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Proportional activation coefficients during stimulation of oxidative phosphorylation by lactate and pyruvate or by vasopressin

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Abstract

A 'proportional activation' approach designed to deal with the influence of external effectors within biochemical systems is described. The proportional activation coefficient, which enables the quantitative determination of the relative stimulation of different parts of a system by a given effector, is defined. The proportional activation approach was used to calculate the relative activation of Δp-producing and Δp-consuming subsystems during stimulation of the respiration rate of cells by a variety of different effectors. Oxidative phosphorylation was stimulated by the addition of either lactate and pyruvate (10 mM and 1 mM) or vasopressin. The addition of lactate and pyruvate to suspensions of resting hepatocytes increased the respiration rate by about 50%. The Δp-consuming subsystem was stimulated about 60% as much as the Δp-producing subsystem. Quinolinic acid, commonly considered to be a specific inhibitor of gluconeogenesis, was found to block the Δp-producing oxidative subsystem as well as the Δp-consuming subsystem, indicating some nonspecific effects of this inhibitor. Addition of vasopressin to hepatocytes that were incubated in the presence of lactate and pyruvate resulted in an increase of the respiratory rate by up to 35%. The relative stimulation of the Δp-producing and Δp-consuming subsystems was essentially equal. Using the 'proportional activation approach' to analyse these and previously published data, it is shown that substrates (lactate/pyruvate and fatty acids), Ca^{2+}-acting hormones (vasopressin and others) and calcium in muscles (heart muscle and skeletal muscle) activate both subsystems to a similar extent (it concerns especially Ca^{2+}-dependent systems).

Keywords: Oxidative phosphorylation; Proportional activation; Calcium-acting hormone; Gluconeogenesis; Lactate; Pyruvate

1. Introduction

The oxidative phosphorylation system can be divided into two subsystems: a Δp-producing subsystem containing oxidation subsystem (substrate transport, substrate dehydrogenation, respiratory chain), and a Δp-consuming subsystem containing a phosphorylation subsystem (ATP synthase, ATP/ADP carrier, phosphate carrier, ATP usage) and proton leak. Two opposing opinions concerning activation of oxidative phosphorylation by external effectors (hormones and neural stimulation of muscle) exist in the literature. According to the first opinion, one of the aforementioned subsystems is stimulated directly, the other being stimulated by an increase (decrease) in Δp (and other relevant parameters, as ATP/ADP or NADH/NAD). The second opinion is that both subsystems are activated.

It has, for example, been proposed [1–3] that calcium-stimulated ATP utilisation during muscular contraction leads to increases in ADP concentration and decreases in the phosphorylation potential. This, in turn, activates the oxidation subsystem to the extent necessary to maintain sufficient ATP concentration. Similarly, it was proposed that Ca^{2+}-acting hormones (such as vasopressin, adrenaline and glucagon) exclusively activated the substrate dehydrogenation subsystem (i.e., that part of the oxidation system which includes glycolysis, Krebs cycle and fatty acid β-oxidation) resulting in an increased ATP/ADP ratio and the stimulation of cellular processes (such as gluconeogenesis) that are sensitive to this ratio [4]. On the other hand, some other authors discuss a parallel activation of different subsystems [5–10]. However, such models are

Abbreviations: Δp, protonmotive force; $\Delta\Psi_m$, mitochondrial membrane potential; $TPMP^+$, triphenylmethylphosphonium; OX, oxidation subsystem; PH, phosphorylation subsystem; LK, proton leak subsystem; PH + LK, phosphorylation + proton leak subsystem; coefficients; ϵ, elasticity coefficient; κ, special elasticity coefficient (to external effector); P, proportional activation coefficient.

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only intuitive and qualitative. This is clearly seen when we compare the 'latent enzymes' [11] and the 'multi-site activation' [12,13] ideas. Starting from the same fact of small changes in the ATP/ADP ratio during muscle contraction, the first hypothesis says that the PH(+LK) subsystem is activated exclusively and the sensitivity of the OX subsystem to ATP/ADP is great and the second one says that both the OX and PH + LK subsystems are activated to a similar extent. 'Small changes' says nothing until they are compared with kinetic responses to intermediate metabolite concentration of the OX and PH + LK subsystems. Opinions in the recent literature still exist supporting both the opposing ideas [2–4,11] vs. [8,12,13]. In some studies it was shown that both subsystems were activated, but the relative extent of this stimulation has not been estimated. Therefore, a more quantitative, even crude method seems to be advantageous.

