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Effects of the mitogen concanavalin A on pathways of thymocyte energy metabolism

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Abstract

The lectin concanavalin A (Con A) acts as a mitogen that preferentially activates T-cells. It stimulates the energy metabolism of thymocytes within seconds of exposure. We studied short-term effects (< 30 min) of Con A on a conceptually simplified model system of rat thymocyte energy metabolism in the concentration range of 0–2 μg Con A per 10^7 cells, using metabolic control analysis. The model system consisted of three blocks of reactions, linked by the common intermediate mitochondrial membrane potential ($\Delta\psi_m$): the substrate oxidation reactions, which produce the linking intermediate, and the proton conductance (or leak) and ATP turnover pathways which consume $\Delta\psi_m$. Firstly, we used top-down elasticity analysis to establish which subsystems are targeted by Con A. Secondly, we quantitatively analysed the steady-state regulation of the system variables by Con A: how do the subsystem fluxes respond to Con A individually and as a whole? Our results indicate that: (1) steady-state respiration and $\Delta\psi_m$ increase as Con A concentration is raised, but at higher concentrations the increase in respiration is less and $\Delta\psi_m$ falls; (2) Con A independently changes the kinetics of the reactions that produce and consume $\Delta\psi_m$: the $\Delta\psi_m$-producing reactions are inhibited, and the reactions involved in ATP turnover are stimulated; and (3) the overall effects of Con A are mostly mediated by effects on ATP turnover.

Keywords: Concanavalin A; Stimulation of respiration; Oxidative phosphorylation; Mitochondrial proton leak; Regulation of energy metabolism; Metabolic control analysis

1. Introduction

The control and regulation of respiration have been the subject of a large number of studies in the past few years [1,2]. Within resting cells, oxygen consumption rates are fairly stable [2]. However, under certain circumstances (e.g. hyperthyroidism, muscle work, antigenic stimulation) respiration rates may increase dramatically and sometimes very rapidly. It remains uncertain how the bioenergetic machinery brings about such increases in steady-state respiration: are they caused solely by increased ADP, or is activation of electron supply important?

Here, we investigate the concanavalin A-induced increase of respiration in rat thymocytes. This system provides an interesting and convenient model as it features reproducible stimulation which remains at

Abbreviations: Con A, concanavalin A; J_{ox}, J_s, J_p or J_l, flux through the system, the substrate oxidation system, the ATP turnover system or the proton conductance pathway (leak) (measured as oxygen consumption rates); $\Delta\psi_m$, mitochondrial membrane potential; TPMP, triphenylmethylphosphonium cation

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steady state for at least 30 min and therefore opens a way to study how cells adjust their bioenergetic pathways to external stimuli. Up to 80% of cells isolated from rat thymus respond immediately to Con A with an increase of 30–40% in steady-state oxygen consumption [3], depending on the conditions. Another reason for using this cellular system is that a great deal is already known about its bioenergetics. Exposure of quiescent thymocytes to mitogenic lectins such as Con A triggers a prototypical range of biochemical changes which initiate, or coincide with, the metabolic derepression of quiescent cells in the G_0 phase. The principal changes are similar to those that are likely to be involved in the pathogenesis of autoimmmune diseases [4]. Increased transport of Ca^{2+}, Na^+ and K^+, an acceleration of phospholipid turnover, and accelerated rates of amino acid transport and protein synthesis have been described [4,5]. Buttgereit and Brand [5] were able to account for over 80% of the ATP consuming processes in Con A-stimulated thymocytes, whereas in quiescent cells, 50–55% of the oxygen consumption remains unexplained [4]. On the supply side, there are typical rises in cytosolic [Ca^{2+}] after administering Con A [6] which have stimulatory effects on enzymes such as mitochondrial pyruvate dehydrogenase [7].

Con A has a variety of bioenergetic targets that could mediate the stimulation of respiration, both on the supply side of intermediates like mitochondrial membrane potential and on the demand side. It is not clear how the increase in respiration is eventually brought about, for example, it seems that stimulation of mitochondrial dehydrogenases by Ca^{2+} may not be important [8]. To establish the relative importance of the different effects of Con A, a systemic approach such as metabolic control analysis must be chosen.

The first objective of this investigation was to determine the target sites for Con A and to analyse the nature of any interaction qualitatively, i.e. whether there is a stimulation or an inhibition. The analysis was conducted in a conceptually simple model system of thymocyte energy metabolism comprising the subsystems substrate oxidation reactions, ATP turnover and mitochondrial proton conductance pathway ('leak') (see [9], Fig. 1). The method used is referred to as top-down elasticity analysis [10,11]. Secondly, we used top-down regulation analysis [2] to explain quantitatively the Con A induced changes in steady-state respiration in terms of Con A effects on particular subsystems at different mitogen concentrations.

