POLYAMINE EFFECTS ON SUCCINATE-LINKED AND αKETOGLUTARATE-LINKED RAT LIVER MITOCHONDRIAL RESPIRATION

Rowand R.J. Chaffee[1], Leon Salganicoff[2], Robert M. Arine[1], Rene H. Rochelle[1] and Edward L. Schultz[3]

[1]Department of Ergonomics, University of California, Santa Barbara, California 93106, [2]Department of Pharmacology, Health Sciences Center, School of Medicine, Temple University, Philadelphia, Pennsylvania 19140 and [3]Department of Biological Sciences, University of California, Santa Barbara, California 93106.

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SUMMARY: At 0.6-1.17 mM Mg^{++}, physiological spermine levels strikingly enhance respiratory control ratios of rat liver mitochondria with αketoglutarate by suppressing respiration which occurs after added ADP is converted to ATP. Respiration with added ADP is enhanced at 0.6 but not at 1.17 mM Mg^{++}. Conversely, with succinate, spermine at high concentrations depresses respiratory control ratios but only slightly affects respiration. Spermidine with αketoglutarate increases respiratory control ratios but suppresses respiration both during and after added ADP-ATP conversion. However, suppression is greater after this conversion. With succinate, spermidine also increases respiratory control but its effects on respiration during or after added ADP-ATP conversion vary with Mg^{++} concentration. Thus, polyamines seem to affect mitochondrial metabolism.

Many reviews (1-6) have been written on spermine and spermidine, indicating that they affect many cellular processes, sometimes acting like bivalent cations, primarily Mg^{++} (cf. 2). However, only one abstract (7) has been published on the effects of spermidine and spermine on mitochondrial respiration of any tricarboxylic acid cycle intermediate. In that abstract, it was reported that spermidine and spermine enhance mitochondrial succinic acid oxidase activity* in the presence of added ADP**. Moreover, it stated that polyamines do not affect mitochondrial respiration after the added ADP has been converted to ATP*** (ibid).

The information herein presented seems to contradict several of the findings reported in the above mentioned abstract. More importantly, however, the research herein reported explores the combined effects of differ-

* μl O_2 consumed/mg rat liver mitochondrial protein/hr
** Defined as state 3 respiration by some workers
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ent concentrations of Mg^{++} and polyamines on mitochondrial respiration associated not only with succinate but also with αketoglutarate. The evidence obtained strongly indicate that naturally occurring polyamines (i.e., spermine and spermidine) may play an important role in the regulation of mitochondrial respiration and directly affect the respiratory control ratio**** and thus these polyamines may well be directly involved in cellular oxidative metabolic regulation.

METHODS

Mitochondria were prepared from livers of adult male Sprague-Dawley rats by methods described elsewhere (8). The polarographic method of Estabrook (9) was used and all assays were done at 37°C. For assaying succinate oxidation, a modification of the reaction mixture of Harris et al (10) was used so that the results could be directly compared with those previously reported in the above mentioned abstract (7). In the abstract, however, only one level of Mg^{++} was involved. The 3.6 ml reaction mixture contained: 900 μMoles sucrose, 90 μMoles KCl, 18 μMoles KH_2PO_4, 72 μMoles Tris-HCl, 100 μMoles succinate and 1.08 μMoles ADP (pH 7.2).

For assaying αketoglutarate oxidation, a modification of the reaction mixture of Hoch and Lipmann (8) was used, since this method gives good activity with this substrate, unlike the assay system of Harris et al (10). The 3.6 ml reaction mixture contained: 1.44 mMoles glycylglycine, 48 μMoles KH_2PO_4, 50 μMoles αketoglutarate and 1.08 μMoles ADP (pH 7.4).

In each assay, 2-4 mg of mitochondrial protein was used. Protein content was determined by the spectrophotometric method of Lowry et al (11).

