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THE EFFECTS OF LAURYLAMINE ON RESPIRATION AND RELATED REACTIONS OF LIVER MITOCHONDRIA *IN VITRO*

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SUMMARY

1. The effects of laurylamine on respiration and related reactions of mitochondria from rat liver were studied. At low concentrations (0.1 mM) the amine increased initial, State-3, and State-4 respiration and decreased the acceptor control and ADP:O ratios (succinate or α-ketoglutarate). ADP:O ratios with ascorbate–tetramethyl-*p*-phenylenediamine were also decreased.

2. Laurylamine caused a small stimulation of ATPase activity. ATPase induced by 0.03 mM dinitrophenol was further increased by low and inhibited by higher amine concentrations; the amine similarly influenced respiration stimulated by 0.03 mM dinitrophenol. ATPase activity with 0.15 mM dinitrophenol was only inhibited by the amine.

3. Laurylamine inhibited the P_i–ATP exchange reaction.

4. Chlorpromazine similarly affected respiration and both compounds stimulated oxygen uptake without P_i added or in the presence of oligomycin. It is suggested that the two amines may affect oxidative phosphorylation in the same way.

5. Of the C_9 to C_{15} aliphatic amines, the optimal concentration for a stimulation of respiration was least for the C_{13} and C_{14} compounds. Binding of decylamine compared with laurylamine was sufficiently less to account for the greater concentration required for maximal stimulation.

INTRODUCTION

Previously[1,2] it was shown that laurylamine, chlorpromazine, imipramine, and phencyclidine cause a greater uncoupling and stimulation of respiration with succinate as substrate at an alkaline pH whereas dinitrophenol, laurate, and lauryl sulfate have more effect on oxidative phosphorylation at an acid pH. The present study was undertaken to further define the effects of laurylamine on respiration.

METHODS

Mitochondria were prepared from livers of fasted rats essentially according to SCHNEIDER[3], but washed free of fluffy layer. Respiration was measured by Warburg technique or polarographically (Gilson Medical Electronics Oxygraph with oscillating

Abbreviation: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

platinum electrode). The ascorbate–*N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) system was similar to that employed by PACKER AND MUSTAFA[4].

Phosphorus was measured by the method of LOWRY AND LOPEZ[5], slightly modified, and protein by a biuret method.

Analyses for P_i–ATP exchange were performed essentially as described by GÓMEZ-PUYOU *et al.*[6]. After precipitation of protein with trichloroacetic acid an aliquot of the supernatant was chromatographed on Whatman No. 1 paper using methanol–1 M ammonium acetate (75:35, v/v). After elution, ATP was determined spectrophotometrically (259 mμ) and radioactivity measured with a Nuclear-Chicago gas-flow counter with a thin window. Counts were corrected for radioactivity obtained with trichloroacetic acid added before the mitochondria, as well as background, and for changes in specific activity during the exchange[7].

Binding was studied using ^{14}C-labeled amines as previously described[2].

Chlorpromazine–HCl was donated by Smith Kline and French Laboratories and laurylamine–HCl by General Mills Chemical Division. The other amines were obtained from Aldrich Chemical Co. and dissolved as hydrochloride salts. ADP, ATP, antimycin A and oligomycin (85 % B, 15 % A) were from Sigma Chemical Co.; [1-^{14}C]laurylamine–HCl and [1-^{14}C]decylamine–HCl from Nuclear Research Chemicals; [^{32}P]orthophosphate from Nuclear-Chicago.

RESULTS AND DISCUSSION

Previously the effects of laurylamine on respiration by mitochondria from rat liver were measured manometrically with succinate and ATP added[2]. The amine had little effect at low levels but with increasing concentrations respiration was stimulated and then inhibited. These effects have been further studied polarographically. As the concentration of amine was raised, respiration with α-ketoglutarate (Table I) or

TABLE I

EFFECT OF LAURYLAMINE ON RESPIRATION MEASURED POLAROGRAPHICALLY

Expt. 1. Medium contained in 1.4 ml: 87 μmoles KCl, 10 μmoles potassium phosphate, 10 μmoles $MgCl_2$, 33 μmoles Tris–HCl buffer, 17 μmoles potassium α-ketoglutarate, and mitochondria (2.3 mg protein) in 0.2 ml 0.25 M sucrose; ADP added was 0.2 μmole. The pH was 7.5, temp. 23°. *Expt. 2.* In 1.51 ml: 30 μmoles KCl, 10 μmoles potassium phosphate, 7.5 μmoles $MgCl_2$; 33 μmoles Tris–HCl, 100 μmoles sucrose, 0.4 μmole TMPD, 10 μmoles ascorbate, 0.25 μg antimycin A, 1.5 mg mitochondrial protein; 0.15 μmole ADP added. The pH was 7.5, temp. 23°.

