The signal transduction function for oxidative phosphorylation is at least second order in ADP

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For isolated mitochondria at constant, high [Pi] (5, 6, 12, 13), and in situ canine cardiac muscle (14) and ex situ transgenic mouse liver (15) were analyzed analogously by curve-fitting analysis of the specific velocity function $v(S^*)$ (11). Data points were obtained by graphical extraction in all cases except (14) and transformed to $v'(S'*)$ format, where specific velocity $v' = vV_{\max}$ and specific substrate concentration $S' = S/[S]_0$, were obtained from the experimental maximal velocity $V_{\max}$ and $[S]_0$, given in each study. Data on the kinetics of [ADP] stimulation of MOP during pacing and inotropic stimulation in vivo canine heart muscle (14) were obtained from Tables 2 and 4 in Ref. 14 and transformed to $v'(S'*)$ format using a maximal oxygen consumption of $v$ dog heart of 0.45 ml/min/g (16) and 0.074 mM for [ADP]$_{0.5}$ corresponding to the [ADP] in cardiac muscle at half this rate (14).

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(Received for publication, August 20, 1996)

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RESULTS

Dynamic Range of Energy Balance in Skeletal Muscle—The studied range of energy balance states in forearm muscle included the maximal sustainable steady state of energy balance in all subjects as judged from the physiological responses. Typically a maximum occurred at a twitch frequency of 1.6 Hz; higher twitch frequencies resulted in acidosis (8). The calculated rate of contraction coupled ATP hydrolysis in forearm flexor muscle cells at this state was 0.25 ± 0.01 mmol ATP liter⁻¹ s⁻¹ (mean ± S.E., n = 6). The matching ATP synthesis flux was mostly mitochondrial (maximally 0.22 mmol ATP liter⁻¹ s⁻¹), by increases in the average ADP concentration (in mm) in forearm muscle cells during contraction (pooled data, n = 6). Variables were calculated from 31P NMR spectroscopic data as described elsewhere (8). Over the experimentally achievable range of energy balance states in the muscle, the covariation of [ADP] and \( p_{\text{MOP}} \) was equally well predicted by any of three relations: (i) \( y = 0.15 \cdot x - 0.03 \) (solid line, \( r^2 = 0.91 \)); (ii) \( y = 0.51 \cdot (x/0.079)/(1 + (x/0.079)) - 0.10 \) (dashed and dotted line, \( r^2 = 0.93 \)); (iii) \( y = 0.29 \cdot (x/0.048)^2(1 + (x/0.048)^2) - 0.02 \) (dashed line, \( r^2 = 0.93 \)). Inset, extrapolated covariation of [ADP] and \( p_{\text{MOP}} \) as predicted by each of the three fitted functions over a (nonphysiological) [ADP] range of 0–0.225 mm.

Fig. 1. Stimulation of mitochondrial ATP synthesis flux, \( J_{\text{MOP}} \) (in mmol ATP liter⁻¹ s⁻¹), by increases in the average ADP concentration (in mM) in forearm muscle cells during contraction (pooled data, \( n = 6 \)). Variables were calculated from 31P NMR spectroscopic data as described elsewhere (8). Over the experimentally achievable range of energy balance states in the muscle, the covariation of [ADP] and \( p_{\text{MOP}} \) was equally well predicted by any of three relations:

Analysis of Kinetics of [ADP] Stimulation of MOP in Other Systems—To test the generality of this in vivo result, we also analyzed the in vitro kinetics of [ADP] stimulation of MOP reported for isolated mitochondria (5, 6, 12, 13) (Fig. 2B, inset). Likewise, stimulation of MOP by [ADP] reported in these in vitro studies required in each case a Hill coefficient significantly greater than 1 to explain the kinetics. The range of \( n_H \) values was 2.1–2.9 (2.6 ± 0.2, mean ± S.E., \( n = 4 \)) and not significantly different from the value we obtained for mitochondria studied in situ in skeletal muscle. This result was surprising because the description of approximately first order control characteristics of extramitochondrial [ADP] (2, 3) had been formulated based on just these studies (12).

To next test if this apparent kinetic order (i.e. between 2 and 3) for transduction of cytosolic [ADP] to intramitochondrial \( F_\text{1-ATPase} \) explains the covariation of cytosolic [ADP] and MOP flux also in other mammalian cell types, we analyzed the reported kinetics of [ADP] stimulation of MOP in intact cardiac muscle (14) and liver (15) cells pooled with skeletal muscle data (Fig. 3). The covariation of [ADP] and the rate of MOP in the pooled data from all three cell types was adequately explained by a transduction function for cytosolic [ADP] with \( n_H = 2.2 ± 0.4 \) (± S.E. of regression, \( r^2 = 0.73 \)).