2. Materials and methods

2.1. Isolation of thymocytes

Female white Wistar rats (4–7 weeks old) were kept under artificial light at 19–21°C and fed a high-carbohydrate diet with access to water and food ad libitum. Thymocytes were isolated as described in [12]. Briefly, rats were killed by cervical dislocation. The thymus was pressed through a nylon mesh and the cells were washed twice by centrifugation, diluted to a final concentration of $4.5–5.5\times10^7$ cells/ml and kept at 37°C in RPMI 1640 with glutamine, without glucose, buffered with HEPES (pH 7.4).

2.2. Determination of mitochondrial membrane potential and oxygen consumption rate

$\Delta\psi_m$ was estimated in situ using [^{3}H]TPMP. Aliquots (1.7 ml) of cell suspension were incubated for 2 min with different amounts of Con A (0, 0.15, 0.5, 0.8, 1.0 and 2.0 μg per 10^7 cells), stirred under CO_2/air (1:19) at 37°C in a plastic vial. Subsequently, carriers (TPMP, tetraphenylboron) and inhibitors were added as described in [12] and below. A 0.5-ml amount of cell suspension was transferred to a 1-ml thermostatted and stirred Clarke oxygen electrode chamber and oxygen consumption rate was measured 20 min later. [^{3}H]TPMP was added to the remainder of the cell suspension. After 20 min, [^{3}H]$TPMP^+$ distribution was measured. Two 500-μl aliquots were transferred into microcentrifuge tubes and centrifuged for 20 s in an MSE Micro Centaur bench centrifuge. Samples of supernatant (120 μl) were transferred into scintillation vials and mixed with 3 ml of scintillant. The remaining supernatant was removed from the pellets, which were then resuspended in 40 μl of Triton X-100 (20% w/v), transferred into scintillation vials and immediately mixed with 3 ml of scintillant. [^{3}H]$TPMP^+$ distribution and $\Delta\psi_m$ were determined by calculating [^{3}H]isotope spaces and using the equation given in [12].

Plasma membrane potential was increased by about 3 mV in the presence of Con A, in agreement with [13]; this was accounted for when calculating $\Delta\psi_m$ (see also [12]). Brand and Felber [14] found that Con A did not change $\Delta\psi_m$. In agreement with this, our results (Fig. 1) show that at Con A levels where stimulation is just below maximal, the mitochondrial membrane potential may be the same as in the quiescent condition.

Glycogen was measured as glucose after amyloglucosidase (E.C. 3.2.1.3) digest in perchloric acid extracts from thymocytes and (positive control) hepatocytes. Glucose was assayed using the Sigma glucose assay kit 510-A.

2.3. Experimental approach to top-down elasticity and regulation analysis in lymphocytes

2.3.1. Top-down elasticity analysis

We conceptually divided bioenergetic reactions into three blocks: a block of reactions that generate $\Delta\psi_m$ (the substrate oxidation subsystem), and two blocks that consume it, either producing ATP for consumption in the cell (ATP turnover subsystem), or uncoupling oxidative reactions from phosphorylation of ADP (the proton conductance pathway or proton leak subsystem) (see [9]).

The kinetic responses of the subsystems to $\Delta\psi_m$ were determined by specific manipulations that changed the activity of other blocks in the system and therefore changed $\Delta\psi_m$ (see [15] for a more detailed description of elasticity analysis). The following conditions were used (brackets denote the subsystems whose kinetics were examined): 0, 5 and 80 ng/ml oligomycin (substrate oxidation), 0, 8.2 and 16.4 nM myxothiazol ($\Delta\psi_m$-consumers), and 0, 8.2 and 16.4 nM myxothiazol in the presence of 80 ng/ml oligomycin (proton conductance pathway). The kinetics of the ATP turnover subsystem were calculated as the difference between consumers and proton leak rates at appropriate values of $\Delta\psi_m$. The kinetic profiles of the subsystems were represented in plots of subsystem rate versus $\Delta\psi_m$.

2.3.2. Response coefficients to Con A

Partial response coefficients describe how much an infinitesimal change in Con A changes the rate of a subsystem through direct effects on each of the blocks in the system. They were determined from the elasticities of subsystems to Con A and the appropriate control coefficients. The determination of group control coefficients (which are estimated from flux data and group elasticities of the subsystems to $\Delta\psi_m$) and group elasticities to external effectors is described by Kesseler and Brand [16,17].

