Data article

Data supporting the involvement of the adenine nucleotide translocase conformation in opening the Ti⁺-induced permeability transition pore in Ca²⁺-loaded rat liver mitochondria

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ABSTRACT

There we made available information about the effects of the adenine nucleotide translocase (ANT) ‘c’ conformation fixers (phenylarsine oxide (PAO), tert-butylhydroperoxide (tBHP), and carboxytrotyloside) as well as thiol reagent (4,4'-diisothiocyanostilbene-2,2'-disulfonate (DIDS)) on isolated rat liver mitochondria. We observed a decrease in A540 (mitochondrial swelling) and respiratory control rates (RCRADP [state 3/state 4] and RCRDNP [2,4-dinitrophenol-uncoupled state/basal state or state 4]), as well as an increase in Ca²⁺-induced safranin fluorescence (F485/590, arbitrary units), showed a dissipation in the inner membrane potential (∆Ψmito), in experiments with energized rat liver mitochondria, injected into the buffer containing 25–75 mM TlNO₃, 125 mM KNO₃, and 100 mM Ca²⁺. The fixers and DIDS, in comparison to Ca²⁺ alone, greatly increased A540 decline and the rate of Ca²⁺-induced ∆Ψmito dissipation. These reagents also markedly decreased RCRADP and RCRDNP. The MPTP inhibitors (ADP, cyclosporin A, bongkrekic acid, and N-ethylmaleimide) fixing the ANT in ‘m’ conformation significantly hindered the above-mentioned effects of the fixers and DIDS. A more complete scientific analysis of these findings may be obtained from the manuscript “To involvement the conformation of the adenine nucleotide translocase in opening the Ti⁺-induced

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permeability transition pore in Ca\textsuperscript{2+}-loaded rat liver mitochondria” (Korotkov et al., 2016 [1]). © 2016 The Author. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

### Specifications Table

| Subject area | Biology |
|-------------|---------|
| More specific subject area | Biochemical toxicology |
| Type of data | Table |
| How data was acquired | Observational data, swelling assay as a decline in $A_{\text{540}}$, oxygen consumption assay, mitochondrial membrane potential assay as safranin fluorescence intensity change at 485/590 nm |
| Data format | Raw and analyzed |
| Experimental factors | Temperature and concentration of TlNO\textsubscript{3} in buffers |
| Experimental features | Liver was extracted from Wistar male (250–300 g). Rat liver mitochondria were isolated by a dual sequential isolation, and the resulting protein was used for the observational data assay |
| Data source location | St. Petersburg, Russian Federation |
| Data accessibility | Data is within the article. |

### Value of the data

- The scientific data can be referenced by other scientists investigating the effects of Tl\textsuperscript{+} on cells and mitochondria.
- The findings can provide comprehensive toxicological analysis of the effects of thallous salts on animal organisms.
- Effects of tBHP, PAO, and DIDS in the new in vitro model of the K\textsuperscript{+} surrogate Tl\textsuperscript{+}-induced MPTP can be the basis in searching new inducers and inhibitors of mitochondrial permeability transition pores in the inner membrane.
- These data may be helpful in evaluating the combined action of thallium and other sulphydryl toxicants such as heavy metals and industrial oxidants.

1. **Data**

   This manuscript contains additional information to the research of [1]. The use of swelling technique as the change in $A_{\text{540}}$ tests changes in mitochondrial volume. The respiratory control ratios (RCR\textsubscript{ADP}=state 3/state 4 and RCR\textsubscript{DNP}=DNP-uncoupled respiration/basal state or state 4) give information about enzymes, involved in oxygen consumption and oxidative phosphorylation processes, correspondingly. The safranin uptake of energized rat liver mitochondria allows to do assertion about the change in the inner membrane potential ($\Delta\Psi_{\text{mito}}$).

2. **Experimental design, materials and methods**

   The research was used male Wistar rats (250–300 g) of 9–12 months old which were kept at 20–23 °C under 12-h light/dark cycle with free access to water ad libitum and the standard rat diet. All
treatment procedures of rats were carried out according to the Animal Welfare act and the Institute Guide for Care and Use of Laboratory Animals.