Lactate and pyruvate (10 mM and 1 mM respectively) are commonly added to incubation media for hepatocytes. This leads to an increase in the respiration rate. It is thought that lactate + pyruvate activate gluconeogenesis [18]. It would be interesting to check whether these substrates stimulate also Δp-producing processes.

Quinolinic acid, through its effect on phospho*enol*pyruvate carboxykinase, is thought to be a specific inhibitor of gluconeogenesis [20]. However, the specificity of its inhibitory action has not been rigorously assessed. Therefore, it would be important to determine whether there are nonspecific effects of this inhibitor on the oxidative phosphorylation system (i.e., beyond the expected inhibition of the phosphorylation + proton leak subsystem).

The proportional activation of the Δp-producing and Δp-consuming subsystems by Ca^{2+}-acting hormones, like vasopressin, glucagon and adrenaline is also interesting. Ca^{2+}-acting hormones have been shown to stimulate the respiration rate, increase the mitochondrial ATP/ADP [21] and NADH/NAD^+ [22] ratios and increase the cytochrome *c* reduction level [22]. The simplest interpretation of these findings is that the oxidative subsystem is activated [4,16]. This opinion is supported in Ref. [19]. On the other hand, quantitative calculations using a dynamic mathematical model of oxidative phosphorylation [15] suggest that both subsystems are activated to a very similar extent. Therefore, the problem requires additional studies.

The purpose of the work described here was to use the proposed method, the proportional activation approach, to identify the important sites of action (and to determine the relative extent of the effects at these sites) of lactate plus pyruvate, of the gluconeogenic inhibitor, quinolinate, and of vasopressin on oxidative phosphorylation.

2. Proportional activation approach

Let us consider the simple biochemical system presented in Fig. 1a. The concentration of its substrate *S* and product *P* is kept constant. The system is divided into two subsystems: subsystem *A*, producing an intermediate metabolite, *M*, and subsystem *B*, consuming this metabolite. Let us assume that these subsystems affect each other only through changes in the concentration of *M*. The system is activated by an external effector, *X*, leading to a net increase of flux, *J*, through the system. At the beginning the system is treated as a 'black box' (Fig. 1a), as the mechanism of the activation is unknown.

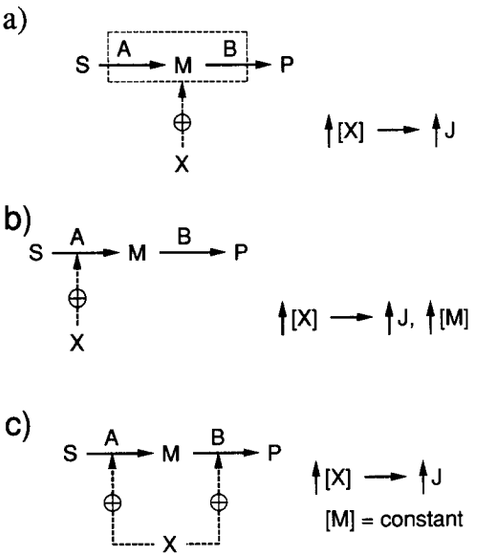

Fig. 1. (a) The system. S, substrate, P, product, M, intermediate metabolite, A, M-producing subsystem, B, M-consuming subsystem, J, flux through the system, X, external effector. X affects the system as a 'black box', causing a net increase in the flux J through the system. (b) X activates subsystem A directly and exclusively. Subsystem B is activated by the increase in M concentration. (c) X activates subsystems A and B to a similar extent. [M] remains approximately the same.

There are two possible extreme cases. The first is presented in Fig. 1b. In this case the external effector stimulates subsystem *A* exclusively and directly; the increase in flux through *B* is solely a consequence of an increase in the concentration of the common metabolite *M*. Similarly, we could consider the case where the external effector stimulates *B*, and the increase in flux through *A* is due to a decrease in *M*.

In the second case, the external effector *X* stimulates both subsystems *A* and *B* to a similar extent, such that *M* remains approximately constant (Fig. 1c).

The relevant question for a given effector in the cell is whether this effector acts on only one side of M, or there is equal activation on each side of M, or whether a case in between these two extremes occurs.