DISCUSSION

The main result and novel finding of this study is that the kinetic function for [ADP] stimulation of MOP in skeletal muscle is approximately a second order function of the form \( p_{\text{MOP}} = f([\text{ADP}]^{n_H}) \) where \( n_H = 2 \) and not 1 as has hereto been assumed (1–4). This implies that the scaled sensitivity of mitochondria to variations in cytosolic [ADP] is at least 1 order of magnitude greater than has been assumed. This result impacts the understanding of the biochemistry of mitochondria and its integration in the physiology of mammalian cells.

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Apparent Kinetic Order of \([\text{ADP}]\) Transduction: Analysis—

The crucial piece of information in the analysis of the apparent kinetic order of the transduction function of cytosolic \([\text{ADP}]\) to the mitochondrial matrix was knowledge of the maximal and minimal sustainable MOP fluxes in the muscle cells. Dense sampling of the full physiological domain of the \(([\text{ADP}], J_p^{\text{MOP}})\) relation in itself did not allow for discriminating between first or second order (or higher order, for that matter) of the transduction function (Fig. 1). We obtained estimates of the flux asymptotes from analysis of the thermodynamic flow-force relation of MOP (Fig. 2A). This is a well established and valid description of the relation between the flux through a reaction and the concentration of its substrates and products (19, 20).

The flow-force relation predicts that under the condition of constant sum of substrate and product concentrations, the flux (or flow) \(J\) through a reaction varies in sigmoidal fashion with the thermodynamic driving force \(\Delta G\) between maximal forward and reverse rates (19, 20). It was previously shown that this description applies to MOP in muscle (21).

Of utmost importance to the analysis, this approach is by definition unbiased toward the specific kinetic mechanism of a reaction (20). The only prior knowledge about the nature of the flow-force relation of MOP in muscle that was used in the analysis was that this relation is innately sigmoidal (19, 20). The curve fitting of a four parameter sigmoidal function (Equation 1) to the \((\Delta G_p, J_p^{\text{MOP}})\) covariation was performed fully unconstrained. The performance of the curve fitting of this function was enhanced by its symmetrical properties.

The fitted estimate of the minimal flux \((-0.03 \text{ mmol ATP liter}^{-1} \text{s}^{-1})\) predicted that there would be net ATP hydrolysis by the mitochondrial ATPase over a nonphysiological range of \([\text{ADP}]\) in muscle \(\text{(i.e.} \, 0 < [\text{ADP}] < 13 \mu\text{M})\) if such conditions were to be achieved experimentally in intact cells. This is not unprecedented. Net ATP hydrolysis has been demonstrated in intact isolated mitochondria (20, 22, 23) and significant ATP hydrolysis flux even at maximal net synthetic flux (22, 23), which is entirely consistent with this relation. The flow-force relation predicted net ATP synthesis by MOP over the entire physiological range of \([\text{ADP}]\) and \([P_i]\) in muscle corresponding to a \(\Delta G_p\) range of approximately \(-64 \text{ to } -54 \text{ kJ/mol}\). Of course, this was fully expected and consistent with mitochondrial function as the primary source of ATP in the eukaryotic cell. On basis of these considerations, we conclude that the estimates of the flux asymptotes from the analysis of the flow-force relation provided a sound basis for analysis of the apparent kinetic order of cytosolic \([\text{ADP}]\) transduction in muscle in \(\text{in situ}\).

Higher Order of \([\text{ADP}]\) Transduction: Implications—The result that the apparent kinetic order of cytosolic \([\text{ADP}]\) transduction is at least 1 order of magnitude higher than has hereto been assumed is dramatic and impacts both the understanding of the biochemistry of mitochondria and integrative physiology of mitochondrial function in the cell. According to the formalism proposed by Koshland et al. (24), a Hill coefficient greater than 1 implies amplified sensitivity of mitochondria to variations in cytosolic \([\text{ADP}]\). Sensitivity amplification of enzymes and entire metabolic pathways may be achieved by any of a number of kinetic mechanisms but not a Michaelis-Menten
mechanism (24). Therefore a fundamentally different molecular transduction mechanism for cytosolic ADP must now be considered.