2.1. Isolation of rat liver mitochondria

Rat liver mitochondria were isolated accordance the standard protocol [2]. Male rat was decapitated and the liver was quickly extracted and placed into ice-cold isolation buffer containing 250 mM sucrose, 3 mM Tris–HCl (pH 7.3), and 0.5 mM ethylene glycol tetraacetic acid (EGTA). The decapitation procedure of fasted animals is mandatory in isolating rat liver mitochondria. Then the liver was minced with scissors, washed out by the medium, transferred into a Potter-Elvehjem glass homogenizer and homogenized using a teflon pestle. The liver homogenate was centrifuged at 800 x g for 7.5 min, then the pellet has been thrown out and the supernatant was centrifuged at 10,000 x g for 10 min. The mitochondrial pellet was twice washed out with a buffer containing 250 mM sucrose and 3 mM Tris–HCl (pH 7.3) and centrifuged at 10,000 x g for 10 min. The final pellet was resuspended in 950 μl of the wash buffer and kept on ice during the experiment. The whole process of mitochondrial isolation was carried out on ice. The mitochondrial protein content was determined by Bradford [3] and was within the range of 50–60 mg/ml.

2.2. Swelling of mitochondria

The early mention about suitability to use millimolar Tl⁺ concentrations was made in research of Melnick et al. and Saris et al. which applied swelling and polarographic techniques in experiments with isolated mitochondria (see more detail [1]). The applicability of such experimental model in toxicological studies using isolated mitochondria and buffers containing thallous salts has been earlier substantiated by us in more detail [4]. Mitochondrial swelling was measured as a decrease in A₅₄₀ at 20 °C using a SF-46 spectrophotometer (LOMO, St. Petersburg, Russia). Mitochondria (1.5 mg protein/ml) were injected into a 1-cm cuvette with 1.5 ml of 400 mOsm buffer containing 200 mM KNO₃ (Table 1) or 75 mM TlNO₃ and 125 mM KNO₃ (Tables 1–4) as well as 5 mM succinate, 5 mM Tris–NO₃ (pH 7.3), 2 μM rotenone, and 1 μg/ml of oligomycin. The following chemicals were added into the medium before mitochondria: phenylarsine oxide (PAO), tert-butyl hydroperoxide (tBHP), N-ethylmaleimide (NEM), 4,4’-disothiocyanostilbene-2, 2’-disulfonate (DIDS), ADP, cyclosporin A (CsA), bongrekic acid (BKA), and carboxyatractyloside (CATR). Ca²⁺ (where indicated) was injected to the buffer at one min after mitochondria. The swelling, oxygen consumption rates, and ΔΨ_mito were carried out in 400 mOsm media in order to verify the comparability and consistency between findings in different experiments.