Titration of both subsystems with specific inhibitors and simultaneous measurements of changes in flux *J* intensity and intermediate metabolite *M* concentration enables the determination of elasticity coefficients of subsystems *A* and *B* to the common metabolite *M* at a steady-state point under physiological conditions. If the titration curves are

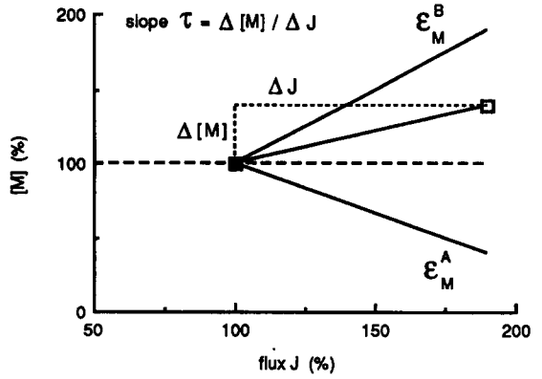

Fig. 2. Changes in flux J intensity and metabolite M concentration after external effector X addition, representing transition from the old steady state (full square) to a new one (open square). The lines representing the kinetic responses (in the 'linear' case corresponding to elasticity coefficients) of subsystems A and B to metabolite M are also shown. This set of data is the basis for calculation of the P coefficient.

linear (or nearly linear), the elasticity coefficient values can be extrapolated for a broader range of conditions, and finite changes (Δ) instead of infinitesimal ones (d) can be considered. Such near-linear titration curves were obtained, when the 'top-down' approach to Metabolic Control Analysis [25] was used with intact hepatocytes, for Δp-producing reactions (OX subsystem) and Δp-consuming reactions (PH + LK subsystem) [17,19,23,24]. In a simplified oxidative phosphorylation system, A is the OX subsystem following the scheme shown in Fig. 1a, B is the PH + LK subsystem, M is Δp (or $\Delta\Psi_m$), J is the respiration rate and X may be Ca^{2+}, a hormone, a substrate or some other external effector.

After the introduction of an external effector to the system, a new steady state will be achieved with a changed flux J and a changed common metabolite M concentration (Fig. 2). What we are interested in is: activation of B divided by activation of A. A parameter which we will refer to as the proportional activation coefficient, which describes this ratio, is mathematically defined as follows:

$$P_X^{AB} = \left(\frac{dB/B}{dA/A}\right)_X \quad (1)$$

where A and B are activities of subsystems A and B. In other words, the proportional activation coefficient is the infinitesimal relative change in the subsystem B activity caused by external effector X divided by such a change in the subsystem A activity.

It is necessary to relate this coefficient to the elasticity coefficients of subsystems A and B to common metabolite M and to changes in this metabolite concentration and flux J intensity after external effector X addition (see Fig. 2). The slope of the line representing the effector action τ can be defined as follows:

$$\tau = \frac{dM/M}{dJ/J} \quad (2)$$

where M is metabolite M concentration and J is flux J intensity. The relative change in flux J intensity, caused by relative changes in activity of subsystem B is equal to:

$$(dJ/J)_B = \epsilon_M^B \cdot (dM/M) + \kappa_X^B \cdot (dX/X) \quad (3)$$

where ϵ indicates elasticity coefficient to internal metabolite and κ indicates special elasticity coefficient to external effector, respectively. An analogous equation can be written for subsystem A. Dividing one equation by the other we obtain, taking into account that, for a given concentration of X, $\kappa_X^B/\kappa_X^A = (dB/B)/(dA/A)$, the following expression:

$$P_X^{AB} = \frac{1 - \epsilon_M^B \cdot \tau}{1 - \epsilon_M^A \cdot \tau} \quad (4)$$

Eq. (4) exhibits some interesting properties:

1. If there is no change in intermediate metabolite M concentration after external effector addition ($\tau = 0$), then the proportional activation coefficient is equal to unity ($P = 1$). This case occurs when both subsystems A and B are activated to exactly the same extent.

2. If $\tau = 1/\epsilon_M^B$, then only subsystem A is activated and $P = 0$. Alternatively, if $\tau = 1/\epsilon_M^A$, then only B is activated and $P =$ infinity (the last property is maybe not very convenient but it is easy to interpret). Therefore, ϵ_M^A and ϵ_M^B fix the range of possible τ values (see below for a discussion of the situation where neither subsystem is inhibited by a given external effector).

