P	G	P	G	P	G	P	G	P
–	20.4	25.6	30.9	24.6	27.6	32.2	29.0	38.2	36.4
M	9.2	12.2	14.6	12.3	14.5	20.3	11.5	14.8	18.0
M + NAD	13.9	15.2	19.2	25.4	16.9	23.1	25.5	27.4	27.4
M + TDP	10.6								19.3
M + NAD + TDP	15.7								27.9
M + NAD + TDP + CoA									28.8

(Ref.[7] and Table 2). Except with mitochondria from cerebellum, this is of the same order as when pyruvate is the substrate.

One immediate precursor of methylglyoxal is aminoacetone. According to Urata and Granick [16] the degradation of aminoacetone in liver preparations is supposed to go principally via deamination to methylglyoxal. The formation of aminoacetone from glycine and acetyl-CoA, pyruvate or Krebs cycle intermediates has been demonstrated in liver mitochondria.[16] Arnstein and Keglevic [17] have shown that the major portion of tissue glycine arises from serine, which is formed from carbohydrates or fats via hydroxypyruvate and with alanine as an amino group donor.

Thus threonine, glycine and several other compounds, but mainly serine, by giving rise to glycine, can be transformed into methylglyoxal. Methylglyoxal is metabolized further to lactate by a glyoxylase system which is dependent on reduced glutathione. The glyoxylase system, however, seems sluggish or inactive in isolated liver and brain mitochondria, as the mitochondria only slowly recover from the inhibition (Fig. 2).

Conditions resulting in an altered methylglyoxal metabolism thus might cause changes in the oxidation rate of certain substrates in the tissues. These are now being investigated.

Acknowledgements. This is part of investigations made possible by grants from the *Swedish Medical Research Council.* I am indebted to Miss Margareta Skoglund for skillful technical assistance.

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Received May 6, 1963.