2.3. Oxygen consumption assay

Respiration (oxygen consumption rate) was tested using Expert-001 analyzer (Econix-Expert Ltd., Moscow, Russia) in a 1.3-ml closed thermostatic chamber with magnetic stirring at 26 °C. Mitochondria (1.5 mg protein/ml) were administrated into 400 mOsm buffer containing 25 mM TlNO₃, 100 mM sucrose, 3 mM Mg(NO₃)₂, and 3 mM Tris-PO₄ (Table 5) or 75 mM TlNO₃ and 1 μg/ml of oligomycin (Table 6) as well as 125 mM KNO₃, 5 mM Tris–NO₃ (pH 7.3), 5 mM succinate, and 2 μM rotenone. In some cases, we used buffers containing glutamate with malate and free of rotenone (Fig. 1). The following reagents (Table 5) were added in the buffer at one minute after mitochondria: PAO, tBHP, DIDS, and NEM. ADP at 130 μM and DNP at 30 μM were correspondingly injected into the buffer after 2 min to induce state 3 and after 4 min to record DNP-uncoupled respiration. The following reagents (Table 6) were added in the buffer one min after mitochondria: PAO, tBHP, and DIDS. If the MPTP inhibitors (ADP plus CsA or NEM alone) were injected into the buffer one min after mitochondria, the first reagents (PAO, tBHP, and DIDS) and Ca²⁺ at 100 μM were correspondingly added into the buffer one and two min latter the inhibitors. Further, DNP at 30 μM was administered one minute later the reagents or Ca²⁺ (Table 6). The respiratory control ratio (RCR_ADp=state 3/state 4) that shows the quality of rat liver mitochondria (RLM) was measured in standard buffer containing 100 mM KCl, 20 mM Tris–HCl (pH 7.3), 3 mM MgCl₂, and 3 mM Tris–PO₄, 5 mM Tris–succinate, and
| PAO ($\mu$M) | 200 mM KNO$_3$ | 75 mM TINO$_3$ + 125 mM KNO$_3$ | tBHP ($\mu$M) | 200 mM KNO$_3$ | 75 mM TINO$_3$ + 125 mM KNO$_3$ |
|---|---|---|---|---|---|
| | $\Delta$A$_{540}$ ± SEM | P value | $\Delta$A$_{540}$ ± SEM | P value | $\Delta$A$_{540}$ ± SEM | P value |
| 0 | -0.030 ± 0.003 (3) | - | -0.027 ± 0.001 (3) | - | 0 | -0.031 ± 0.002 (3) | - |
| 1 | -0.094 ± 0.012 (3) | P < 0.05 | 50 | -0.248 ± 0.022 (3) | P < 0.01 | 75 |
| 2 | -0.092 ± 0.018 (3) | P < 0.03 | -0.534 ± 0.011 (3) | P < 0.01 | 100 |
| 5 | -0.447 ± 0.040 (3) | P < 0.01 | -0.656 ± 0.025 (3) | P < 0.01 | 200 |
| 10 | -0.632 ± 0.019 (3) | P < 0.01 | -0.715 ± 0.023 (3) | P < 0.01 | 500 | -0.049 ± 0.005 (3) | P < 0.03 |
| 20 | -0.447 ± 0.040 (3) | P < 0.01 | -0.656 ± 0.025 (3) | P < 0.01 | -0.418 ± 0.007 (3) | P < 0.01 |

The absorbance changes ($\Delta$A$_{540}$) were detected within seven minute interval after addition of mitochondria and presented as Means ± SEM. The number of experiments showed in parentheses. P-values were accordingly calculated to experiments free of PAO or tBHP (a dash in the P value columns).
Table 2
Effects of PAO, DIDS, and tBHP on change of A540 in suspension of Ca²⁺-loaded succinate-energized rat liver mitochondria.

| PAO (μM) ↓ | − ADP | + 500 μM ADP | tBHP (μM) ↓ | − ADP | + 500 μM ADP |
|-----------|-------|-------------|-------------|-------|-------------|
|           | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value |
| 0         | −0.197 ± 0.006 (8) | − | −0.013 ± 0.001 (8) | P < 0.01 | 0 | −0.203 ± 0.010 (9) | − | −0.015 ± 0.002 (9) | P < 0.01 |
| 1         | −0.241 ± 0.014 (3) | P < 0.01 | −0.025 ± 0.002 (3) | P < 0.01 | 25 | −0.213 ± 0.015 (3) | * | −0.017 ± 0.003 (3) | P < 0.01 |
| 2         | −0.255 ± 0.006 (6) | P < 0.01 | −0.078 ± 0.007 (8) | P < 0.01 | 50 | −0.219 ± 0.009 (8) | * | −0.143 ± 0.027 (7) | P < 0.04 |
| 5         | −0.305 ± 0.018 (3) | P < 0.01 | −0.279 ± 0.015 (3) | * | 100 | −0.235 ± 0.010 (4) | * | −0.248 ± 0.011 (4) | * |
| 10        | −0.363 ± 0.013 (3) | P < 0.01 | −0.349 ± 0.016 (3) | * | 500 | −0.230 ± 0.017 (3) | * | −0.249 ± 0.015 (3) | * |