Added spermidine, spermine and Mg^{++} concentrations were varied in order to see how these alterations affect mitochondrial respiration and respiratory control. Spermidine concentration was varied between 1.25 and 2.5 mM and spermine between 1.0 and 2.0 mM. These concentration ranges of polyamines are close to those reported for rat liver (cf. 1), if one assumes the liver to be 60% or less water (12). The levels of Mg^{++} ranged from 0.5 to 1.17 mM. These levels were selected from data reported by Veloso et al (13) who found that the liver contained 0.6-1.17 μMoles/gm wet weight of Mg^{++} free in the cytoplasm (i.e. 0.6-1.17 mM), here again based on rat liver being about 60% water.

The times of additions of substrate, polyamine, Mg^{++} and ADP were kept constant throughout these studies. Mg^{++} and/or polyamine were added to the reaction mixture and allowed to equilibrate. Next mitochondria were added and 3.5 min. later, the substrate was added. ADP was added 2 min after this and respiration was allowed to proceed for 6-8 min.

RESULTS AND DISCUSSION

Spermine Effects on αKetoglutarate Oxidation at Various Mg^{++} Concentrations

Spermine strikingly enhances the respiratory control ratio at all levels of Mg^{++} used (Table 1) and thus acts as a mitochondrial metabolic

**** respiration rate with ADP/respiration rate after conversion of ADP to ATP

Table 1. Spermine effects on α ketoglutarate-linked rat liver mitochondrial respiration and respiratory control ratio.

Mg^{++} (mM)	Spermine (mM)	Q_{O_2}* during ADP-ATP conversion	p	Q_{O_2}* after ADP-ATP conversion	p	Respiratory Control Ratio	p
0.60	0**	27.60 ± 3.42	---	21.42 ± 2.52	---	1.29 ± 0.07	---
0.60	1.00	39.19 ± 3.50	0.001	15.15 ± 0.85	0.02	2.56 ± 0.14	0.001
0.60	1.50	40.98 ± 3.67	0.001	15.71 ± 0.84	0.05	2.60 ± 0.15	0.001
0.60	2.00	40.52 ± 2.58	0.001	16.74 ± 1.25	0.05	2.49 ± 0.17	0.001
0.93	0**	34.27 ± 3.61	---	22.95 ± 1.97	---	1.51 ± 0.13	---
0.93	1.00	40.54 ± 3.46	0.01	14.27 ± 1.11	0.001	2.91 ± 0.22	0.001
0.93	1.50	39.36 ± 3.38	0.02	14.25 ± 0.97	0.001	2.76 ± 0.11	0.001
0.93	2.00	38.03 ± 2.79	0.10 (NS)	13.76 ± 0.96	0.001	2.80 ± 0.16	0.001
1.17	0**	36.90 ± 4.13	---	21.95 ± 1.67	---	1.69 ± 0.14	---
1.17	1.00	39.90 ± 3.01	0.20 (NS)	14.76 ± 0.95	0.001	2.72 ± 0.12	0.001
1.17	1.50	36.80 ± 3.37	0.99 (NS)	13.42 ± 1.14	0.001	2.76 ± 0.10	0.001
1.17	2.00	35.50 ± 3.34	0.70 (NS)	12.95 ± 1.14	0.001	2.80 ± 0.16	0.001

The reaction mixture contained: 1.44 mMoles glycylglycine, 48 μmoles KH_2PO_4, 50 μmoles α ketoglutarate and 1.08 μmoles ADP (pH 7.4).

* IN: μl O_2 consumed/mg rat liver mitochondrial protein/hr

** control

controlling compound. Moreover, spermine markedly suppresses respiration after added ADP has been converted to ATP (Table 1). This suppression (between 22 and 41%) is most noticeable at 1.17 mM Mg^{++}. Hence, spermine and Mg^{++} seem to act synergistically in suppressing mitochondrial respiration after ADP-ATP conversion.

Mitochondrial respiration during ADP-ATP conversion is enhanced at all levels of spermine with 0.6 mM Mg^{++}, and at 1.0 and 1.5 mM spermine with 0.93 mM Mg^{++}. There is no enhancement at 2.0 mM spermine with 0.93 mM Mg^{++} or at any of the spermine levels with 1.17 mM Mg^{++}. This indicates that Mg^{++} at high concentrations antagonizes the respiratory enhancing effects of spermine on mitochondria.