Briefly, elasticities of subsystem fluxes to $\Delta\psi_m$ were quantified as the scaled slopes of the inhibitor titration curves near the resting condition, i.e. with no inhibitor present (see Section 2.3.1). We assumed linearity between the resting point and the first inhibitor point. Group flux control coefficients were calculated from these elasticities and the resting system fluxes [18].

Elasticities to Con A describe the change in flux through a particular subsystem upon small changes in Con A around a reference level when no relaxation to a new steady-state $\Delta\psi_m$ is allowed (see [17]). First, the subsystem flux was plotted against $\Delta\psi_m$ at each of six different Con A concentrations (including zero). For each Con A concentration, the rate of the subsystem at the same value of $\Delta\psi_m$ at the next higher and next lower concentration of Con A was interpolated. Then the three rates were plotted against Con A concentration, and the slope was calculated as the mean of the slopes on each side of the reference Con A concentration. The elasticity to Con A was determined as the normalised value of this slope. A total of four elasticities could be determined. For the substrate oxidation subsystem, the plots of flux against $\Delta\psi_m$ were constructed using linear regression to the data points at 0 and 5 ng/ml oligomycin only. For the phosphorylation subsystem, the plots of flux against $\Delta\psi_m$ were constructed using linear regression to the consumer and leak data points at 0 and 6.4 nM myxothiazol only. Elasticities of the proton leak subsystem to Con A were shown to be zero (see Section 3).

2.4. Integrated responses to a single large change in Con A

Ainscow and Brand [19] recently devised a method to quantify, under certain conditions, the effect of single large changes of external parameters within the framework of metabolic control analysis. For a single step change in Con A concentration from 0 to

0.8 μg per 10^7 cells (representing optimal stimulation of respiration), we calculated the integrated response of respiration rate a to Con A through each subsystem i using the same raw data as above and the following equation:

$$IR^{a}_{\Delta[\mathrm{ConA}]} = \sum_{\text{all } i} C^{a}_{i} \cdot IE^{i}_{\Delta[\mathrm{ConA}]} \quad (1)$$

where

$$IE^{i}_{\Delta[\mathrm{ConA}]} = \Delta J_i - \varepsilon^{i}_{\Delta\psi_m} \cdot \Delta\,(\Delta\psi_m)$$

(see [19]). The partial integrated response coefficients appearing in the right hand term of Eq. 1 describe the contribution of primary effects on each of the three subsystems to the overall change in respiration rate caused by the finite step change in Con A concentration.

2.5. Statistics

Titration data (in plots of oxygen consumption against $\Delta\psi_m$) are presented as means ± S.E.M. (for four independent determinations unless otherwise stated). Two-sided simultaneous confidence intervals with 90% confidence limit were calculated to assess differences in the proton leak curves for different Con A concentrations.

To assess the significance of coefficients, we used Monte Carlo analysis [20]; program code was written and executed in Matlab. The significance of calculated coefficients was expressed as the percentage of 2000 simulated values that had opposite sign to the value determined from the averaged data and are given as pseudo-P-values ($\sim p$).

2.6. Materials

Con A was from ICN Biomedicals, Aurora, OH, USA. [^{3}H]TPMP$^+$ was from NEN Life Science Products, Boston, MA, USA. Scintillant (Wallac Opti-Phase 'HiSafe' 2) was from Fisher Chemicals, Loughborough, UK. RPMI 1640 (with glutamine, without glucose) and all other chemicals were from Sigma–Aldrich, Poole, Dorset, UK.

3. Results and discussion

3.1. Effect of Con A on respiration and $\Delta\psi_m$

Fig. 1 shows the effects of Con A on oxygen consumption and mitochondrial membrane potential. Con A stimulated respiration by about 26%, with maximal effect at 0.8 μg Con A per 10^7 cells. At higher concentrations, respiration was stimulated less. Addition of Con A increased $\Delta\psi_m$ by about 4% at 0.5 μg Con A per 10^7 cells. At higher Con A concentrations, $\Delta\psi_m$ was lower than in quiescent cells.

3.2. Elasticity analysis: the effect of Con A on the kinetics of the subsystems

Elasticity analysis was carried out to determine which blocks of reactions were directly affected by addition of Con A, and hence were possible mediators of the effect of Con A on respiration rate and $\Delta\psi_m$. Fig. 2 shows the results. For clarity, only the lines representing 0.15, 1.0 and 2.0 μg Con A per 10^7 cells are shown; however, these lines show the general trends.