Expt. No.	*Substrate*	*Concentration laurylamine–HCl (mM)*	*O_2 uptake (μatoms/min)*			*Acceptor control ratio*	*ADP:O ratio*
			Initial	*With ADP*	*After ADP*		
1	α-Keto-glutarate	—	0.024	0.085	0.014	6.1	2.47
		0.05	0.030	0.112	0.022	5.1	2.13
		0.10	0.043	0.115	0.022	5.2	2.03
		0.15	0.047	0.090	0.033	2.7	1.95
2	TMPD + ascorbate	—	0.115	0.180			0.95
		0.06	0.119	0.138			0.54
		0.07	0.127	0.117			0.29

TABLE II

EFFECTS OF LAURYLAMINE AND CHLORPROMAZINE ON RESPIRATION WITH AND WITHOUT INORGANIC PHOSPHATE OR WITH OLIGOMYCIN ADDED

Medium as in Table I (Expt. 1) except with 33 μmoles succinate as substrate; in Expt. 1 mitochondrial protein was 1.9 mg, temp. 24°; in Expt. 2: 2.0 mg, 23°.

Expt. No.	*Compound added*	*O_2 uptake (μatoms/min)*			*ADP:O*	*Initial O_2 uptake*	
		Initial	*With ADP*	*After ADP*		*Without P_i*	*With 6 μg oligomycin*
1	None	0.037	0.144	0.020	1.93	0.028	
	0.05 mM laurylamine–HCl	0.052	0.181	0.054	1.78	0.062	
	0.10 mM	0.104	0.169	0.062	1.60	0.080*	
	0.12 mM	0.089	0.144	0.050	1.40	0.084*	
	0.05 mM chlorpromazine–HCl	0.065	0.180	0.055	1.54	0.053	
	0.10 mM	0.100	0.182	0.080	1.28	0.074	
	0.12 mM	0.122	0.182	0.083	1.17	0.092	
2	None	0.039					0.038
	0.10 mM laurylamine–HCl	0.074					0.068
	0.16 mM						0.092
	0.20 mM						0.060
	0.10 mM chlorpromazine–HCl	0.079					0.076
	0.20 mM						0.106
	0.25 mM						0.102
	0.03 mM 2,4-dinitrophenol						0.173

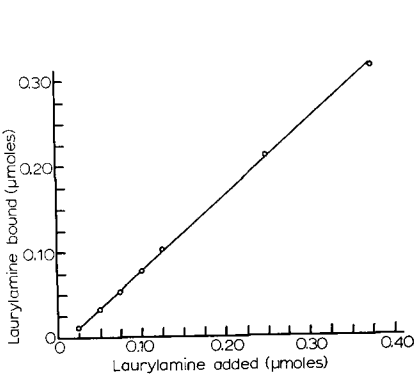
* Values were measured during first minute (less during second minute).

succinate (Table II) first increased—including the initial rate, respiration with ADP added and after it was used (States 3 and 4, respectively[8])—then dropped off; at higher amine levels (data not shown) the addition of ADP depressed oxygen uptake below the initial rate. Acceptor control ratios and ADP:O ratios decreased. At higher amine concentrations respiration tended to decrease after 1 min, particularly in State 4. The concentration of amine employed was limited because as the level was increased, the mitochondria tended to clump which interfered with the electrode, and also to settle out, probably because of neutralization of the net negative charge on the mitochondrial surface[9]. The concentration range of amine that stimulated respiration seemed to be more narrowly defined when measured manometrically than polarographically, perhaps because of differences in technique such as shaking or timing. It was not due to amount of ATP as results with the oxygen electrode remained similar with 3 μmoles ATP added (data not shown). The optimal concentration for the stimulatory effect has been found to vary with the mitochondria (0.09–0.10 μmole/mg protein with manometric technique) which could be expected since most of the compound is bound to the mitochondria and in proportion to the concentration of amine added (Fig. 1). With ascorbate–TMPD (Table I) laurylamine decreased the ADP:O ratios. The concentrations shown stimulated initial respiration but were inhibitory with ADP present; higher levels inhibited both.