| DIDS (μM) ↓ | − Ca²⁺ | + 500 μM ADP | DIDS (μM) ↓ | + 100 μM Ca²⁺ | − ADP | + 500 μM ADP |
|------------|--------|-------------|------------|--------------|-------|-------------|
|            | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value |
| 0          | −0.021 ± 0.002 (3) | − | −0.016 ± 0.001 (3) | * | 0 | −0.288 ± 0.007 (3) | − | −0.016 ± 0.001 (3) | P < 0.01 |
| 2.5        | −0.021 ± 0.001 (3) | * | −0.022 ± 0.001 (3) | * | 2.5 | −0.303 ± 0.010 (3) | * | −0.016 ± 0.002 (3) | P < 0.01 |
| 5          | −0.031 ± 0.001 (3) | P < 0.03 | −0.020 ± 0.001 (3) | * | 5 | −0.314 ± 0.016 (3) | * | −0.132 ± 0.007 (3) | P < 0.01 |
| 12.5       | −0.044 ± 0.001 (3) | P < 0.02 | −0.032 ± 0.001 (3) | P < 0.03 | 12.5 | −0.376 ± 0.012 (3) | P < 0.04 | −0.272 ± 0.018 (3) | * |
| 25         | −0.064 ± 0.002 (3) | P < 0.01 | −0.064 ± 0.002 (3) | P < 0.01 | 25 | −0.376 ± 0.012 (3) | P < 0.04 | −0.272 ± 0.018 (3) | * |
| 50         | −0.273 ± 0.012 (3) | P < 0.01 | −0.126 ± 0.006 (3) | P < 0.01 | 50 | −0.376 ± 0.012 (3) | P < 0.04 | −0.272 ± 0.018 (3) | * |
| 100        | −0.332 ± 0.018 (3) | P < 0.01 | −0.318 ± 0.018 (3) | P < 0.01 | 100 | −0.376 ± 0.012 (3) | P < 0.04 | −0.272 ± 0.018 (3) | * |

The absorbance changes (ΔA540) were accordingly detected within three minute interval after administration of mitochondria (−Ca²⁺ columns) or 100 μM Ca²⁺ to mitochondria (+100 μM Ca²⁺ columns) and this is presented as Means ± SEM. The number of experiments showed in parentheses. P-values in experiments free of Ca²⁺ (−Ca²⁺ columns) are calculated to experiments free additions (a dash in the P value columns). P-values with Ca²⁺-loaded mitochondria (+100 μM Ca²⁺ columns) are calculated to experiments with Ca²⁺ alone (a dash in the P value columns). Asterisks indicate that statistical difference between appropriate ΔA540 values is not statistically significant.
The absorbance changes (ΔA540) were detected within three minute interval after administration of 100 μM Ca2+ to mitochondria and presented as Means ± SEM. The number of experiments showed in parentheses and corresponding P-values calculated to experiments free of above additions (a dash in the P value columns). Asterisks indicate that statistical difference between appropriate ΔA540 values is not statistically significant.

### Table 4
Effect of NEM on A540 in suspension of Ca2+-loaded succinate-energized rat liver mitochondria.

| NEM (μM)  | 0 | 50 | 250 | 500 |
|-----------|---|----|-----|-----|
| +100 μM Ca2+ | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value |
| 0         | -0.009 ± 0.001 (3) | - | -0.192 ± 0.008 (4) | - | -0.014 ± 0.002 (4) | P < 0.01 |
| 50        | -0.093 ± 0.007 (3) | P < 0.01 | -0.095 ± 0.009 (3) | P < 0.01 | -0.021 ± 0.004 (3) | P < 0.01 |
| 250       | -0.298 ± 0.009 (3) | P < 0.01 | -0.206 ± 0.006 (3) | * | -0.220 ± 0.008 (3) | * |
| 500       | -0.298 ± 0.009 (3) | P < 0.01 | -0.288 ± 0.007 (4) | P < 0.01 | -0.265 ± 0.023 (4) | P < 0.03 |

The absorbance changes (ΔA540) in experiments free of Ca2+ (−Ca2+ columns) were detected within six minute interval and P-values are calculated to experiments free of NEM (a dash in the P value columns). The absorbance changes with CaRLM (+100 μM Ca2+ columns) were detected within three minute after administration of Ca2+ to mitochondria and they are presented as Means ± SEM. P-values with Ca2+-loaded mitochondria are calculated to experiments with Ca2+ alone (a dash in the P value columns). Asterisks indicate that statistical difference between appropriate ΔA540 values is not statistically significant. The absorbance changes in experiments free of NEM (2*) were detected within six minute after Ca2+ administration to mitochondria. P-values (2*) are calculated to experiments free of additions (a dash with asterisk in the P value columns).

