The Mitochondrial Complex V–Associated Large-Conductance Inner Membrane Current Is Regulated by Cyclosporine and Dexpramipexole

Kambiz N. Alavian, Steven I. Dworetzky, Laura Bonanni, Ping Zhang, Silvio Sacchetti, Hongmei Li, Armando P. Signore, Peter J. S. Smith, Valentin K. Gribkoff, and Elizabeth A. Jonas

Department of Internal Medicine (K.N.A., P.Z., S.S., H.L., E.A.J.) and Department of Neurobiology (E.A.J.), Yale University School of Medicine, New Haven, Connecticut; Division of Brain Sciences, Department of Medicine, Imperial College London, London, United Kingdom (K.N.A.); Department of Neuroscience, Imaging and Clinical Sciences, University G.d’Annunzio of Chieti-Pescara, Chieti-Pescara, Italy (L.B.); Knopp Biosciences LLC, Pittsburgh, Pennsylvania (S.I.D., A.P.S., V.K.G.); and Biocurrents Research Center, Marine Biological Laboratory, Woods Hole, Massachusetts (P.J.S.S.)

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ABSTRACT

Inefficiency of oxidative phosphorylation can result from futile leak conductance through the inner mitochondrial membrane. Stress or injury may exacerbate this leak conductance, putting cells, and particularly neurons, at risk of dysfunction and even death when energy demand exceeds cellular energy production. Using a novel method, we have recently described an ion conductance consistent with mitochondrial permeability transition pore (mPTP) within the c-subunit of the ATP synthase. Excitotoxicity, reactive oxygen species–producing stimuli, or elevated mitochondrial matrix calcium opens the channel, which is inhibited by cyclosporine A and ATP/ADP. Here we show that ATP and the neuroprotective drug dexpramipexole (DEX) inhibited an ion conductance consistent with this c-subunit channel (mPTP) in brain-derived submitochondrial vesicles (SMVs) enriched for F$_1$F$_0$ ATP synthase (complex V). Treatment of SMVs with urea denatured extramembrane components of complex V, eliminated DEX- but not ATP-mediated current inhibition, and reduced binding of [14C]DEX. Direct effects of DEX on the synthesis and hydrolysis of ATP by complex V suggest that interaction of the compound with its target results in functional conformational changes in the enzyme complex. [14C]DEX bound specifically to purified recombinant b and oligomycin sensitivity–conferring protein subunits of the mitochondrial F$_1$F$_0$ ATP synthase. Previous data indicate that DEX increased the efficiency of energy production in cells, including neurons. Taken together, these studies suggest that modulation of a complex V–associated inner mitochondrial membrane current is metabolically important and may represent an avenue for the development of new therapeutics for neurodegenerative disorders.

Introduction

The efficient regulation of cellular energy production by mitochondria is dependent on the integrity of the inner and outer mitochondrial membranes and the maintenance of the proton–motive force that drives the production of ATP by complex V, the ATP synthase complex (Caviston et al., 1998; Brand, 2005; Watt et al., 2010; Jonas et al., 2014). Under physiologic conditions, mitochondrial membranes contain a number of ion conductance pathways, including homologs of “classic” ion channels, with diverse regulatory and homeostatic functions, as well as conductance pathways that appear to be functionally expressed only under pathophysiological conditions (Pavlov et al., 2001; Kirichok et al., 2004; O’Rourke, 2004, 2007; Dejean et al., 2005; Bernardi, 2013; Elrod and Molkentin, 2013; Raffaello et al., 2013).