Some stimulation of ATPase activity was obtained with laurylamine (Fig. 2) but much less than with 2,4-dinitrophenol. There was a greater effect on dinitrophenol-induced ATPase activity. In the presence of 0.03 mM dinitrophenol, which does not give optimal stimulation, the amine at low concentrations stimulated and at higher

concentrations inhibited the ATPase activity, whereas with 0.15 mM dinitrophenol the amine was inhibitory throughout. These results correlated with the effects of the amine on respiration stimulated by 0.03 mM dinitrophenol with further stimulation by low and inhibition by higher amine levels (Table III).

Laurylamine also inhibited the P_i–ATP exchange reaction (Fig. 2). A minimal concentration was required and inhibition was about 50 % at 0.09 μmole/mg mitochondrial protein which corresponded to the amount for maximal stimulation of respiration.



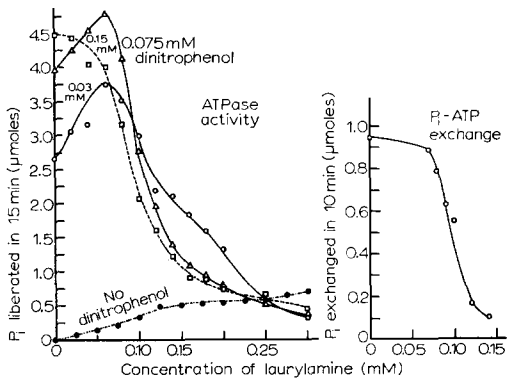


Fig. 1. The binding of laurylamine to the mitochondria. Medium contained in 2.5 ml: 108 μmoles KCl, 30 μmoles potassium phosphate, 50 μmoles Tris–HCl buffer, 15 μmoles $MgCl_2$, 50 μmoles potassium succinate, and mitochondria (4.8 mg protein) in 0.5 ml 0.25 M sucrose; pH was 7.2.

Fig. 2. The effects of laurylamine on ATPase activity and the P_i–ATP exchange reaction. For ATPase activity the medium contained in 1.5 ml: 112 μmoles KCl, 75 μmoles Tris–HCl buffer, 0.9 μmole EDTA, 10 μmoles ATP, and mitochondria (0.8 mg protein) in 0.5 ml 0.25 M sucrose. The pH was 7.2, temp. 25°. Two experiments are shown, one with and one without dinitrophenol. After 15 min incubation, 0.5 ml 20 % trichloroacetic acid was added. An aliquot of the supernatant was analyzed for inorganic phosphate; initial values were subtracted. For the P_i–ATP exchange reaction the medium contained in 1 ml: 5 μmoles potassium orthophosphate labeled with ^{32}P (500000 counts per min), 5 μmoles ATP, 25 μmoles Tris–HCl buffer, and mitochondria (1.1 mg protein) in 0.1 ml 0.25 M sucrose; pH was 7.3. After 10 min incubation at 21°, 0.2 ml 30 % trichloroacetic acid was added.

TABLE III

EFFECT OF LAURYLAMINE ON RESPIRATION STIMULATED BY DINITROPHENOL

Medium as in Table I (Expt. 1) with 33 μmoles succinate as substrate, 2.1 mg mitochondrial protein, and temp. 22°.

Concentration of laurylamine–HCl (mM)	*Initial O_2 uptake (μatoms/min)*	
	Without dinitrophenol	*0.03 mM dinitrophenol*
—	0.030	0.172
0.05		0.224
0.10	0.083	0.226
0.15	0.115	0.206
0.20	0.094	0.109
0.25	0.065	0.081
0.30	0.062	0.072

Chlorpromazine affects the ATPase activity of liver mitochondria[10,11] much like laurylamine, inhibits the P_i–ATP exchange reaction[12], and the effects of both on respiration and oxidative phosphorylation are similar and influenced in the same way by pH[1,2]. It was therefore included in some of these experiments. The two amines similarly affected respiration measured polarographically (Table II). Furthermore, without inorganic phosphate added or in the presence of oligomycin, both amines stimulated respiration at low concentrations; however, the maximal rate obtainable was only 50 to 60 % of the oxygen uptake with 0.03 mM dinitrophenol because of the inhibition by higher amine levels. The response was always immediate whether the mitochondria were added to a medium containing both oligomycin and amine or if the amine were added after respiration had been measured with oligomycin present. Stimulation of respiration by chlorpromazine at a low phosphate level has been reported by DAWKINS, JUDAH AND REES[12].

These results suggest further that chlorpromazine and laurylamine probably do affect respiration in the same way. According to the mechanism of oxidative phosphorylation supported by many workers[13–15], the amines, at low concentrations at least, probably do not affect the same site as oligomycin (indicated by LARDY[16]) but one independent of P_i and closer to the respiratory chain. The site could be the same as that influenced by dinitrophenol (respiration and ATPase stimulated by dinitrophenol were similarly affected by laurylamine) but the effects may be caused in a different manner, considering the difference in concentration required and the opposite effects of pH on uncoupling by the phenol compared with the amines[1]. Also binding of dinitrophenol[17] is greater at acid pH, whereas binding of the amines[2] is about the same at pH 6 to 8.