3.2.1. Effect of Con A on the substrate oxidation system

As Con A concentration was increased, the lines in Fig. 2a shifted left and down, indicating an inhibition of the substrate oxidation system (i.e. J_s decreased at any given $\Delta\psi_m$ when Con A was increased). We did observe a small but statistically insignificant stimulation of this block at lower Con A concentrations (not shown). Calcium-dependent stimulation of mitochondrial dehydrogenases is a conceivable consequence of Con A stimulation [21,8,22] and activation of reactions that form part of our substrate oxidation subsystem has been demonstrated [7]. However, the inhibition of the substrate oxidation reactions at optimal Con A concentrations was unexpected.

3.2.2. Effect of Con A on the $\Delta\psi_m$-consumers

Up to 0.5 μg Con A per 10^7 cells, the kinetics of

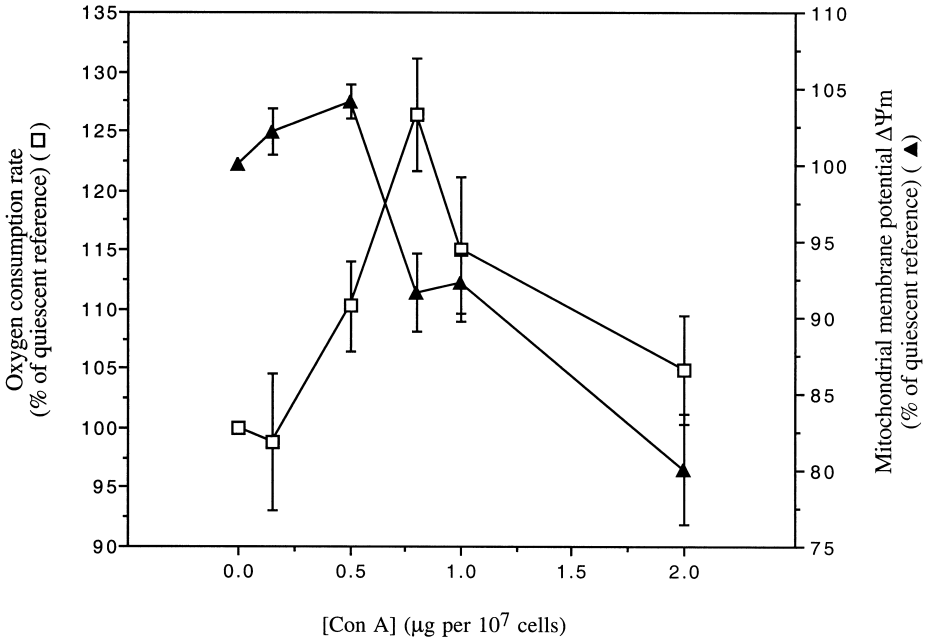

Fig. 1. Effects of Con A on oxygen consumption and mitochondrial membrane potential in thymocytes. Thymocytes were incubated with different concentrations of Con A and oxygen consumption (□) and mitochondrial membrane potential (▲) were measured after 20 min as described in Section 2. Values were normalized to the quiescent condition (with no inhibitors or Con A present) which was set as 100%. Data points represent means ± S.E.M. ($n = 8$). Note that the data points in Fig. 2 where inhibitors were absent represent a subset of the data shown in this figure. Basal respiration was 5.99 ± 0.15 nmol O_2/min per 5×10^7 cells, in agreement with previous results [4].

the $\Delta\psi_m$-consumers were largely unaffected (not shown). Above that concentration, respiration was higher for any value of $\Delta\psi_m$, showing that the $\Delta\psi_m$-consumers were directly stimulated by Con A addition (Fig. 2b).

3.2.3. Effect of Con A on the proton leak

The leak curves at different Con A concentrations (Fig. 2c) appeared to be similar. Statistical analysis of linear regressions of the curves supports the view that the leak subsystem did not change with Con A (at 90% confidence level). In particular, the leak curves did not significantly change their slope and regression value around $\Delta\psi_m = 110$ to 120 mV when compared with the quiescent condition. Note that linear regression does not necessarily represent the most appropriate fit to the leak data, but it is a sufficient approximation in the light of the more drastic changes induced by Con A in other subsystems.

