# Effect of Bovine Serum Albumin on Mitochondrial Respiration in the Brain and Liver of Mice and Rats

**A. V. Panov, V. A. Vavilin*, V. V. Lyakhovich*, B. R. Brooks, and H. L. Bonkovsky**

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We studied the effect of BSA (in the isolation medium) on the oxidation rate of succinate, glutamate, pyruvate, and α-ketoglutarate by mitochondria of the brain and liver from C57Bl/6g mice and Taconic Sprague Dawley rats. BSA had no effect on liver mitochondrial respiration, but increased oxidation of substrates (particularly of succinate) in brain mitochondria. Therefore, the major effect of BSA on brain mitochondria is manifested in activation of SDH. The improvement of mitochondrial properties in the brain after treatment with BSA is associated with antioxidant activity of this agent. Our results confirm the hypothesis that inhibition of SDH in brain mitochondria is not the artifact. This process serves as a mechanism protecting neurons from free oxygen radicals during succinate oxidation.

**Key Words:** *mitochondria of the brain and liver; respiration; bovine serum albumin; succinate dehydrogenase*

Defatted BSA is extensively used for isolation of mitochondria from the heart, brain, and other organs. BSA improves the mitochondrial properties due to removal of natural uncouplers (free fatty acids) [13]. It should be emphasized that BSA can bind a variety of bioactive substances, including hormones, organic anions, and bivalent cations [4,5]. Therefore, the effect of BSA on mitochondrial function can be mediated by a mechanism that differs from binding of fatty acids. For example, the presence of BSA in the isolation medium abolishes the specific effect of the mutated protein huntingtin on brain mitochondria (BM) and liver mitochondria from YAC72 transgenic mice (animal model of Huntington's disease) [7]. Our recent studies showed that BM have a unique metabolic property. Oxidation in the presence of malate and pyruvate or glutamate is accompanied by the formation of considerable amounts of succinate [10]. Increased formation of reactive oxygen species (ROS) in BM is associated with activation of succinate-dependent electron transfer in the respiratory chain [10]. This metabolic property is not typical of mitochondria from other organs and tissues. Moreover, the property of BM did not depend on BSA [10]. It should be noted that SDH activity in BM and skeletal muscle mitochondria depends on conditions of their isolation (the presence or absence of BSA) [6,10]. However, SDH activity in heart mitochondria did not depend on BSA [10].

Here we studied the effect of defatted BSA on respiratory activity of mitochondria from the liver and brain of C57Bl/6g mice and Taconic Sprague Dawley rats.

## MATERIALS AND METHODS

Mitochondria were isolated from 8-week-old Taconic Sprague Dawley rats and C57Bl/6g mice. The animals were maintained in accordance with the requirements of NIH and international rules (Helsinki) for the care and use of laboratory animals. The animals were deprived of food for 12 h (during the nighttime) before the experiment. Liver mitochondria and BM were

Cannon Research Center, Carolinas Medical Center, Charlotte, North Carolina, USA; *Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** alexander.panov@carolinashealthcare.org. A. V. Panov

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isolated as described elsewhere [5,9]. The isolation medium consisted of mannitol (75 mM), sucrose (175 mM), (4-morpholino)propanesulfonic acid (MOPS; 20 mM, pH 7.2), EGTA (1 mM), and 0.1% BSA (if present). The mitochondrial protein was assayed with Pierce reagents.

Mitochondrial respiratory activity was studied in a 765-μl original plastic chamber equipped with a standard Clark microelectrode (Yellow Spring Instrument Co., Inc.), a tetraphenylphosphonium-sensitive ($TPP^+$) mini electrode, and a reference electrode (via a KCl-agar bridge). The devices were connected with a two-channel self-recording unit and data-processing computer system. The highest degree of oxidative phosphorylation was reached by using incubation medium containing 125 mM KCl, 10 mM MOPS (pH 7.2), 2 mM $MgCl_2$, 2 mM $KH_2PO_4$, 10 mM NaCl, 1 mM EGTA, and 0.7 mM $CaCl_2$. Free $Ca^{2+}$ concentration was 1 μM at the $Ca^{2+}$/EGTA ratio of 0.7. We used the following respiratory substrates: 5 mM succinate (without rotenone), 10 mM glutamate, 2.5 mM pyruvate, 2 mM malate, and 10 mM α-ketoglutarate. Glutamate, pyruvate, and α-ketoglutarate were used in combination with malate. Oxidative phosphorylation was stimulated by 150 μM ADP. Uncoupled respiration was induced by gradual addition of 0.05 μM chloro-(carbonyl cyanide)phenylhydrazone (CCP) to complete uncoupling of respiration and phosphorylation. Fluorescence of mitochondrial pyridine nucleotides during oxidation of various substrates was estimated in the respiratory medium (1 ml) in the presence of 0.5 mg mitochondrial protein. The measurements were performed on a C&L fluorometer (Middletown) at the excitation and emission wavelengths of 340 and 480 nm, respectively. The data were recorded and analyzed with C&L devices and software (Middletown). The data shown in Table 1 had normal distribution. The results are presented as the mean and standard error (4-6 procedures of mitochondria isolation). The significance of between-group differences was evaluated by Student's *t* test.