There is considerable in vitro experimental evidence for allosteric instead of Michaelis-Menten kinetics of adenine nucleotide translocation (13, 25–27). Allosteroism of the translocator ANT could be the mechanistic basis for ultrasensitivity of mitochondria to cytosolic [ADP] (24). First of all, our analysis showed that ultrasensitivity of isolated mitochondria to ADP under physiologically comparable conditions of limiting [ADP] and saturating [Pi] is abolished upon bypassing the enzyme-catalyzed translocation of ADP, ATP, and Pi. We analyzed the published kinetics of [MOP] stimulation of MOP for intact versus digitonin-treated mitochondria (13) and found Hill coefficients of 2.9 ± 0.61, and 1.2 ± 0.05, respectively (± S.E. from regression). MOP flux in each preparation could be 100 and 33% inhibited by ANT inhibition, respectively (13), suggesting that in the latter case cytosolic ADP now had direct access to F1-ATPase in a major fraction of the preparation. This suggests that the origin of the ultrasensitivity is at the level of the translocation step in MOP, and not the phosphorylation step. Second, Sluse-Goffart et al. (27) found second order rate dependence of the ADP-MOP homoexchange on extramitochondrial [ADP] over a wide range of concentrations. Other evidence from studies of the ANT suggested positive cooperativity of adenine nucleotide exchange across the intramitochondrial membrane (25, 26) that would result in allosteroism of the ANT (25, 26) not Michaelis-Menten behavior as was originally proposed (28, 29). There is no such evidence for the Pi carrier (18).

The apparent kinetic order of ADP transduction, nADP, may well be different (but ≥ 2) for different cell types (e.g. skeletal versus cardiac muscle cells (Fig. 3). The mechanistic basis for these possible differences is that specific conditions that affect ANT function such as membrane potential and phospholipid composition (30) may well differ between mitochondria in different cell types. Therefore, although the mechanistic value of n (i.e. the number of strongly cooperative binding sites on the enzyme (11)) for ANT in mitochondria in different cell types may be the same, the kinetic, apparent n value, nADP (i.e. the actual operation of the translocation) could differ. It is nADP and not n that is measured in kinetic studies.

The new understanding of mitochondrial detection of variations in cytosolic [ADP] proposed in the present study integrates mitochondrial biochemistry into the physiology of mammalian cells. Second or greater instead of first order of the kinetic function for cytosolic [ADP] transduction has broad explanatory power with respect to ATP homeostasis in intact cells (Fig. 3). Energy balance in skeletal and cardiac muscle and liver cells was sufficiently explained by one and the same kinetic function \( f_{\text{MOP}} = f(\text{ADP}^{n_{\text{ADP}}}) \) with \( n_{\text{ADP}} = 2.2 \) (Fig. 3). Importantly, explicit consideration of proposed \([Ca^{2+}]\) effects on MOP flux (31) was not required in any of these cell types to explain the energy balance. This implies that \([Ca^{2+}]\) may not be a necessary signal by which cellular ATP utilization flux is transduced to the mitochondria, contrary to what has been proposed (1, 31).\(^2\) This conclusion fits the hypothesis that mitochondrial detect variations in cellular ATP utilization during work via reciprocal changes in cytosolic [ADP], a biochemical concept originally proposed by Chance (2). What is a fundamentally novel insight is that the apparent kinetic order of the transduction function of this signal is at least 1 order of magnitude higher than was proposed (2).

Based on current knowledge, it now appears that two amplification mechanisms effectuate ATP homeostasis in the cell: magnitude and sensitivity amplification (24). The first mechanism has been early recognized (1, 31, 33) and involves increases in absolute MOP flux (e.g. mitochondrial density) to match absolute cellular capacity for ATP utilization flux. The second and newly recognized mechanism is the here described sensitivity amplification by which the relative ATP synthesis flux is matched to ATP utilization flux. This mechanism operates independent of mitochondrial density, but the amplification factor may likewise be cell type-specific because of particular conditions that affect the apparent kinetic order of ANT operation. The new challenge in understanding the integration of mitochondrial biochemistry into mammalian cell physiology will be to test this hypothesis.

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\(^2\) \([Ca^{2+}]\) can play a role in altering the absolute MOP flux to match ATP utilization flux via “feed forward” (32) modulation of the absolute value of \( V_{\text{max}} \) (or “gain” of MOP (32) and \([\text{ADP}]_{\text{eq}} \) (or “operating point” of MOP (32)). Such effects are normalized and thus implicit in the reduced transduction function \( v(S)^n \). The magnitude of these effects is, however, not constant but subject to specific conditions such as substrate selection (1).