Mitochondrial dysfunction is particularly problematic in excitable cells, which have exceptional energy requirements (Nguyen et al., 1997; David and Barrett, 2003; Chouhan and Peltier, 2012). In the case of neurons, their unique anatomic characteristics often require the physical translocation of mitochondria and metabolic precursors long distances along axons to distal synapses, providing numerous additional opportunities for disruption of critical energy supplies (Glater et al., 2006; Courchet et al., 2013; Sun et al., 2013; Sheng, 2014). Neuronal mitochondrial stress and injury result from environmental and genetic factors such as heteroplasy, increasing the risk of cell death as increasing proportions of mitochondria become inefficient or unavailable (Albers and Beal, 2000; Nicholls, 2008). Large-conductance leak currents that disrupt mitochondrial osmotic gradients may develop...
during stress and contribute to mitochondrial dysfunction and bioenergetic inefficiency (Damiano et al., 2006; Rao et al., 2014). It had been shown previously that mitochondrial permeability transition may be brought on by calcium- or reactive oxygen species–induced opening of a nonsselective inner membrane channel mitochondrial permeability transition pore (mPTP) (Petronilli et al., 1989; Szabo et al., 1992; Zorov et al., 2000; Bernardi, 2013). We have recently found that this conductance is located within the c-subunit of the ATP synthase (Alavian et al., 2014). We seek to understand more about the biophysical characteristics and reversibility of the opening of this channel and the possible implications for increasing bioenergetic efficiency by increasing the probability of channel closure. Understanding structural substrates for inhibition of these conductance pathways may provide insight into new avenues for the discovery of effective therapies for a variety of neuronal diseases.

Recently we presented evidence that cyclosporine A (CsA), a compound that is a known modulator of mitochondrial function (Szabo and Zoratti, 1991; Giorgio et al., 2009), and deoxpramipexole (DEX) (Gribkoff and Bozik, 2008) inhibited stress- and injury-induced large-conductance currents recorded from whole neuronal mitochondria (Alavian et al., 2012). DEX is the nondopaminergic $R^+$ enantiomer of the high-affinity dopamine agonist and Parkinson’s disease therapeutic pramipexole (Mirapex; Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT) (Gribkoff and Bozik, 2008). Pramipexole is neuroprotective by a nondopaminergic mechanism (Gu et al., 2004), likely involving inhibition of mitochondrial permeability transition pore (mPTP) (Cassarino et al., 1998; Sayed et al., 2006). Because DEX is not a high-affinity dopamine agonist, its use avoids the side effects of dopaminergic agonists, and it is neuroprotective (Bozik et al., 2011). Mitochondrial effects of both drugs have not been completely characterized. DEX has been found to increase the efficiency of energy production in cells, including neurons, at concentrations that inhibit large-conductance mitochondrial currents (Alavian et al., 2012).

In the current study, we demonstrate that currents suppressed by CsA and DEX are located at the inner mitochondrial membrane and are also inhibited by ATP. We find that DEX binds to the oligomycin sensitivity-conferring protein (OSCP) and b-subunits of the F$_1$/F$_0$ ATP synthase, suggesting that it may indirectly inhibit the c-subunit (mPTP) pore by producing a conformational change in complex V that places F$_1$ over the pore, inhibiting its conductance.

Materials and Methods

**DEX.** DEX was prepared by contract with Albany Molecular Research Inc. (Albany, NY; commercially available from Sigma-Aldrich, St. Louis, MO) and was determined to have chemical purity of >99.99% and enantiomeric ($R^+$) purity relative to pramipexole of >99.95%.

**Submitochondrial Vesicle Preparation.** Preparations of submitochondrial vesicles (SMVs) were adapted from earlier methods (Sacchetti et al., 2013). The Lubrol-insoluble fraction (SMVs) was prepared from isolated mitochondria by resuspension in isolation buffer (IB) [250 mM sucrose, 20 mM HEPES (pH 7.2), 1 mM EDTA, and 0.5% bovine serum albumin] and combined with an equal volume of 1% digitonin on ice for 15 minutes. The pellet was washed twice in IB, resuspended in 200 µl of IB and 2 µl of 10% Lubrol PX (Calbiochem, San Diego, CA) on ice for 15 minutes, then layered onto IB and centrifuged at 39,000 rpm for 1 hour, followed by an IB wash. Final protein concentration was ~1–4 mg/ml of protein as determined by Pierce BCA Protein Assay (Thermo Scientific, Rockford, IL).

