Vol. 86, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1979 Pages 293-299

THE POSSIBLE ROLE OF INTRACELLULAR POLYAMINES IN MITOCHONDRIAL METABOLIC REGULATION

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Received November 28,1978

SUMMARY: Studies were made on the effects of very low concentrations of the polyamine, spermine, on rat liver mitochondrial metabolism associated with β-hydroxybutyrate. The respiratory control ratio and the rate of respiration during ADP-ATP conversion are significantly altered with shifts in spermine concentrations of as little as 15.7 nMoles/ml within the physiological Mg^{++} concentration range. These spermine concentration changes are small compared to the estimated hepatic intracellular levels of spermine which have been reported to be between 200 and 1200 nMoles/gm wet weight under normal conditions. There is now evidence that exposure of an animal to certain environmental conditions induces changes of 164 nMoles/gm wet weight in intracellular levels of liver spermine in a few hours. Also there is evidence that the concentration of intracellular polyamines is influenced by endocrines since the levels of the enzymes responsible for their synthesis are markedly affected by hormonal changes. Therefore, alterations of polyamine levels may play a role in mitochondrial metabolic regulation *in vivo*.

It has long been recognized that polyamines such as spermine can affect RNA synthesis and thus protein synthesis (1-3) and therefore would indirectly affect mitochondrial respiration. Also, Huunan-Seppala (4) made the very interesting observation that during the oxidation of succinate, spermine causes a change in mitochondrial swelling and the mean respiratory rate. However, he did not design his assay procedures for the purpose of studying the polyamine effects on mitochondrial respiration during and after the conversion of ADP to ATP and thus did not obtain any information on mitochondrial respiratory control ratios. In fact, there is very little information on the effects of naturally occurring intracellular polyamines on these mitochondrial metabolic parameters. The few papers we have written on this subject (5-7) show that with certain substrates, 1.0 mM spermine and 1.25 mM spermidine affect the respiratory control ratio and respiration during the conversion of ADP to ATP. These polyamine levels are within the concentration ranges found in rat liver (8). However, the data in our previous papers (5-7) might well be subject to

0006-291X/79/020293-07$01.00/0

critcism because it took such high concentrations of a polyamine to cause any significant changes in mitochondrial metabolism with those substrates. And one might argue that any intracellular regulatory system requiring such gross polyamine changes would at best represent an insensitive metabolic control mechanism. However, in the data herein presented with β-hydroxybutyrate as substrate, we have found that a spermine concentration as low as 0.0157 mM (or 15.7 nMoles/ml) significantly affects liver mitochondrial respiratory control ratios and respiration during ADP-ATP conversion. Furthermore, the latter two parameters show incremental changes with extra additions of small amounts of spermine over a wide range. Thus, the data herein presented is the first evidence that such small changes in concentration of a polyamine can cause very significant effects on mitochondrial respiration during ADP-ATP conversion and the respiratory control ratio. The significance of these findings is that they suggest the polyamines have possible regulatory effects on cellular respiration which is the hypothesis presented in this paper.

METHODS

Mitochondria were isolated from livers of adult male Sprague-Dawley rats by the method described by Hoch and Lipmann (9). All assays were made at 37° C, using the polarographic apparatus described by Estabrook (10). In the liver *in vivo*, acetoacetate is reduced to β-hydroxybutyrate (11), but if there is an excess of β-hydroxybutyrate *in vitro*, the reaction is reversed and the oxidation of NADH thus generated can be followed polarographically. For assaying β-hydroxybutyrate oxidation, a modification of the reaction mixture of Harris et al (12) was used. The 3.6 ml reaction mixture contained: 900 μMoles sucrose, 90 μMoles KCl, 18 μMoles KH_2PO_4, 72 μMoles Tris-HCl, 1.08 μMoles ADP, 90 μMoles β-hydroxybutyrate and the appropriate concentrations of spermine and Mg^{++}.

In each assay, 2-4 mg of mitochondrial protein was used. Protein concentration was determined spectrophotometrically by the method of Lowry et al (13).