## RESULTS

In various metabolic states (MS), the succinate and glutamate oxidation rates of in mouse liver mitochondria were higher than in rat liver mitochondria (by 30 and 50%, respectively). For succinate, the respiration rate in MS-4 with or without BSA was 60.2±4.0/59.2±10.0 (mouse liver mitochondria) and 41.7±7.0/38.5±5.0 ng×atom O/mg protein/min (rat liver mitochondria). During oxidation of pyruvate or α-ketoglutarate by liver mitochondria, addition of ADP or uncoupling agent was followed by a slight increase in respiration. These changes probably result from the fact that the liver of fasting animals is characterized by gluconeogenesis. Fatty acids and amino acids serve as an energy source for the liver. Therefore, liver mitochondria in mice and rats oxidize only succinate and glutamate. Experiments with various substrates showed that the presence or absence of BSA in the isolation medium has no effect on respiratory activity of liver mitochondria from rats and mice.

Table 1 shows the respiration rate of BM that were isolated from Taconic Sprague Dawley rats and C57Bl/6g mice in the presence or absence of BSA.

As distinct from BM isolated with BSA, the respiration rate of BM isolated in the absence of BSA depended on the substrate, MS, and species of animals. The inhibition of succinate oxidation was particularly

**TABLE 1.** Effect of BSA on BM Respiration in Experimental Animals

| Animals, substrates | | BM isolated with 0.1% BSA | | | BM isolated without 0.1% BSA | | |
|---|---|---|---|---|---|---|---|
| | | MS-4 | MS-3 | MS-3R | MS-4 | MS-3 | MS-3R |
| C57Bl/6g mice | succinate | 163±10 | 620±76 | 589±67 | 64±13*** | 63±13*** | 64±13*** |
| | glutamate+malate | 70±4 | 382±91 | 443±91 | 85±3** | 181±15*** | 243±16*** |
| | pyruvate+malate | 72±5 | 452±28 | 614±85 | 98±5*** | 292±10*** | 559±45 NS |
| | α-ketoglutarate+malate | 64±4 | 272±31 | 256±26 | 78±5* | 141±11** | 177±21 NS |
| Taconic Sprague Dawley rats | succinate | 143±6 | 486±13 | 506±8 | 121±13*** | 165±13*** | 65±13*** |
| | glutamate+malate | 66±4 | 388±12 | 486±36 | 98±3** | 278±12** | 364±26** |
| | pyruvate+malate | 71±7 | 421±21 | 557±35 | 105±5*** | 297±10*** | 564±42 NS |
| | α-ketoglutarate+malate | 63±6 | 296±15 | 323±16 | 79±4* | 177±20*** | 262±21* |

**Note.** Respiration rate is expressed in ng×atom O/mg mitochondrial protein/min. *$p$<0.05, **$p$<0.01, and ***$p$<0.001 compared to the corresponding control with 0.1% BSA.