3.2.4. Effect of Con A on the ATP turnover system

If the leak does not change significantly, the observed effect of Con A on the $\Delta\psi_m$-consumers must reflect a direct effect the kinetics of ATP turnover. This is shown in Fig. 2d: addition of Con A shifted the lines left and up, showing that the ATP turnover reactions were directly stimulated and went faster at any given value of $\Delta\psi_m$. The increase in activity of some individual ATP consumers has been established previously (e.g. [5,23]) and was not unexpected.

We were aware of the possibility that the three blocks might communicate through intermediates other than $\Delta\psi_m$. If such crosstalk was large it could invalidate the analysis described here, although it could be dealt with either by measuring the new intermediate and analysing the resulting two-intermediate system, or by using slightly different experimental protocols [24,25]. Crosstalk could arise if ATP produced in substrate oxidation affected the ATP turnover reactions or if ATP generated in oxidative phosphorylation affected the substrate oxidation reactions. This was tested for both blocks (Fig. 3) by assessing whether the kinetic response to $\Delta\psi_m$ changed in the presence of small amounts of FCCP, which will change the relationship between

ATP and $\Delta\psi_m$. There was no change in the kinetics of either the substrate oxidation or the phosphorylation system around the resting points within the error of our analysis, showing that the problem of crossover was negligible in our glucose-free system. Preliminary experiments in the presence of glucose (not shown) indicated that the equivalent curves diverged greatly near the resting point, presumably because of greater glycolytic ATP production, so glucose was omitted in the experiments reported here. No glycogen was detectable in these thymocytes (compared to 3.9 μmol glucose per 10^7 cells for hepatocytes in parallel control experiments, $n=2$), suggesting that glycolysis in the absence of added glucose was low.

The elasticity analysis describes qualitatively how the kinetics of the system change as it progresses from quiescent to maximum stimulation and beyond: the proton leak does not change its kinetic response to $\Delta\psi_m$ but the phosphorylation system is stimulated as Con A is increased. For the substrate oxidation system, the gross effect with increasing Con A concentration is inhibition. Despite this, the substrate oxidation system has a higher steady-state rate after Con A administration. Because oxygen consumption is increased for all Con A concentrations, stimulation of the ATP turnover system must overcome the inhibitory effect on the substrate oxidation subsystem and the overall system relaxes to increased steady-state rates.

The changes in mitochondrial membrane potential and respiration shown in Fig. 1 are obviously linked and can be viewed in the light of the elasticity analysis. $\Delta\psi_m$ initially rises as the substrate oxidation system is (mildly) stimulated. However, with further increases in Con A towards the optimum concentration, the substrate oxidation subsystem becomes increasingly inhibited, and ATP turnover is stimulated. This results in a decrease in $\Delta\psi_m$ whilst respiration remains stimulated well above the quiescent level. Finally, the stimulatory effect of Con A on ATP turnover becomes relatively weaker, resulting in respiration rates which are below maximum, but still above the quiescent level.

3.3. Control analysis: group flux control coefficients

The elasticities of each block to $\Delta\psi_m$ were calculated from the full dataset summarised in Fig. 2 as described in Section 2. Group control coefficients over the rate of each subsystem at each concentration of Con A were calculated from the fluxes and elasticities [16,18]. Control over substrate oxidation rate was mainly shared between the substrate oxidation subsystem (0.3–0.6) and the ATP turnover subsystem (0.2–0.5); less control was exerted by the proton leak (0.1–0.3). ATP turnover rate was controlled by the substrate oxidation subsystem (0.3–1.2), by the ATP turnover subsystem (0.3–0.8) and, negatively, by proton leak (−0.1 to −0.5). Proton leak rate was controlled mostly by the activity of the proton leak subsystem (0.9–1.0); there were small contributions by the substrate oxidation (0.1–0.3) and ATP turnover subsystems (−0.15 to −0.05).

Although the control coefficients changed with Con A concentration, the general pattern found in quiescent cells was largely conserved: control was shared, and if a block exerted significant control over a variable, this was true at different Con A concentrations. For optimal Con A levels, our control coefficients agree with values published for Con A stimulated thymocytes [12] or indeed for other systems, such as hepatocytes or perfused muscle [26,9].

3.4. Regulation analysis: partial response coefficients to Con A through each of the subsystems

Partial response coefficients were calculated from the elasticities of the subsystems to Con A and the appropriate control coefficients as described in Section 2. Fig. 4 shows partial and overall (or group) response coefficients of substrate oxidation rate and ATP turnover rate to Con A acting through the substrate oxidation and ATP turnover subsystems for small finite changes around four different Con A concentrations. The proton leak kinetics were not affected by Con A (Fig. 2c), so there were no partial responses to Con A through this pathway.