The inhibition of respiration by higher levels of laurylamine could be due to a further exaggeration of the effect of low concentrations or might, perhaps in part, be a separate effect. If the optimal concentration for a stimulatory effect is any indication of the level where inhibition begins, it may be significant that neither the optimal concentration nor the binding of laurylamine were affected by the pH when the respiration rate was[2].

With respect to the possible mechanism of action of chlorpromazine, YAGI, OZAWA AND NAGATSU[18] showed the amine can complex with flavins and, as pointed out by LÖW[10] and DAWKINS, JUDAH AND REES[11], it might interfere with the flavoproteins of the respiratory chain to inhibit respiration. Oxidative phosphorylation is uncoupled with ferrocytochrome *c* as substrate[19–21] but no evidence of uncoupling was found between α-ketoglutarate or β-hydroxybutyrate and cytochrome *c*[19,21]. In addition, cytochrome oxidase activity is inhibited by the amine[21,22] (also by laurylamine, unpublished work) but this could occur in a different way, *e.g.*, by limiting access of cytochrome *c*. The suggestion by SPIRTES AND GUTH[23] that chlorpromazine may act primarily by affecting the mitochondrial membranes has been supported by LØVTRUP[24]. The effects of imipramine, included in previous studies[1,2], are similar and probably occur in the same way[24–26]. BORG AND COTZIAS[27] and BORG[28] have proposed that the biological effects of chlorpromazine and imipramine may depend on the formation of a semiquinone or similar radical. This would not account for the effects of the aliphatic amine on respiration.

The influence of chain length (C_9 to C_{15}) on the effects of aliphatic amines on succinoxidase activity was also studied (Fig. 3). As the chain length was increased

from C_9 to C_{13}, the concentration required for optimal stimulation decreased and differed less from that for the amine with one carbon less. From C_{12} to C_{15} the optimal concentration did not vary greatly but appeared to be least for the C_{13} and C_{14} compounds; C_{15} tended to exhibit the broadest curve and was least inhibitory.

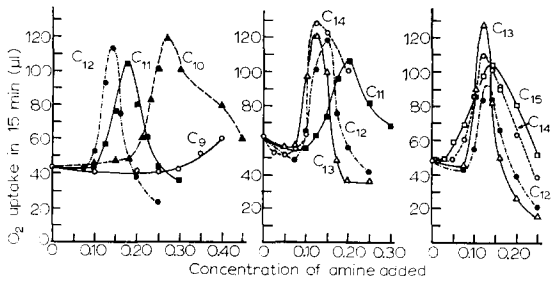


Fig. 3. The effects of C_9 to C_{15} aliphatic amines on succinoxidase activity. Manometric technique was employed with 2.5 ml medium as in Fig. 1 except 3 µmoles ATP were also added, mitochondrial protein was 3.4 to 3.6 mg in the three experiments, and the pH 7.4. Center wells contained 0.2 ml 3 M KOH and filter paper. Temperature was 37°, equilibration period 8 min. Concentration mM.

It is interesting that of the saturated fatty acids of even carbon number, BJÖRNTORP, ELLS AND BRADFORD[29] found myristic acid required the least concentration to affect mitochondrial respiration with α-ketoglutarate. Sufficiently less decylamine than laurylamine was bound (Table IV) to account for the difference in amount required for maximal stimulation. In this case, at least, the variation in optimal concentration with chain length seems to depend simply on the degree of hydrophobic bonding. The differences in the shape of the curves for the longer-chain amines might relate to an effect of molecular size on the binding. Chlorpromazine, which would be bulkier, exhibits a broader curve[2] than the aliphatic amines and could conceivably be bound in a less organized manner.

TABLE IV

THE BINDING OF DECYLAMINE AND LAURYLAMINE TO MITOCHONDRIA

Medium as in Fig. 1; mitochondrial protein was 5.8 and 6.5 mg for Expts. 1 and 2, respectively; pH 7.5. Samples were in duplicate.

Expt. No.	*Compound*	*Concentration (mM)*	*Per cent bound*
1	Decylamine–HCl	0.15	58
	Laurylamine–HCl	0.15	79
2	Decylamine–HCl	0.10	52
		0.20	39
	Laurylamine–HCl	0.10	83
		0.20	81

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