**F$_1$ Removal from SMVs.** F$_1$ subunits were removed from SMVs by adapting previously established methods (Pedersen et al., 1981). Sixty micrograms of SMVs per 1 ml of IB was treated with 1 µl of 6 M urea for 5 minutes on ice, then centrifuged at 21,000g for 10 minutes. The pellet was washed three times in IB (centrifugation at 21,000g for 10 minutes) and stored in IB.

**$^{14}$CDEX Binding to SMVs.** Urea-treated or control SMVs were incubated in $^{14}$CDEX (56 µCi/µmol; GE Healthcare UK, Pollards Wood, UK) overnight (4°C) in an agitator, then applied to a Centrifugal Filter Unit with Ultracel YM-10 membrane (Millipore Corp., Billerica, MA) and centrifuged at 4000g for 1 hour. SMVs were washed twice with IB. Filter units were incubated in Ultima Gold scintillation liquid (PerkinElmer Health Sciences, Inc., Waltham, MA) overnight. Samples were counted for $^{14}$C with a Beckman Coulter LS 5000TD scintillation counter (GMI, Inc., Ramsey, MN).

**$^{14}$CDEX Interaction with Heterologously Expressed F$_1$/F$_0$ ATP Synthase Subunits.** The human open reading frame constructs for α, β, c, δ, ε, γ, and OSCP ATP synthase subunits, tagged with Myc or DDK (Flag) tags, were purchased from OriGene Technologies (Rockville, MD). 293T cells were transfected with the above constructs, using the calcium phosphate method (Li et al., 2008). On day 3 post-transfection, the cells were lysed and the fusion proteins were bound to the EZview Red Anti-FLAG M2 Affinity Gel (Sigma-Aldrich), according to the manufacturer’s protocol. The proteins on the beads were identified by immunoblot analysis, using mouse anti-Myc antibodies (Cell Signaling Technology, Danvers, MA). The protein-bound beads were incubated in Ultima Gold scintillation liquid overnight. Samples were counted for $^{14}$C using a Beckman Coulter LS 5000 TD scintillation counter.

**Patch-Clamp Recordings from SMVs.** Patch-clamp recordings were made from SMVs in intracellular solution (120 mM potassium chloride, 8 mM NaCl, 0.5 mM EGTA, and 10 mM HEPES; pH adjusted to 7.3) at room temperature (22–25°C). Pipettes (80–100 MΩ) were filled with the same solution; recordings were made using a HEKA 8 amplifier (HEKA Instruments, Bellmore, NY) with a +20 mV holding potential. Data were recorded at 20 kHz and filtered at 500–1000 Hz. DEX (Knopp Biosciences LLC, Pittsburgh, PA) was prepared as a 10 mM aqueous stock and diluted in intracellular recording solution; CsA (Sigma-Aldrich) was prepared as an 8 mM stock solution in ethanol and diluted in buffer. Reagents were rapidly fused into the recording chamber. Peak membrane conductance was measured as the peak amount of current (in picoamperes) from zero, converted to picoammperes by assuming a linear current-voltage relationship.

**Western Blot Analysis.** Western blot analysis was performed using standard protocols (Li et al., 2008). Primary antibodies were mouse anti-β-subunit 1:1000 (Mitosciences, Eugene, OR) and mouse anti-adenine nucleotide transporter 1:1000 (Santa Cruz Biotechnology, Dallas, TX).

**Measurement of ATP Hydrolysis Using a Luciferin-Luciferase Assay.** ATP hydrolysis in SMVs was measured with the BioVision Assays. ATP Kit in a 96-well plate with a plate reader (VICTOR3 Multilabel; PerkinElmer). SMVs were suspended in mitochondrial IB plus bovine serum albumin (0.5%) (Bonanni et al., 2006), ATP solution (final concentration, 0.5 mM), and 1 µl of reconstituted ATP-monitoring enzyme. To initiate, 100 µl of nucleotide-releasing agent containing Triton X was added and luminescence measured and displayed as percentage change in luminescence over...
time. Three wells were used for each condition, repeated at least three times on different SMV isolations.