The rates of substrate oxidation (Fig. 4e) and ATP turnover (Fig. 4f) responded positively to small increases in Con A at lower concentrations, but negatively at the highest concentration. These overall responses to Con A were made up of partial responses through the two subsystems that were sensi-

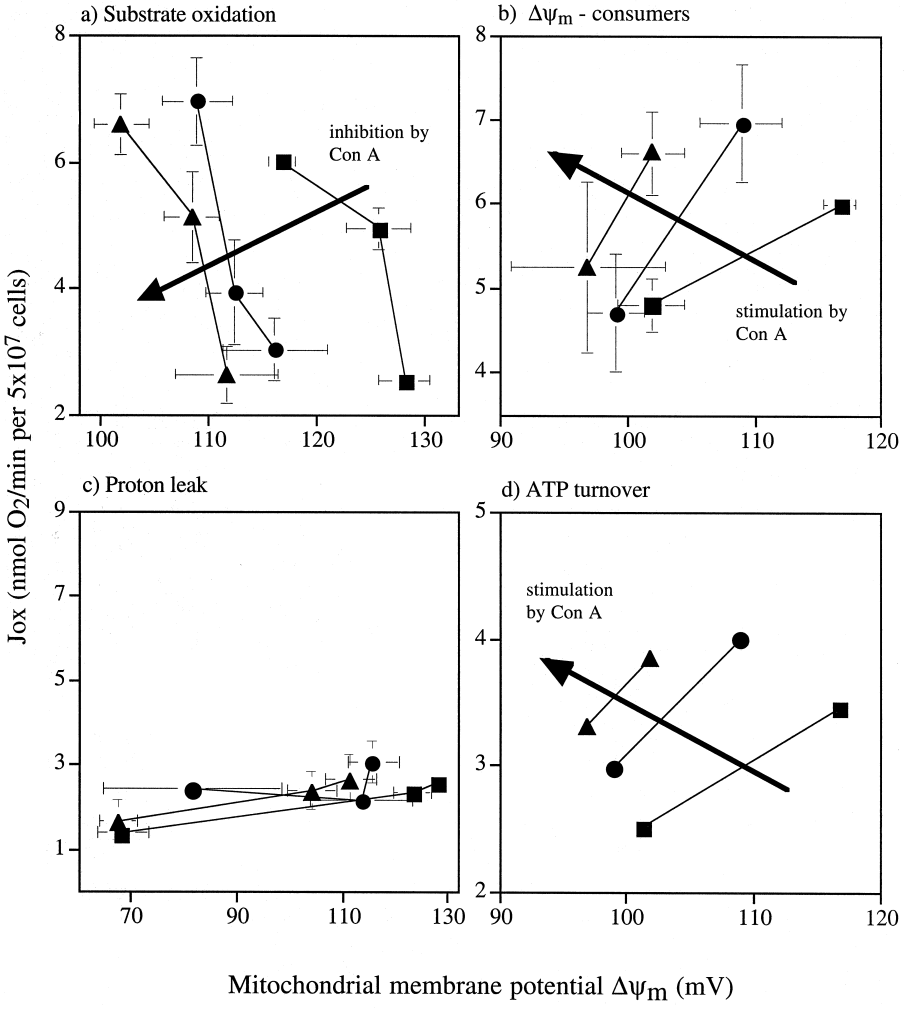

Fig. 2. Kinetic responses of subsystems to $\Delta\psi_m$. Thymocytes were incubated in the presence of different inhibitors (see Section 2). The relationship between subsystem flux and $\Delta\psi_m$ is shown for (a) the substrate oxidation subsystem, (b) the $\Delta\psi_m$-consumers, (c) the proton leak subsystem and (d) the ATP turnover subsystem. Points are averages ± S.E.M. for four cell preparations. For clarity, results for only three Con A concentrations are shown: ■, quiescent cells, ●, 1.0 μg Con A per 10^7 cells, ▲, 2.0 μg Con A per 10^7 cells. The kinetics of the ATP turnover subsystem in d were calculated by subtracting the interpolated rates in c from those in b at the appropriate membrane potentials, so no errors are shown.

tive to the mitogen, thus primary responses (Fig. 4a,b) and secondary responses via the other subsystem (Fig. 4c,d) combine to give the overall response coefficients (Fig. 4e,f). Overall response coefficient profiles were the same whether we calculated them directly from flux data (not shown) or from the partial response coefficients.