2 μM rotenone. The RCRADP for succinate-energized RLM was equal 6.65 ± 0.21 (n=14) (Fig. 1). Wherein, the DNP-dependent respiratory control ratio (RCRDNP) was calculated as a ratio of DNP-uncoupled respiration to state 4 (Fig. 1). The RCRDNP for succinate-energized RLM was equal 9.18 ± 0.49 (n=14) (Fig. 1). Table 5 shows the RCRADP under above experimental conditions in TiNO3.

### Table 3
Effects of PAO, DIDS, and tBHP on A540 in suspension of Ca2+-loaded succinate-energized rat liver mitochondria in the presence of ADP, CsA, and NEM.

| Before mitochondrial additions of MPTP inhibitors | 2 μM PAO | 50 μM tBHP | 5 μM DIDS |
|-------------------------------------------------|---------|------------|---------|
| ΔA540 ± SEM | P value | ΔA540 ± SEM | P value | ΔA540 ± SEM | P value |
| Free additions | -0.259 ± 0.007 (7) | - | -0.229 ± 0.011 (8) | - | -0.314 ± 0.016 (3) | - |
| ADP | -0.073 ± 0.007 (9) | P < 0.01 | -0.086 ± 0.029 (5) | P < 0.01 | -0.132 ± 0.007 (3) | P < 0.01 |
| CsA | -0.248 ± 0.011 (3) | * | -0.220 ± 0.034 (3) | * | -0.276 ± 0.018 (3) | * |
| NEM | -0.157 ± 0.030 (5) | P < 0.03 | -0.119 ± 0.027 (3) | P < 0.01 | -0.235 ± 0.039 (3) | * |
| ADP + NEM | -0.028 ± 0.003 (5) | P < 0.01 | -0.023 ± 0.006 (3) | P < 0.01 | -0.065 ± 0.028 (3) | P < 0.02 |
| CsA + NEM | -0.070 ± 0.015 (4) | P < 0.01 | -0.111 ± 0.016 (3) | P < 0.01 | -0.122 ± 0.008 (3) | P < 0.01 |
| ADP + CsA | -0.032 ± 0.006 (3) | P < 0.01 | -0.019 ± 0.005 (3) | P < 0.01 | -0.015 ± 0.003 (3) | P < 0.01 |
| ADP + CsA + NEM | -0.013 ± 0.002 (3) | P < 0.01 | -0.013 ± 0.001 (3) | P < 0.01 | -0.046 ± 0.011 (4) | P < 0.01 |

The absorbance changes in experiments free of NEM (2*) were detected within six minute after Ca2+ loaded succinate-energized rat liver mitochondria.
### Table 5
Effects of PAO, DIDS, tBHP, and NEM on RCR\textsubscript{ADP} and RCR\textsubscript{DNP} in energized rat liver mitochondria.

| PAO (µM) | RCR\textsubscript{ADP} ± SEM | P value | RCR\textsubscript{DNP} ± SEM | P value | tBHP (µM) | RCR\textsubscript{ADP} ± SEM | P value | RCR\textsubscript{DNP} ± SEM | P value |
|----------|-------------------------------|---------|-------------------------------|---------|-----------|-------------------------------|---------|-------------------------------|---------|
| 0        | 2.57 ± 0.11 (3)               | –       | 3.92 ± 0.27 (3)               | –       | 0         | 2.47 ± 0.09 (3)               | –       | 3.73 ± 0.16 (3)               | –       |
| 1        | 2.22 ± 0.06 (3)               | P < 0.05| 3.77 ± 0.29 (3)               | *       | 50        | 2.47 ± 0.10 (3)               | *       | 3.88 ± 0.17 (3)               | *       |
| 2        | 2.19 ± 0.05 (3)               | P < 0.04| 3.57 ± 0.29 (3)               | *       | 100       | 2.32 ± 0.06 (3)               | *       | 3.16 ± 0.22 (3)               | P < 0.04|
| 5        | 1.83 ± 0.08 (3)               | P < 0.05| 2.67 ± 0.30 (3)               | P < 0.04| 200       | 2.40 ± 0.06 (3)               | *       | 2.94 ± 0.12 (3)               | P < 0.02|
| 10       | 1.42 ± 0.14 (3)               | P < 0.03| 1.01 ± 0.34 (3)               | P < 0.03|           |                               |         |                               |         |