Spermine concentration was varied between 0.0157 and 2.0 mM whereas Mg^{++} concentrations were 0.6 and 0.93 mM. Mg^{++} levels used were those estimated to be free in rat liver cytoplasmic fluid (14-16).

RESULTS AND DISCUSSION

In figure 1 is shown the striking effects of increasing the spermine concentration from 0 up to 0.25 mM on the respiratory control ratio with β-hydroxybutyrate as substrate. As was mentioned previously, such concentrations of spermine within this range are at or below the concentration levels found

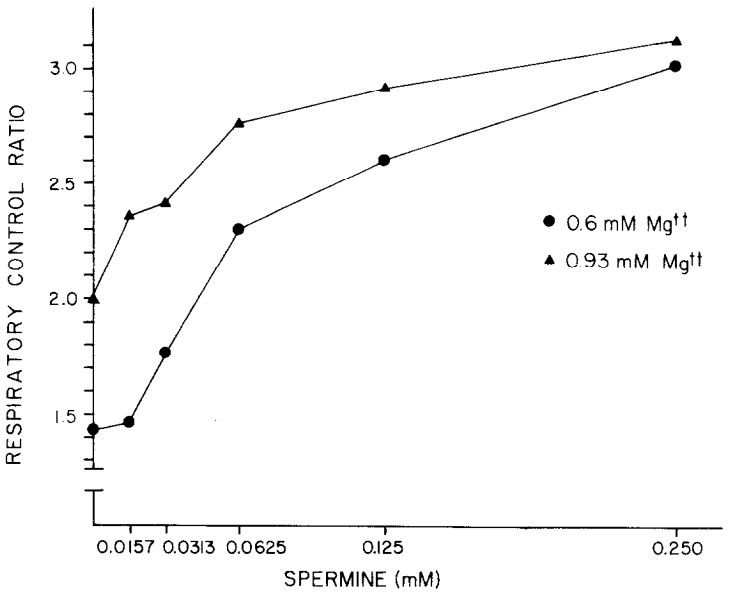

Figure 1. Effects of increasing the concentration of spermine on the respiratory control ratio with β-hydroxybutyrate as substrate at 0.6 and 0.93 mM Mg^{++}.

in rat liver homogenates (17-20). Therefore, it is quite likely that concentrations of at least these amounts are present in the cell and therefore would influence mitochondrial metabolism in vivo. Table 1 shows how small increases in spermine concentration elevate the rate of oxidation of β-hydroxybutyrate during ADP-ATP conversion and how they influence the respiratory control ratio. The above evidence is particularly important in light of the fact that spermine levels have been shown to fluctuate much in excess of 0.0157 mM in vivo under certain environmental conditions (20). Tsvetnenko and Shugaley (ibid) reported that when rats are returned to normoxia after exposure to hyperoxia, liver spermine levels increase from 213 nMoles/gm wet weight up to 379 nMoles/gm wet weight which is a change of 164 nMoles/gm wet weight (or a concentration change of approximately 0.164 mM) in a matter of hours.

There is evidence that indicates that polyamines may be subject to relatively rapid concentration changes in the cell since the enzymes responsible for their synthesis have remarkably short half-lives. For example, S-adenosyl-

Table 1. Spermine effects on rat liver mitochondrial respiration and metabolic control associated with β-hydroxybutyrate.