3. The proportional activation approach applies to inhibitors of biochemical pathways as well. For example, it allows the estimation of the relative inhibition of Δp-producing and Δp-consuming reactions by a given inhibitor.

4. If the value of the P coefficient is negative, it means that one of the subsystems is activated and the other is inhibited by a given external effector.

5. If the absolute value of the elasticity coefficient of one subsystem is significantly greater than the absolute value of the elasticity coefficient of the other subsystem, then the former coefficient will be mainly responsible for the deviation of the P coefficient from unity.

The proportional activation approach is valid for the oxidative phosphorylation system only under some assumptions:

1. Δp is the only 'connection' between the Δp-producing and Δp-consuming subsystem. This has been shown for hepatocytes [19]. However for, for example, muscle it can be only an approximation. However, such an approximation is supported by the fact that Krebs cycle in muscle is only weakly dependent on the ATP/ADP ratio, being predominantly regulated by the NADH/NAD^+ ratio [26].

2. An external effector does not affect significantly the elasticities of the both considered subsystems to Δp. However, this assumption is not important when we consider infinitesimal changes in the flux intensity and M concentration.

3. The kinetic responses of the OX and PH + LK subsystems to Δp are near linear. This was shown for hepatocytes in a broad range of the respiration rate and Δp [17,19], but for other conditions and cells it can be only an approximation.

4. The kinetic responses of the both considered subsystems to Δp do not change significantly from experiment to experiment. As different experiments are performed under slightly different conditions, it can be true only approximately.

Because of these limitations, the proportional activation approach is surely crude. However, even a semiquantitative method seems to be an advance in relation to purely intuitive considerations.

3. Materials and methods

The experimental procedures used, including the preparation and incubation of rat hepatocytes and measurements of respiration rate and $\Delta\Psi_m$ were as described by Harper and Brand [17]. Hepatocytes were prepared from fed male Wistar rats (240–400 g). The viability of cells as assessed by the exclusion of Trypan blue (0.3 w/v) was greater than 96%. Cellular respiration rates were measured at 37° C in duplicate, using two Clark-type oxygen electrodes. Non-mitochondrial oxygen consumption was identified as the oxygen consumption that was insensitive to 1 μM myxothiazol and was subtracted from respiration rates. $\Delta\Psi_m$ was calculated from the distribution of TPMP with corrections for plasma membrane potential, cytoplasmic and mitochondrial binding of TPMP and other factors, as described in [17].

The elasticity coefficients to $\Delta\Psi_m$ for hepatocytes incubated without lactate and pyruvate are about $\epsilon^{OX}_{\Delta\Psi_m} = -7$ and $\epsilon^{PH+LK}_{\Delta\Psi_m} = 3$ (assessed in [19]); those determined in the presence of lactate/pyruvate are about $\epsilon^{OX}_{\Delta\Psi_m} = -11$ and $\epsilon^{PH+LK}_{\Delta\Psi_m} = 7$ (assessed in [17]).

4. Results and discussion

4.1. Average absolute values of measured parameters

Because absolute values of such parameters as the respiration rate, mitochondrial membrane potential ($\Delta\Psi_m$) and plasma membrane potential ($\Delta\Psi_c$) differed somewhat between different preparations, in the following discussion we deal with relative values and changes. Moreover, for the 'proportional activation' approach only relative changes in parameter values are needed. However, the absolute values which were used as the 100% values (i.e., 0% change) which are shown in Figs. 3, 4 and 6 are given below.

The average respiration rate of hepatocytes incubated in the presence of lactate and pyruvate was 6.18 ± 0.15 (S.E.M., $n = 18$) nmol O_2/min per mg dry weight. The average plasma membrane potential (assessed using distribution of ^{36}Cl) was 32.3 ± 13.5 (S.E.M., $n = 5$) mV and the average $\Delta\Psi_m$ was 173.4 ± 14.1 (S.E.M., $n = 5$) mV. The values for the respiration rate and mitochondrial membrane potential were slightly greater than those obtained in [17]; perhaps this is due to a slightly higher cell viability in the present study than in the earlier study. The average respiration rate for hepatocytes that were incubated in the absence of lactate and pyruvate, and isolated from younger rats (up to 300 g, see below), was 4.42 ± 0.20 (S.E.M., $n = 6$) nmol O_2/min per mg dry weight. These values were used as the 100% values in all of the following results. There were no changes in plasma membrane potential under any of the incubation conditions used.