In summary, the respiratory control ratio is a function of the spermine and Mg^{++} concentrations, and spermine has a different effect on mitochondrial respiration depending on whether or not respiration is being influenced by added ADP. Thus, spermine may well be an active *in vivo* intracellular metabolic regulating component.

Spermidine Effects on αKetoglutarate Oxidation at Various Mg^{++} Concentrations

Table 2 shows that at 0.6 and 0.93 mM Mg^{++}, 1.25 and 1.8 mM spermidine significantly enhance the respiratory control ratio. This enhancement also occurs at 1.17 mM Mg^{++} and 2.5 mM spermidine. It would appear that for the respiratory control ratio to increase, there must be a balance between Mg^{++} and spermidine levels. Also, all levels of spermidine markedly suppress respiration after ADP-ATP conversion at 0.6 and 0.93 mM Mg^{++} whereas at 1.17 mM Mg^{++}, it takes 2.5 mM spermidine to suppress respiration (Table 2).

At 0.6 mM Mg^{++}, it takes 2.5 mM spermidine to suppress respiration during ADP-ATP conversion. At 0.93 mM Mg^{++}, spermidine at 1.8 mM and above suppresses respiration whereas at 1.17 mM Mg^{++}, all levels of spermidine suppress respiration (Table 2).

In summary, 2.5 mM spermidine always suppresses respiration during

Table 2. Spermidine effects on α ketoglutarate-linked rat liver mitochondrial respiration and respiratory control ratio.

Mg^{++} (mM)	Spermidine (mM)	Q_{O_2}* during ADP-ATP Conversion (% of Control)	p	Q_{O_2}* after ADP-ATP Conversion (% of Control)	p	Respiratory Control Ratio	p
0.60	0**	100.0	---	100.0	---	1.26 ± 0.08	---
0.60	1.25	92.00 ± 3.81	0.09 (NS)	80.63 ± 4.87	0.02	1.46 ± 0.14	0.05
0.60	1.80	91.47 ± 5.09	0.18 (NS)	77.98 ± 5.84	0.02	1.50 ± 0.15	0.05
0.60	2.50	86.30 ± 1.70	0.001	76.24 ± 4.80	0.01	1.46 ± 0.19	0.10 (NS)
0.93	0**	100.0	---	100.0	---	1.44 ± 0.11	---
0.93	1.25	95.72 ± 2.46	0.10 (NS)	82.92 ± 4.78	0.02	1.69 ± 0.17	0.05
0.93	1.80	90.34 ± 2.56	0.01	71.23 ± 5.40	0.01	1.99 ± 0.30	0.05
0.93	2.50	87.09 ± 2.43	0.001	72.92 ± 4.09	0.001	1.91 ± 0.31	0.07 (NS)
1.17	0**	100.0	---	100.0	---	1.22 ± 0.10	---
1.17	1.00	89.75 ± 2.97	0.05	81.76 ± 9.88	0.15 (NS)	1.35 ± 0.17	0.75 (NS)
1.17	1.25	87.88 ± 3.17	0.01	78.75 ± 8.99	0.07 (NS)	1.37 ± 0.18	0.35 (NS)
1.17	1.80	90.33 ± 3.94	0.05	75.65 ± 11.41	0.13 (NS)	1.45 ± 0.13	0.09 (NS)
1.17	2.50	86.67 ± 3.41	0.01	65.98 ± 8.97	0.05	1.74 ± 0.24	0.02

The reaction mixture contained: 1.44 mmoles glycylglycine, 48 μmoles KH_2PO_4, 50 μmoles α ketoglutarate and 1.08 μmoles ADP (pH 7.4).

* given as % of control

** Control

and after conversion of added ADP to ATP. This suppression is always greater after conversion of ADP to ATP, and causes the enhancement in the respiratory control ratio. Thus, spermidine and Mg^{++} appear to act synergistically.