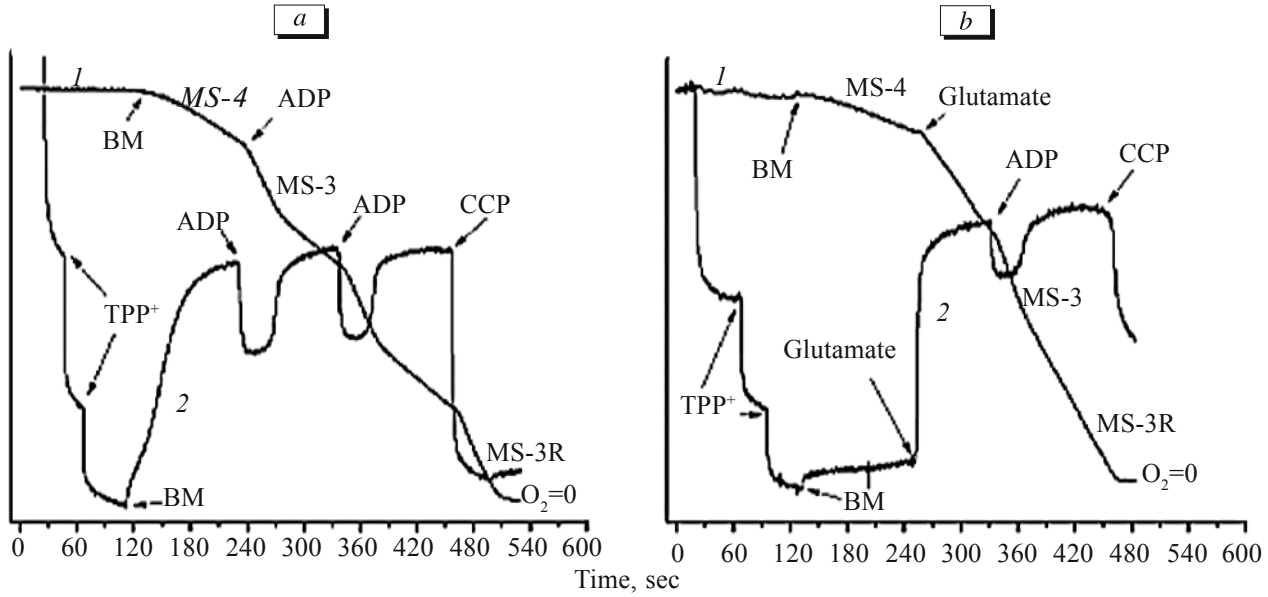


**Fig. 1.** Respiration and membrane potential during succinate oxidation by mouse BM isolated in the presence (*a*) and absence of BSA (*b*). Oxygen consumption (*1*); membrane potential (*2*). Succinate, 5 mM; BM, 0.3 mg. Additives: $TPP^+$, 0.5 μM (final concentration 1.5 μM); ADP, 150 μM; CCP, 0.3 μM; glutamate, 10 mM.

pronounced after isolation of mouse BM in the absence of BSA. Addition of ADP or uncoupling agent was not followed by activation of respiration (Table 1). The membrane potential of mouse BM did not increase in MS-4 (Fig. 1, *b*). Studying the oxidation of succinate by rat mitochondria after isolation in the absence of BSA revealed only a 15% decrease of respiration in MS-4. The inhibition of respiration for mouse BM without BSA was 61% (as compared to BM isolated in the presence of BSA). In MS-3, the oxidation rate of glutamate, pyruvate, and α-ketoglutarate for BM from rats and mice after isolation without BSA was 40-50 and 30-40% lower, respectively, than for BM isolated in the presence of BSA. It should be noted that pyruvate oxidation by uncoupled BM from mice and rats did not depend on BSA (as differentiated from glutamate and α-ketoglutarate; Table 1). Succinate oxidation by uncoupled BM was significantly reduced after isolation in the absence of BSA. The degree of inhibition was shown to increase progressively. Our results are consistent with published data that mitochondrial deenergization is followed by a 10-fold increase in affinity of SDH to oxaloacetate [14]. When the basic incubation medium contained glutamate (Fig. 1, *b*) or pyruvate and glutamate, the rate of succinate oxidation by BM did not differ after isolation in the absence and presence of BSA.

Mouse BM isolated in the presence of BSA can be characterized by high membrane potential in MS-4. However, the maximum value of $\Delta\Psi$ can be achieved over 2 min. In the presence of substrates (glutamate or pyruvate), the maximum value of $\Delta\Psi$ was achieved over 15-20 sec. Similar results were obtained for rat BM after isolation in the presence of BSA. Our previous experiments showed that BM from Taconic Sprague Dawley rats have an interesting property. The spontaneous inhibition of succinate oxidation was observed 1-2 h after isolation of BM in the presence of BSA (similarly to mouse BM isolated in the absence of BSA; Fig. 1, *b*). The oxidation of succinate returned to normal after addition of glutamate or pyruvate. However, the oxidation rate of glutamate and pyruvate in MS-3 and value of the respiratory control remained high even 4-5 h after isolation of rat BM in the presence of BSA [10].