**Measurement of ATP Hydrolysis Using an NADH Assay.**
ATP hydrolysis was also measured using an NADH-ATP-synthase kit (Mitosciences), according to the manufacturer’s protocol with modifications (Lotscher et al., 1984). SMVs were added 20 minutes prior to addition of the reagent mix. The rate of change in fluorescence over time as NAD was oxidized was measured as a decrease in absorbance at 340 nm.

**Kinetic ATP Synthesis Assay.**
Synthesis of ATP in liver mitochondria was measured using an ATP determination kit (Molecular Probes, Eugene, OR), supplemented with 1 mM sodium succinate and 500 nM rotenone. Assays were performed in 96-well microplates (Becton Dickinson, Franklin Lakes, NJ) in a final volume of 10 μl consisting of 10 μl of mitochondria (0.1 mg protein/ml final concentration), 10 μl of drug or control buffer, and 90 μl of ATP-determination reaction mixture. After a 5-minute recording of basal luciferase-generated luminescence, ADP (final concentration, 10 μM; 10 μl) was injected into each well. Luminescence was recorded every 6 seconds for 15–20 minutes.

**Statistical Analyses and Curve Fitting.** For comparisons involving two groups, paired or unpaired Student’s t tests (two-tailed) were used. For more than two groups, one-way or two-way analyses of variance (ANOVA) and two-factor multiple ANOVAs were performed; in the case of a significant F test, the P value is provided in the figure legend, and preplanned post hoc comparisons (Bonferroni-corrected t tests or Tukey’s HSD test) were performed and significance levels displayed in figures and exact P values provided in the legends. Where possible, P values for tests are presented to two significant digits. All statistical analyses were performed using GraphPad Prism.

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**Results**

**Patch-Clamp Recordings of SMVs Containing F1F0 ATPase Revealed an ATP-, CsA-, and DEX-Sensitive Leak Conductance.** In a previous study (Alaviani et al., 2012), patch-clamp recordings were made from whole mitochondria; drug-induced current inhibition induced by DEX and CsA recorded in those experiments could have reflected changes in mitochondrial currents localized at either or both the inner and outer mitochondrial membrane (Alaviani et al., 2012). An increase in inner membrane leak conductance would be particularly effective at decreasing mitochondrial metabolic coupling. To test if the DEX- and CsA-inhibited conductance was expressed at the inner mitochondrial membrane, and was functioning directly as a “metabolic leak,” we patch-clamped brain-derived SMVs. This preparation consists of isolated inverted inner membrane vesicles containing detergent-resistant mitochondrial components, and in particular F1F0 ATP synthase (Chan et al., 1970; Sacchetti et al., 2013). In the absence of ATP, gigaohm seals were formed on SMVs and high levels of conductance (peak levels, 600–1200 pS) were recorded. Currents in SMVs were modestly enhanced by high [Ca2+] and were inhibited by both DEX and CsA (Fig. 1, A and B). Maximal levels of inhibition by these

![Figure 1](https://www.aspetjournals.org/doi/abs/10.1124/mol.115.107622)