| DIDS (µM) | RCR\textsubscript{ADP} ± SEM | P value | RCR\textsubscript{DNP} ± SEM | P value | NEM (µM) | RCR\textsubscript{ADP} ± SEM | P value | RCR\textsubscript{DNP} ± SEM | P value |
|-----------|-------------------------------|---------|-------------------------------|---------|-----------|-------------------------------|---------|-------------------------------|---------|
| 0         | 2.31 ± 0.09 (3)               | –       | 4.18 ± 0.13 (3)               | –       | 0         | 2.47 ± 0.09 (3)               | –       | 3.73 ± 0.16 (3)               | –       |
| 12.5      | 1.73 ± 0.23 (3)               | *       | 3.08 ± 0.36 (3)               | P < 0.05| 50        | 2.16 ± 0.01 (3)               | P < 0.03| 3.45 ± 0.10 (3)               | *       |
| 25        | 1.27 ± 0.12 (3)               | P < 0.02| 2.20 ± 0.25 (3)               | P < 0.02| 100       | 1.94 ± 0.04 (3)               | P < 0.01| 3.28 ± 0.11 (3)               | *       |
| 50        | 1.00 (3)                      | P < 0.01| 1.73 ± 0.21 (3)               | P < 0.01| 200       | 1.93 ± 0.05 (3)               | P < 0.01| 3.14 ± 0.02 (3)               | P < 0.02|
| 100       | 1.00 (3)                      | P < 0.01| 1.06 ± 0.01 (3)               | P < 0.01|           |                               |         |                               |         |
| 0**       | 1.88 ± 0.13 (3)               | –       | 2.34 ± 0.12 (3)               | –       |           |                               |         |                               |         |
| 12.5**    | 1.57 ± 0.16 (3)               | *       | 2.20 ± 0.31 (3)               | *       |           |                               |         |                               |         |
| 25**      | 1.20 ± 0.04 (3)               | P < 0.01| 2.19 ± 0.13 (3)               | *       |           |                               |         |                               |         |
| 50**      | 1.09 ± 0.03 (3)               | P < 0.01| 1.96 ± 0.22 (3)               | *       |           |                               |         |                               |         |
| 100**     | 1.02 ± 0.02 (3)               | P < 0.01| 2.05 ± 0.07 (3)               | *       |           |                               |         |                               |         |

Values of RCR\textsubscript{ADP} and RCR\textsubscript{DNP} in succinate energized mitochondria are presented as Means ± SEM. The number of experiments showed in parentheses. P-values are calculated to experiments free additions of PAO, DIDS, tBHP, or NEM. Asterisks indicate that difference between appropriate values is not statistically significant. Concentrations of DIDS for mitochondria energized by glutamate and malate are marked by two asterisks.
buffers. The DNP-dependent respiratory control ratio (RCRDNP) in above TlNO₃ buffers was accordingly determined as a ratio of DNP-uncoupled respiration to state 4 (Table 5) or a basal state respiration (Table 6).

### 2.4. Mitochondrial membrane potential

The ΔΨmito induced in succinate-energized on the IMM of RLM (Table 7) was evaluated according to Waldmeier [5] by the intensity of safranin fluorescence (arbitrary units) in the mitochondrial suspension with magnetic stirring at 20 °C using a Shimadzu RF-1501 spectrofluorimeter (Shimadzu, Japan) at 485/590 nm wavelength (excitation/emission). Mitochondria (0.5 mg protein/ml) were placed into a quartz cuvette of four clear walls with 3 ml of a buffer containing 20 mM TlNO₃, 125 mM KNO₃, 110 mM sucrose, 5 mM Tris–NO₃ (pH 7.3), 1 mM Tris–Pi, 3 μM safranin, 2 μM rotenone, and 1 μg/Ca²⁺

### Table 6

Effects of PAO, DIDS, and tBHP on RCRDNP in succinate-energized and Ca²⁺-loaded rat liver mitochondria.