Spermidine Effects on Succinate Oxidation at Various Mg^{++} Concentrations

At the lowest level of Mg^{++} used (0.5 mM), all levels of spermidine enhance the respiratory control ratio up to 25%. With 0.93 mM Mg^{++} and 1.25 mM spermidine, the respiratory control ratio is also increased but to a lesser extent (12%). However, at 0.93 mM Mg^{++} and 1.8 mM spermidine and above, as well as at 1.17 mM Mg^{++} and all spermidine levels, the respiratory control ratio is unchanged. Therefore, spermidine increases the respiratory control ratio markedly at low intracellular levels of Mg^{++}, moderately at 0.93 mM Mg^{++} and not at all at 1.17 mM Mg^{++}.

The previously mentioned abstract (7) indicated that at 0.5 mM Mg^{++}, spermidine enhances respiration during ADP-ATP conversion. In the study herein presented (Table 3), spermidine at that Mg^{++} concentration has no enhancing effect but instead significantly suppresses respiration after ADP-ATP conversion. Thus, at this particular Mg^{++} concentration, the respiratory control ratio enhancement is caused by a suppression of respiration after ADP-ATP conversion. At 0.93 mM Mg^{++} and 1.25 mM spermidine (Table 3), there is an enhancement of respiration during ADP-ATP conversion and a suppression after this conversion.

Thus, it appears that Mg^{++} and spermidine, at least at some combinations of concentrations, act synergistically to increase mitochondrial respiratory control. If the above effects do occur *in vivo*, they could well be of considerable physiological significance because cytoplasmic limits of Mg^{++} and polyamines are subject to change.

Spermine Effects on Succinate Oxidation with Various Mg^{++} Concentrations

Unlike the previously reported findings (7), spermine has little or no effect on respiration during ADP-ATP conversion; but in accordance

Table 3. Spermidine effects on succinate-linked rat liver mitochondrial respiration and respiratory control ratio.

Mg^{++} (mM)	Spermidine (mM)	Q_{O_2}* during ADP-ATP Conversion	p	Q_{O_2}* after ADP-ATP Conversion	p	Respiratory Control Ratio	p
0.50	0**	119.91	---	39.44	---	3.14	---
0.50	1.25	128.72	0.11 (NS)	32.78	0.001	3.94	0.01
0.50	1.80	125.36	0.32 (NS)	33.38	0.02	3.82	0.01
0.50	2.50	121.11	0.80 (NS)	33.17	0.01	3.69	0.02
0.93	0**	116.97	---	34.32	---	3.44	---
0.93	1.25	125.17	0.02	32.85	0.05	3.85	0.01
0.93	1.80	121.81	0.12 (NS)	33.89	0.55 (NS)	3.58	0.08 (NS)
0.93	2.50	121.57	0.12 (NS)	34.69	0.75 (NS)	3.52	0.35 (NS)
1.17	0**	109.56	---	31.73	---	3.48	---
1.17	1.25	111.75	0.55 (NS)	31.17	0.45 (NS)	3.60	0.35 (NS)
1.17	1.80	112.05	0.40 (NS)	32.47	0.35 (NS)	3.47	0.95 (NS)
1.17	2.50	109.46	0.99 (NS)	32.99	0.25 (NS)	3.33	0.22 (NS)

The reaction mixture contained: 900 µmoles sucrose, 90 µmoles KCl, 18 µmoles KH_2PO_4, 72 µmoles Tris-HCl, 100 µmoles succinate, and 1.08 µmoles ADP (pH 7.2).

* In: µl O_2 consumed/mg rat liver mitochondrial protein/hr
** Control

with that report (ibid) spermine has no effect on respiration after ADP-ATP conversion. However, an important new finding is that spermine suppresses the respiratory control ratio, but this occurs only with 2.0 mM spermine at all Mg^{++} levels used. This effect on the respiratory control ratio has not so far been found to be related to any consistent statistically significant changes in respiratory rates during or after ADP-ATP conversion.

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