**Fig. 1.** DEX and CsA decreased conductance in SMVs. (A) Example patch-clamp recording from a brain-derived SMV; holding potential, +60 mV, before and after addition of the indicated agents to the bath (Ca2+ = 100 μM, DEX = 2 μM, CsA = 1 μM). (B) Histograms represent group data (mean ± S.E.M.) of peak conductance of experiments in (A). Current was measured from 0 pA and presented as peak conductance assuming a linear current-voltage relationship. (Left) Level of conductance before and after switching to high [Ca2+], Ca2+ plus 2 μM DEX (n = 13 SMVs), followed by Ca2+. DEX, and 1.0 μM CsA (n = 5 SMVs). One-way ANOVA, P = 0.0002; preplanned post hoc Bonferroni-corrected t tests, Ca2+ versus 2 μM DEX, P = 0.00041; Ca2+ versus CsA, P = 0.0048. (Right) Reversal of order of DEX and CsA addition: level of conductance in SMVs in control medium, after switching to high [Ca2+] and were inhibited by both DEX and CsA (Fig. 1, A and B). Maximal levels of inhibition by these
compounds were approximately equal, and they showed only insignificant levels of additivity (Fig. 1B). The effects of the drugs were also observed in the absence of added Ca\(^{2+}\) (see all other figures), and the effects of DEX were reversible. ATP, a known blocker of mPTP as well as a metabolic modulator, also decreased peak membrane conductance of SMVs (Fig. 1C). The maximal effect of ATP was greater than the effect of DEX or CsA (Fig. 1, C and D), and both ATP and DEX decreased the conductance of SMV membrane patches in a concentration-dependent manner (Fig. 2, A and B). DEX was potent, with an EC\(_{50}\) = 111 nM, very similar to the previously published value obtained in whole, brain-derived mitochondria (Alavian et al., 2012), and the curve was, as previously observed for this compound, quite shallow, with a Hill slope of <1. ATP was much less potent (EC\(_{50}\) = 224 \(\mu\)M), but the Hill slope was >1, and ATP was much more effective, consistently blocking a greater percentage of the total conductance.

**DEX Modulated the Enzymatic Activity of Complex V.** Interaction of DEX with inner mitochondrial membrane components resulted in current inhibition. We had previously observed a small but significant increase in ATP production in some cells in the presence of DEX, even when oxygen consumption rates were decreased or unaffected. This suggested the possibility that DEX directly interacted with complex V, an action that could concomitantly produce an alteration of its enzymatic function. Three different techniques were employed to measure the ability of complex V to hydrolyze or synthesize ATP in the presence and absence of DEX. SMVs can hydrolyze ATP (Alavian et al., 2011), and we found that DEX significantly enhanced ATP hydrolysis in SMVs in a concentration-dependent manner in an assay in which complex V enzymatic activity was estimated by the change in NADH signal (Fig. 3A); CsA also significantly enhanced ATP hydrolysis (Fig. 3A). In a different assay utilizing SMVs, DEX increased ATP hydrolysis, measured by ATP-luciferase levels in the medium, with an EC\(_{50}\) = 163 nM. The ATP synthase inhibitor oligomycin effectively inhibited ATP hydrolysis in both of these assays. In isolated liver mitochondria, where the presence of a proton gradient allows for ATP synthesis, DEX modestly increased ATP synthesis in response to an ADP pulse (Fig. 3B). The effect of DEX was significant and potent (EC\(_{50}\) = 166 nM), and like other measures of DEX’s action, the concentration-response curve had a Hill slope of <1.

**DEX Binding and Current Inhibition Were Reduced by Removal or Denaturation of F\(_{1}\) or Other Extramembrane Components from the F\(_{1}\)F\(_{0}\) Mitochondrial ATP Synthase in SMVs.** Complex V has an inside-out orientation in SMV membranes (Chan et al., 1970; Ko et al., 2003), with the F\(_{1}\) head on the external side of the membrane.