Mg^{++} (mM)	Spm (mM)	Q_{O_2}* during ADP-ATP Conversion	p	Q_{O_2}* after ADP-ATP Conversion	p	Respiratory Control Ratio	p
0.6	0**	54.60	-----	38.76	-----	1.44	-----
0.6	0.0157	60.37	0.05	42.00	0.02	1.47	NSD***
0.6	0.0313	63.65	0.02	38.39	NSD	1.76	NSD
0.6	0.0625	66.85	0.01	29.16	0.05	2.30	0.02
0.6	0.125	69.89	0.001	26.74	0.01	2.62	0.001
0.6	0.250	74.27	0.001	24.65	0.001	3.03	0.001
0.6	0.50	82.23	0.001	23.37	0.001	3.61	0.001
0.6	1.0	82.69	0.001	24.37	0.001	3.50	0.001
0.6	1.5	84.62	0.001	26.81	0.01	3.24	0.001
0.6	2.0	85.80	0.001	29.86	0.05	2.97	0.001
0.93	0**	59.51	-----	31.34	-----	2.00	-----
0.93	0.0157	66.24	0.05	30.71	NSD	2.35	0.05
0.93	0.0313	68.71	0.001	31.12	NSD	2.41	0.05
0.93	0.0625	70.43	0.01	25.69	NSD	2.76	0.01
0.93	0.125	73.61	0.001	25.79	NSD	2.91	0.01
0.93	0.250	75.09	0.001	24.26	0.05	3.13	0.001
0.93	0.50	78.36	0.001	23.36	NSD	3.41	0.001
0.93	1.0	79.02	0.001	24.47	NSD	3.31	0.01
0.93	1.5	79.17	0.001	25.88	NSD	3.12	0.01
0.93	2.0	83.00	0.001	27.15	NSD	3.14	0.01

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

* In: μl O_2 consumed/ mg rat liver mitochondrial protein/hr
** Control
*** Not significantly different from control.

methionine decarboxylase has a half-life of about 60 minutes (1, 21, 22) whereas ornithine decarboxylase has a half-life between 10 and 24 minutes (1, 8, 23-25). Furthermore, anterior pituitary hormones increase ornithine decarboxylase activity in appropriate target organs (26-30). Thus, physiological changes in response to stressful environmental conditions can, via effects on the anterior pituitary, mediate changes in ornithine decarboxylase activity and thus influence the rate of polyamine synthesis in vivo.

Other evidence that stressful environmental conditions can cause mitochondrial metabolic changes due to the action of polyamines is that mitochondria from heat-acclimated rats are more sensitive to a given concentration of spermine than are those of controls (6). That is, mitochondria from the heat-acclimated animals had a consistently greater elevation of the respiratory control ratio than did mitochondria from the controls in the presence of spermine (ibid).

Therefore, since the rate of polyamine synthesis can respond to environmental changes (20) and since small changes in levels of polyamines can significantly affect mitochondrial metabolism in vitro, the possible role of polyamines in mitochondrial metabolic control would seem to be an area which warrants further investigation.

One interesting possibility is that spermine can function in a capacity similar to that of Mg^{++} in mitochondrial metabolic control since it has been reported to function like Mg^{++} in some respects in affecting protein metabolism (3). From the data presented in figure 1 and table 1, it is obvious that the levels of Mg^{++} can alter the effects of spermine on mitochondrial metabolism of β-hydroxybutyrate. As shown in table 1, with 0.6 mM Mg^{++} and no spermine, the respiratory control ratio is only 1.44 whereas with as little as 0.0625 mM spermine, the respiratory control ratio is elevated to 2.30. At 0.93 mM Mg^{++}, the respiratory control ratio is considerably above that at 0.6 mM Mg^{++} and is significantly elevated with as little as 0.0157 mM spermine. Thus, Mg^{++} and spermine within certain concentration ranges seem to be synergistic in

elevating the respiratory control ratio associated with mitochondrial oxidation of β-hydroxybutyrate. But the concentrations of spermine involved are much smaller than those of Mg^{++}. This synergism is interesting because it is thought that the range of variation of free cytoplasmic Mg^{++} is very narrow (14-16) while the spermine concentration may be relatively more variable. Different workers report between 0.2 and 1.2 mM spermine in rat liver (17-20). Thus, changes in levels of a polyamine such as spermine may well play a more flexible role in alterations of cellular metabolic control than do changes in Mg^{++} concentration.

ACKNOWLEDGMENTS

This work was supported by ONR Grant N00014-75-C-0506, NIH Grant AM 18895-01 and NASA-Ames NCA 2-OR-608-601.

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