4.2. Effect of lactate and pyruvate

The addition of 10 mM lactate and 1 mM pyruvate to the incubation medium resulted in an increase of the respiration rate by about 50% in hepatocytes isolated from younger rats (up to 300 g). The stimulation of respiration in hepatocytes from older rats (more than 300 g) was about 10–25%. The difference could be due to differences in hepatic gluconeogenic capacities between younger and older rats. Because the changes in respiration rate were higher and more repeatable in hepatocytes from younger rats compared with older rats all subsequent experiments involved cells from younger rats. The relative changes in $\Delta\Psi_m$ plotted against the relative changes in the respiration rate following the addition of lactate and pyruvate are presented in Fig. 3. It can be seen that changes in mitochondrial membrane potential are small. The calculated

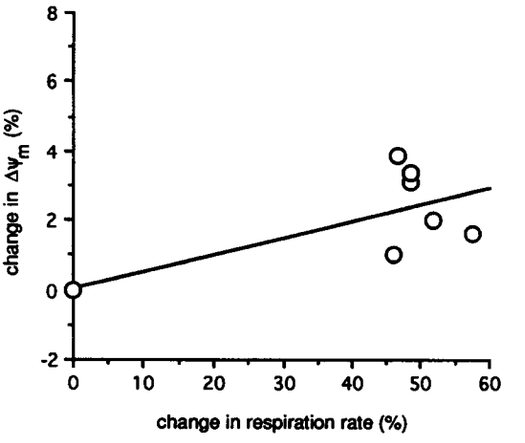

Fig. 3. Effect of lactate and pyruvate on oxidative phosphorylation. Changes in mitochondrial membrane potential ($\Delta\Psi_m$) are compared with changes in the respiration rate after the addition of lactate/pyruvate. Points are from six measurements. Each $\Delta\Psi_m$ determination was carried out in triplicate and each respiration rate determination was carried out simultaneously in duplicate. The 100% values (i.e., 0% change) were determined for hepatocytes incubated in the absence of lactate and pyruvate.

value of proportional activation coefficient (0.64) indicates that the addition of lactate and pyruvate activates the phosphorylation + proton leak subsystem by about 2/3 of the extent to which it stimulates the oxidation subsystem.

Which parts of these subsystems are likely to be stimulated? Among the Δp-consuming reactions, gluconeogenesis is the main candidate, since pyruvate is a substrate for the pathway. Mercaptopicolinic acid and quinolinic acid, considered as inhibitors of gluconeogenesis, removed almost all the stimulation of respiration rate by lactate and pyruvate. This supports the idea that lactate and pyruvate directly stimulate gluconeogenesis. However, as described below, quinolinic acid does not appear to be a specific inhibitor of gluconeogenesis. The stimulation of Δp production is likely to be via the provision of NADH.

4.3. Effect of fatty acids

The 'proportional activation' approach can be also used to analyse previously published results. Nobes et al. [27] showed that the addition of fatty acids to hepatocytes (incubated without lactate and pyruvate) increased the respiration rate by 92%, while $\Delta\Psi_m$ changed by 7 mV (from 155 to 162 mV). The calculated value of the proportional activation coefficient is $P = 0.63$. As for the stimulation resulting from the addition of lactate and pyruvate, the stimulation of the Δp-consuming subsystem was about 2/3 of that of the Δp-producing subsystem. The activation of the phosphorylation subsystem could reflect increased lipogenesis or triacylglycerol formation or cycles in fat metabolism among other effects. It has been shown [27] that there is no uncoupling of oxidative phosphorylation by fatty acids, so the proton leak is not affected. The stimulation of the oxidation subsystem could be due to increased reducing equivalent supply.