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**Fig. 2.** Concentration-response relationship for the inhibition of currents in SMVs by ATP and DEX. (A, Top) Effect of ATP on peak conductance levels; holding potential, +40 mV. (Bottom) Group mean data and logistic fit estimate of the effect of ATP concentration on percentage maximal SMV current inhibition (n = 4 SMVs; EC\(_{50}\) = 224 \(\mu\)M; Hill slope = 1.5). One-way ANOVA, P = 0.0001; preselected post hoc Bonferroni-corrected t tests, control versus 0.4 mM ATP, P = 0.010; 0.6 mM, P = 0.0032; 0.8 mM, P = 0.0023; 1.0 mM, P = 0.0016. (B, Top) Effect of DEX on peak conductance levels in SMVs; holding potential, +50 mV. (Bottom) Group mean data and logistic fit estimate of the mean (±S.E.M.) percentage maximal current inhibition at different concentrations of DEX (n = 5 SMVs; EC\(_{50}\) = 111 nM; Hill slope = 0.31). One-way ANOVA, P < 0.0001; preselected post hoc Bonferroni-corrected t tests, control versus 20 nM DEX, P = 0.0375; 200 nM, P = 0.009; 2 \(\mu\)M, P = 0.0025; 20 \(\mu\)M, P = 0.0005. In all cases, recordings are discontinuous; addition of drugs occurred from 30 seconds to 5 minutes prior to recording the steady-state response. *P < 0.05; **P < 0.01; ***P < 0.001 versus control.
allowed us to examine the effects of urea treatment (see Materials and Methods), which removes/denatures non-membrane-residing components from protein supercomplexes and, in particular, the F1 and associated components of complex V. Urea-treated SMVs were unable to perform ATP hydrolysis, and removal of the F1 subunit was confirmed by Western blot immunoanalysis, whereas a known membrane-inserted component, the adenine nucleotide translocator, was unaffected (Fig. 4, A and B). These urea-treated SMVs still displayed leak conductance of similar magnitude under patch clamp (peak conductance, 600–1200 pS), but DEX no longer inhibited the conductance, suggesting that the presence of a non-membrane-inserted component was critical for DEX’s effects (Fig. 4C). ATP, however, still very effectively decreased the leak conductance in the absence of F1 (Fig. 4C). Finally, total binding of [14C]DEX to SMVs was determined before and after treatment with urea. In three independent experiments, the total binding of [14C]DEX was significantly reduced (mean reduction, 42%) with elimination of functional F1 (Fig. 4D). These data demonstrate that a urea-sensitive component in SMVs was responsible for both binding of [14C]DEX and DEX-regulated current inhibition.

Interaction of [14C]DEX with Specific Subunits of Complex V. To further determine if the site of interaction of DEX resulting in inhibition of inner membrane current is localized to one or more subunits of complex V, individual subunits were heterologously expressed in 293T cells (Fig. 5A) and bound to affinity gels. The bound protein was used to directly test for the specific binding of [14C]DEX. When incubated in [14C]DEX, two subunits, b and OSCP, demonstrated levels of [14C]DEX binding that were significantly greater than those incubated with the untransfected control cell lysate or other complex V subunits (Fig. 5B). Coincubation in "cold" DEX (100 μM) significantly reduced binding of these subunits to levels indistinguishable from untransfected controls, suggesting specific and competitive binding to subunits comprising the lateral stalk (stator) of the mitochondrial ATP synthase (Fig. 5C).

Discussion

Evidence has accumulated over the last decade strongly implicating mitochondrial dysfunction in a number of chronic neurodegenerative disorders (Chaturvedi and Beal, 2008).
The slow loss of neurons in these diseases suggests that the contribution of mitochondrial dysfunction to neuronal death involves increasing and cumulative risk of death, rather than acute and immediate causation, as seen with mitochondrial-induced apoptosis (Youle and Strasser, 2008). This suggests that a final common pathway for neuronal degeneration may be energy insufficiency, and an increased understanding of normal and pathologic mitochondrial metabolic regulation may provide significant insights into therapeutic approaches for the treatment of these diseases (Khatri and Man, 2013). The lack of compounds demonstrated to provide benefit in neurodegenerative disease has hampered this effort, inasmuch as a lack of tools has precluded effective experimental approaches.

Recently, we demonstrated that the neuroprotective compound DEX inhibited stress- and injury-induced large-conductance currents in whole, brain-derived mitochondria (Alavian et al., 2012). Additionally, in cultured cells and neurons in culture, DEX enhanced bioenergetic efficiency (Alavian et al., 2012). Specifically, cells incubated in DEX were protected against disease-relevant stressors and had reduced oxygen consumption rates while concomitantly maintaining or increasing cellular ATP levels. While these and other previous experiments indicated that the most likely site of action of the compound was mitochondrial (Alavian et al., 2012), the molecular substrate of these effects remained completely unknown.