Experiments with NAD-dependent substrates in MS-4 showed that the respiration rate of BM from mice and rats isolated in the absence of BSA is much higher compared to BM isolated in the presence of BSA. At first glance, our results seem to be consistent with published data that BSA abolishes the uncoupling effect of fatty acids. However, fatty acids do not serve as a respiratory substrate for BM from adult rodents. By contrast, liver mitochondria from fasting animals oxidize fatty acids. At the same time, BSA had no effect on respiration of liver mitochondria. The isolated liver mitochondria contain a considerable amount of endogenous substrates that maintain respiration in MS-4 over a long time. Moreover, they contribute to relatively high level of mitochondrial NAD(P)H in the absence of exogenous substrates (Fig. 2, *b*). However, the respiration of isolated BM (independently on BSA) is practically undetected in the absence of exogenous substrates. Mitochondrial pyridine nucleotides are nearly completely oxidized (Fig. 2, *a*). In MS-4, the higher oxidation rate of NAD-dependent substrates by

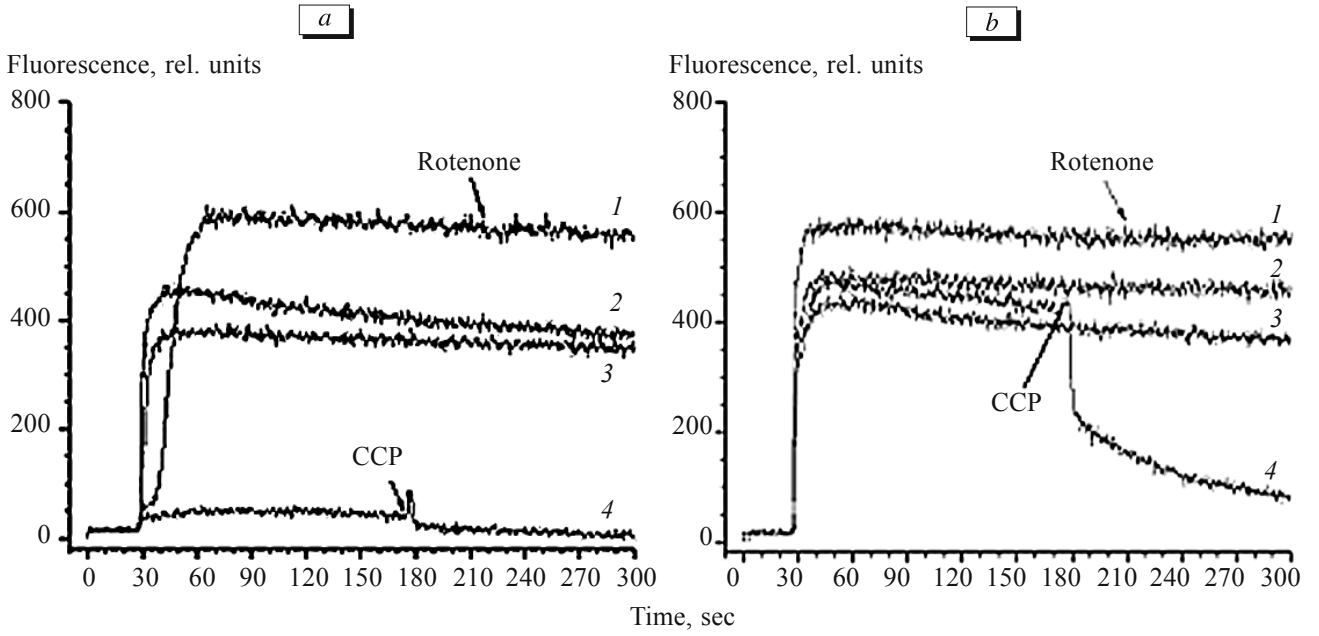


**Fig. 2.** Fluorescence of mitochondrial NAD(P)H in BM (*a*) and liver mitochondria (*b*) from C57Bl/6g mice isolated with BSA during oxidation of various substrates. Succinate (5 mM, *1*); glutamate (10 mM) and malate (2 mM, *2*); pyruvate (2.5 mM) and malate (2 mM, *3*); without substrates (*4*). Additives: CCP, 0.5 μM.

BM isolated without BSA is probably related to oxidative damage to mitochondria during isolation. This hypothesis is confirmed by reduced rate of respiration in MS-3. Published data show that isolation of BM in the presence of antioxidants significantly improves the quality of mitochondria, which is accompanied by a 2-fold increase of the respiration rate in MS-3 [3].

The uncoupling effect of fatty acids is mediated by the complex specific mechanisms for regulation of mitochondrial proton conductance. They differ from the mechanisms for action of standard uncouplers (*e.g.*, CCP) [11,12]. Therefore, BSA can produce a complex effect on BM (as differentiated from the uncoupling action of fatty acids).

Recent studies revealed the unique metabolic properties of BM [10]. The succinate-dependent production of ROS is probably the major cause of oxidative stress in neurons. The inhibition of SDH by oxaloacetate is an important natural mechanism for the prevention of ROS generation in neurons [10]. BSA is capable of binding organic anions [5]. Therefore, removal of oxaloacetate from mitochondria is one of the main effects of defatted BSA on BM.

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