Because the proton leak rate changed only a small amount as Con A was increased, the partial responses through substrate oxidation were similar to each other (Fig. 4a,d), as were those through ATP turnover (Fig. 4b,c). For the same reason, the overall responses of respiration and phosphorylation rate to Con A were similar (Fig. 4e,f).

The set of overall response coefficients for each flux illustrates how the system progresses from zero to optimal and above-optimal stimulation in terms of partial subsystem responses to small changes in Con A concentration. The coefficients indicate the *differential* dose–response behaviour, thus the negative overall response coefficient at high Con A in Fig. 4e describes how respiration rate decreases as Con A is increased around 1 μg per 10^7 cells even though there is an overall stimulation of respiration rate at

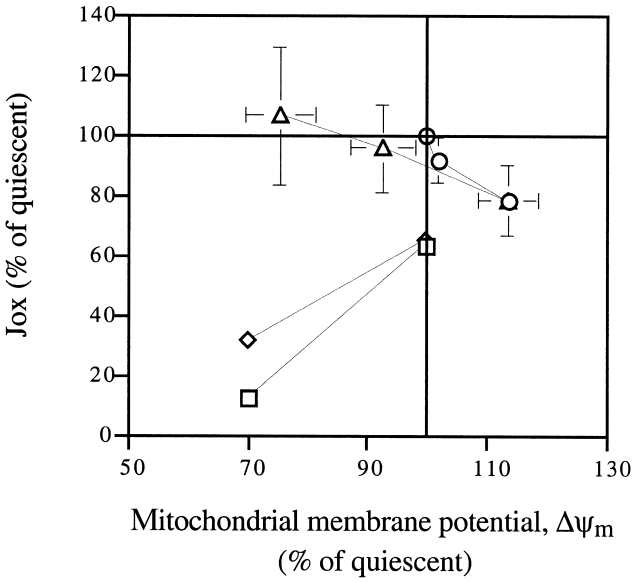

Fig. 3. Experimental assessment of crosstalk between the substrate oxidation and ATP turnover subsystems. Thymocytes were incubated as in Fig. 1 in the presence of 1 μg Con A per 10^7 cells and different inhibitors. ○, Kinetic response of the substrate oxidation subsystem to $\Delta\psi_m$ determined by titration with 0, 3 and 6 ng/ml oligomycin. △, Kinetic response of the substrate oxidation subsystem to $\Delta\psi_m$ determined by titration with 0, 0.3 and 0.8 μM FCCP in the presence of 6 ng/ml oligomycin. □, Kinetic response of the ATP turnover subsystem to $\Delta\psi_m$ determined as in Fig. 2. ◇, Kinetic response of the ATP turnover subsystem to $\Delta\psi_m$ determined as in Fig. 2 in the presence of 0.15 μM FCCP. Consumers were titrated with 0, 6.4 and 12.4 nM myxothiazol and proton leak was titrated with 0, 10 and 20 nM myxothiazol in the presence of 80 ng/ml oligomycin ($n=3$ for all titrations). Regression lines were fitted to the curves and J_p at 70 and 100% $\Delta\psi_m$ was calculated. S.E.M. of the original data was similar to the other titrations shown.

this Con A concentration relative to zero-Con A control cells.

The results show how respiration rate is stimulated by Con A. At 0.5 μg per 10^7 cells there appears to be some primary stimulation through activation of the substrate oxidation subsystem (Fig. 4a), and some secondary stimulation via changes in $\Delta\psi_m$ through the activation of the ATP turnover subsystem (Fig. 4c). These combine to give a modest increase in respiration (Fig. 4e and 1). At 0.8 μg per 10^7 cells there is strong secondary activation through effects on ATP turnover (Fig. 4c), and although this is opposed by negative primary effects through inhibition of the substrate oxidation block (Fig. 4a), the overall response is strongly positive (Fig. 4e). At 1 μg per 10^7 cells the secondary activation through ATP turnover is negative (Fig. 4c) because the stimulation of this block is less as Con A is increased. This effect dominates the overall negative response of respira-