4.4. Effect of quinolinic acid

Quinolinic acid is known as an inhibitor of gluconeogenesis. Our results show that it eliminates the activation by lactate/pyruvate of respiration rate at a concentration of 2 mM. However, at higher concentrations it blocked respiration even more than could be expected from inhibiting gluconeogenesis exclusively. To determine whether quinolinic acid inhibited the oxidation subsystem (including substrate transport, substrate dehydrogenation and respiratory chain) as well as the phosphorylation + proton leak subsystem, we measured changes in the respiratory rate and $\Delta\Psi_m$ after the addition of a moderate concentration of quinolinic acid (2 mM) to hepatocytes incubated with lactate and pyruvate. The results obtained are shown in Fig. 4. The inhibitor caused a decrease in the respiration rate by 15–30%. $\Delta\Psi_m$ also decreased, clearly indicating that the Δp-producing reactions were inhibited even more than the Δp-consuming reactions. The value of the P coefficient (0.51) indicates that the inhibition of the oxidation subsystem was approximately twice that of the phosphorylation + proton leak subsystem. Therefore, quinolinic acid is not a specific inhibitor of gluconeogenesis. Nevertheless, it caused quite significant inhibition of the Δp-consuming subsystem, suggesting a decrease in gluconeogenic flux. Thus the 'proportional activation' approach is a simple and useful method for testing inhibitor specificity in complex systems.

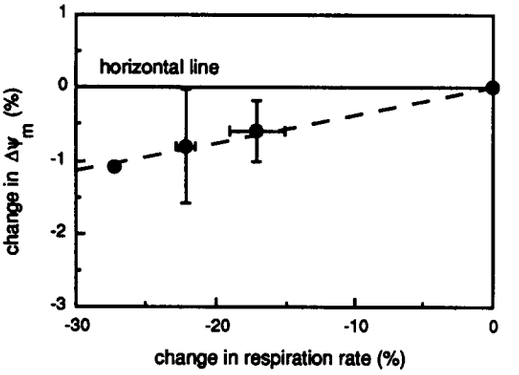

Fig. 4. Changes in mitochondrial membrane potential ($\Delta\Psi_m$) compared to changes in respiration rate after the addition of quinolinic acid. The points represent mean values of four measurements from two cell preparations and a single measurement from third cell preparation. The 100% values (i.e., 0% change) were determined for hepatocytes incubated in the presence of lactate and pyruvate.

4.5. Effect of vasopressin

Both the preincubation of hepatocytes with vasopressin (25 nM) in the presence of lactate and pyruvate and the addition of vasopressin to the oxygen electrode chamber during measurements resulted in increases in the respiratory rate by 10–35%. This activation exhibited a plateau for about 20 min and then respiration returned slowly to its initial rate (Fig. 5). The decreased stimulation over time

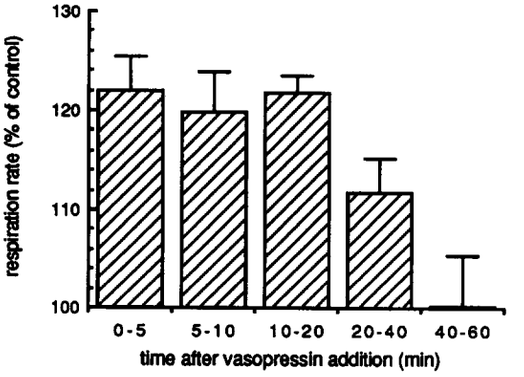

Fig. 5. Time-course of the activation of respiration rate after the addition of vasopressin. Data are from 13 independent preparations. The error bars represent standard errors of the means. 100% of the respiratory rate is relative to values from hepatocytes incubated in the presence of lactate/pyruvate and in the absence of vasopressin.

may be explained by down-regulation of receptors [28]. There were no changes over the same length of time in the respiration rate of control hepatocytes. The time necessary for TPMP equilibration across the mitochondrial inner membrane and other membranes was equal to 10–15 min in presence of vasopressin. Therefore all measurements were performed 15 min or later after the addition of vasopressin (about 2/3 of them were made after 15 min and the remainder were made after 20–60 min). Plasma membrane potential and cellular volume did not change after the addition of vasopressin. Mitochondrial volume was assumed to be constant. Some changes in mitochondrial volume after vasopressin addition have been measured previously [29], using a different incubation medium. However, we found that TPMP distribution was extremely stable after vasopressin addition and it is highly improbable that $\Delta\Psi_m$ decreased exactly to the extent necessary to compensate for changes in mitochondrial volume to keep the TPMP gradient constant. If the changes in volume reported in [29] are correct then we would obtain results which supported the conclusion that the phosphorylation subsystem was activated by vasopressin significantly more than the oxidation subsystem (see below). This would contradict the results of several studies of the effect of Ca^{2+}-acting hormones [19,21,22]; all of these studies show a greater activation of the oxidation subsystem than the phosphorylation + proton leak subsystem.