| PAO (μM) | RCRDNP ± SEM | P value | DIDS (μM) | RCRDNP ± SEM | P value | tBHP (μM) | RCRDNP ± SEM | P value |
|----------|--------------|---------|-----------|--------------|---------|-----------|--------------|---------|
| − Ca²⁺   | 2.48 ± 0.03 (3) | P < 0.01 | − Ca²⁺    | 2.17 ± 0.05 (3) | P < 0.01 | − Ca²⁺    | 2.48 ± 0.03 (3) | P < 0.01 |
| + Ca²⁺   | 0.53 ± 0.06 (3) | −        + Ca²⁺   | 0.68 ± 0.06 (3) | −        + Ca²⁺   | 0.53 ± 0.06 (3) | −        + Ca²⁺   | 0.53 ± 0.06 (3) | −      |
| 1        | 0.42 ± 0.03 (3) | *        2.5      | 1.05 ± 0.02 (3) | P < 0.05 | 50        | 0.39 ± 0.03 (3) | *      |
| 2        | 0.38 ± 0.04 (3) | *        5        | 0.74 ± 0.04 (3) | *        100      | 0.38 ± 0.05 (3) | *      |
| 5        | 0.35 ± 0.02 (3) | P < 0.04 | 12.5      | 0.87 ± 0.13 (3) | *        50+ADP+CsA | 1.43 ± 0.04 (3) | P < 0.01 |
| 2+ADP+CsA| 1.81 ± 0.02 (3) | P < 0.01 | 5+ADP+CsA | 1.47 ± 0.18 (3) | P < 0.02 | 50+NEM    | 1.78 ± 0.09 (3) | P < 0.01 |
| 2+NEM    | 1.54 ± 0.09 (3) | *        12.5+ADP+CsA | 1.69 ± 0.29 (3) | P < 0.03 | 25+NEM | 1.45 ± 0.20 (3) | P < 0.02 |
| 5+NEM    | 0.93 ± 0.03 (3) | P < 0.03 | 12.5+NEM | 1.80 ± 0.29 (3) | P < 0.03 | 25+NEM | 1.45 ± 0.20 (3) | P < 0.02 |

Values of RCRDNP are presented as Means ± SEM. The number of experiments showed in parentheses. P-values are calculated to experiments with Ca²⁺ but free additions of PAO, DIDS, tBHP, or NEM. Asterisks indicate that difference between appropriate values is not statistically significant.

**Fig. 1.** Typical traces in vitro research of rat liver mitochondria. Mitochondria (1.5 mg/ml of protein) were added in medium containing 100 mM KCl, 20 mM Tris–HCl (pH 7.3), 3 mM MgCl₂, and 3 mM Tris–PO₄, 5 mM Tris–succinate, and 2 μM rotenone. Additions of mitochondria (RLM), 130 μM ADP (ADP), and 30 μM DNP (DNP) showed by arrows. Oxygen consumption rates (ng atom O min/mg of protein) are presented as numbers placed near experimental traces.
ml of oligomycin. In addition, the next chemicals were added in the medium before mitochondria: PAO, tBHP, DIDS, ADP, and CsA (where indicated). Succinate, Ca$^{2+}$, and DNP were administrated into the medium after mitochondria. Temperature conditions used in the research were standard for experiments with isolated mitochondria in vitro.

2.5. Statistics

The statistical differences and P-values of experimental results in Tables 1–7 are correspondingly evaluated using the two population t-test (Microcal Origin, Version 6.0, Microcal Software).

2.6. Chemicals

CaCl$_2$, Mg(NO$_3$)$_2$, H$_3$PO$_4$, KNO$_3$, TlNO$_3$, and DNP were of analytical grade from Nevareactiv (St. Petersburg, Russia). Rotenone, oligomycin, PAO, tBHP, NEM, tris–OH, EGTA, ADP, CsA, BKA, CATR, and succinate were from Sigma (St. Louis, MO, USA). DIDS was purchased from Santa Cruz Biotechnology (USA). Sucrose as 1 M solution was refined from cation traces on a column filled with a KU-2-8 resin from Azot (Kemerovo, Russia).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.03.030.
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