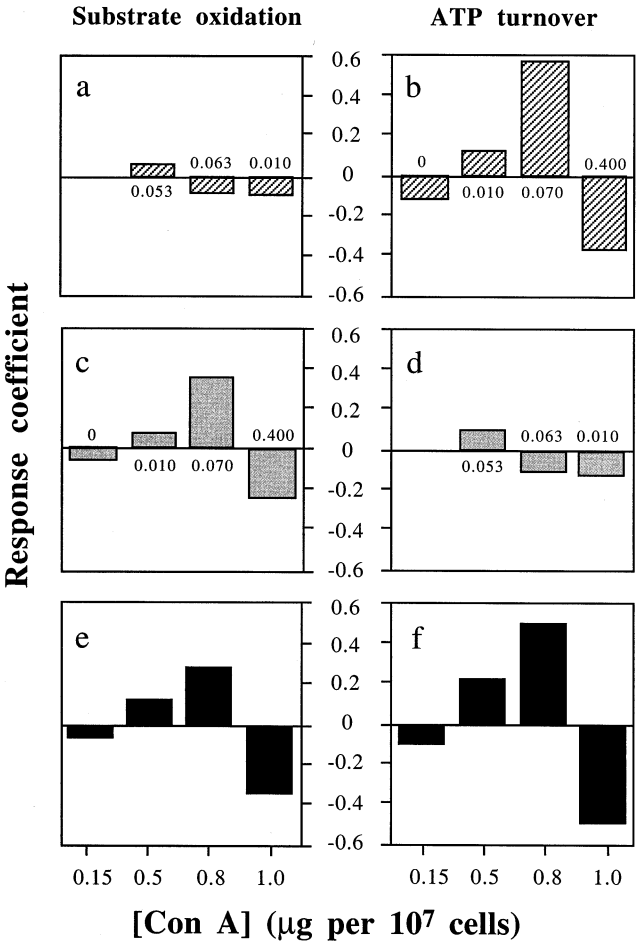

Fig. 4. Partial and group response coefficients of substrate oxidation and ATP turnover rate to Con A. Response coefficients to Con A for small finite changes around the four Con A concentrations shown were calculated from control coefficients and elasticities to Con A as described in Section 2. Left-hand panels show partial response coefficients of substrate oxidation rate to Con A through (a) the substrate oxidation subsystem and (c) the ATP turnover subsystem, and (e) their sum, the overall (group) response coefficients of substrate oxidation rate to Con A. Right-hand panels show partial response coefficients of ATP turnover rate to Con A through (b) the ATP turnover subsystem and (d) the substrate oxidation subsystem, and (f) their sum, the overall (group) response coefficients of ATP turnover rate to Con A. a and b show responses caused by primary effects and c and d show responses caused by secondary effects transmitted via $\Delta\psi_m$ from primary effects on the other block. The figures above or below each bar indicate pseudo-P values ($\sim p$) (see Section 2.5).

tion to increases in Con A concentration in this region (Fig. 4e). Similar analysis could be made of the response of ATP turnover rate to Con A (Fig. 4b,d,f).

3.5. Integrated responses to Con A for a single large change in Con A

When a single large change in Con A from 0 to 0.8 μg per 10^7 cells is considered, the integrated partial responses via each of the subsystems were as follows. The integrated response of substrate oxidation rate to Con A was 0.13, composed of partial integrated responses of −66% via the substrate oxidation system and 166% via ATP turnover, with negligible contribution by the leak subsystem. The integrated response coefficient of phosphorylation was 0.33, breaking down into partial integrated responses of −35% via the substrate oxidation subsystem, 135% via the phosphorylation subsystem and again a negligible (negative) contribution via proton leak.

The partial integrated responses describe the overall effect of a large single-step change in Con A concentration on a particular rate through the effects on each of the subsystems. They indicate a trend: the stimulatory effects of a finite large addition of Con A are due to a direct effect of Con A on ATP turnover, which overcome inhibitory effects on the substrate oxidation system.

4. Conclusions

Biochemical networks are greatly interconnected, and any primary action of an effector on one step will be communicated through the network. Steps that are initially unaffected will adapt to the activity of others by secondary responses to changes in intermediates until a new steady state is reached [2]. This underlines the importance of a systemic approach (as provided by metabolic control analysis) in any investigation of effector-mediated changes in activity of a biochemical system.

Our study shows how the change in a rate induced by Con A can be explained as a consequence of independent activation and/or inhibition of particular groups of reactions which, as whole blocks, are primary targets of the mitogen. Here, top-down elasticity analysis shows that Con A strongly stimulates the ATP turnover reactions. At low concentrations it may mildly stimulate the substrate oxidation reactions; at higher concentration it inhibits them. It has no direct effect on the proton leak reactions. Top-down regulation analysis describes how thymocyte respiration is regulated by Con A by showing how important the different primary effects are in causing the overall stimulatory effects. Under our conditions, the stimulation of respiration and ATP turnover by Con A are mostly caused by the primary effects of Con A on the reactions of ATP turnover.

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