The hypothesis we propose assumes that a leak conductance channel is present at higher frequency in mitochondria isolated from affected brain regions of rodents exposed in vivo to global ischemic injury (Ofengeim et al., 2012), an acute neurologic insult (Ofengeim et al., 2012; Park et al., 2014). Previous studies have suggested that the ischemia channel comprises a complex of proteins including a divalent-sensitive component, Bcl-xL, and voltage-dependent anion channel (Jonas et al., 2004; Miyawaki et al., 2008; Ofengeim et al., 2012). More recently, we determined that a channel sensitive to Bcl-xL resides within the c-subunit of the ATP synthase and has features in common with the mPTP. We determined that components of the F1, including the b-subunit, prevent pore opening by positioning themselves over the pore of a leak conductance within the c-subunit ring (Alavian et al., 2011, 2014; Chen et al., 2011). Our model predicts that cyclophilin D, which is known to bind to OSCP (Giorgio et al., 2009), acts on the pore by facilitating the removal of the F1 from the c-subunit in a CsA-sensitive manner during pore opening (Alavian et al., 2014). Because DEX binds in a similar configuration to that of CsA, we suggest here that it acts similarly to CsA, although how the exact binding domains within F1 differ is as yet undetermined.

In support of our model, we showed that the level of bound radiolabeled DEX and current inhibition by DEX were greatly reduced by denaturation or removal of proteins within the F1 that extend outside the protective lipid bilayer of the inner membrane. Finally, we have shown that radiolabeled DEX binds to the b and OSCP subunits of complex V. These subunits form the stator stalk of complex V and are closely associated with each other, and they would be removed by urea in SMV preparations.

The hypothesis we propose assumes that a leak conductance associated with the mitochondrial inner membrane could lead to shuting of the proton-motive force that provides the energy for oxidative phosphorylation, with negative consequences for bioenergetic efficiency. DEX (and, to the extent tested, CsA) application directly resulted in modulation of ATP synthesis and hydrolysis by mitochondria, and we suggest that the two phenomena, enzymatic modulation and the inner membrane current inhibition by DEX and CsA, are related and result from an interaction with complex V. Specifically, it is possible that the small levels of direct
modulation of enzyme function observed in the presence of DEX reflect a conformational change resulting from the interaction of the drug with complex V. It is unclear which aspect of this action of DEX could prove more relevant to its cellular effects, namely its direct action on enzymatic function or its effect on the associated conductance pathway. It is likely that both will lead to neuroprotection. Regardless, increased or unchanged ATP levels were observed in neurons and other cells after exposure to DEX, accompanied by lower basal oxygen consumption rates (Alavian et al., 2012). This suggests that metabolic protection or even improvement may indeed be a previously unanticipated outcome of enhanced mPTP closure. In the future, drugs that target this channel specifically, potently, and effectively may prove useful in neurodegenerative diseases.

**Authorship Contributions**

**Participated in research design:** Alavian, Dworetzky, Bonanni, Saschetti, Li, Signore, Smith, Gribkoff, Jonas.

**Conducted experiments:** Alavian, Dworetzky, Bonanni, Zhang, Saschetti, Li, Signore, Smith, Gribkoff, Jonas.

**Contributed new reagents or analytic tools:** Dworetzky, Signore, Gribkoff.

**Performed data analysis:** Alavian, Dworetzky, Bonanni, Saschetti, Gribkoff, Jonas.

**Wrote or contributed to the writing of the manuscript:** Alavian, Gribkoff, Jonas.

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Address correspondence to: Elizabeth A. Jonas, Department of Internal Medicine, Yale University School of Medicine, P.O. Box 208020, New Haven, CT 06530. E-mail: Elizabeth.jonas@yale.edu