Fig. 6 presents the effects of the addition of vasopressin on mitochondrial membrane potential and on respiration rate. No changes in $\Delta\Psi_m$ were observed after the addition of vasopressin, while respiration rate increased by up to 35%. This indicates that the Δp-producing and Δp-consuming reactions were activated to similar extents. This result is in agreement with the earlier finding [30] that glucagon, another Ca^{2+} (and cAMP)-acting hormone [4], caused no change in ΔpH. The P coefficient of 1.10 quantifies this conclusion of the almost equal activation by

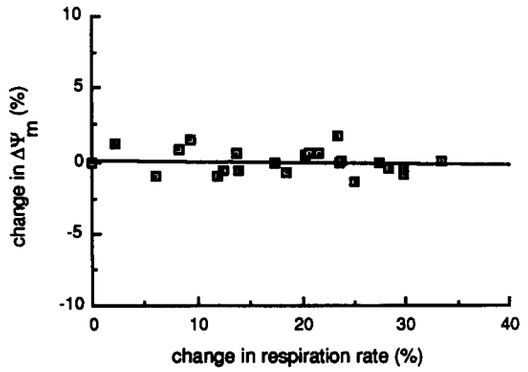

Fig. 6. Changes in mitochondrial membrane potential ($\Delta\Psi_m$) during the stimulation of respiration rate by vasopressin. Each $\Delta\Psi_m$ determination was carried out in triplicate and each respiration rate determination was carried out simultaneously in duplicate. Data are from 11 independent preparations.

vasopressin of both the subsystems in cells that are incubated in the presence of lactate and pyruvate. The main Δp-consuming process affected by Ca^{2+} is probably gluconeogenesis. The ATP/ADP carrier is probably also activated [15,31]. Within the Δp-producing subsystem the most probable and well-characterized targets are the calcium-sensitive Krebs cycle dehydrogenases [32].

Brown et al. [19] found that the addition of vasopressin resulted, almost exclusively, in the stimulation of the NADH-producing subsystem. However, this experiment was performed on hepatocytes incubated without lactate/pyruvate; thus gluconeogenesis, probably the main target for calcium ions in the phosphorylation + proton leak subsystem, was absent. Furthermore, the results were different at 30 and 90 s after the addition of vasopressin, which implies that a steady state had not been achieved. This conclusion is supported by the fact that the transition time between different steady-states in the intact cell is estimated to be a few minutes [33].

The dynamic model of the oxidative phosphorylation system [14,15] predicted that the Δp-producing and Δp-consuming subsystems would be activated to approximately equal extents after the addition of vasopressin. The results of this study support this prediction. The model also predicted that Ca^{2+} would activate the ATP/ADP carrier. Earlier studies have shown that calcium ions probably do stimulate this transporter, although other possibilities were also discussed [31]. Thus experimental findings do provide a support for at least the general properties of this model.

4.6. Effect of glucagon

It was found that glucagon, increasing the respiration rate, does not change ΔpH [30]. Therefore the proportional activation coefficient for this hormone is close to 1, within an experimental error.

4.7. Effect of Ca^{2+}-acting hormones generally

Using the dynamic model of oxidative phosphorylation [14,15], we assessed the value of the proportional activation coefficient for the Ca^{2+}-acting hormones as equal to about 0.8–1.0. The basis for calculations were reported in the literature [21,22] changes in the respiration rate, internal ATP/ADP ratio as well as cytochrome c and the NAD reduction level.

4.8. Effect of neural stimulation in muscle

The kinetic response of the OX subsystem to Δp in heart muscle was assessed from changes in the respiration rate and the internal ATP/ADP ratio in heart mitochondria during State 4 → State 3 transition [26]. The kinetic response of the PH(+LK) subsystem to Δp is most probably much smaller [10] and therefore have little influence on the value of the proportional activation coefficient

Table 1
Proportional activation coefficients for different external effeectors of oxidative phosphorylation

External effector	Proportional activation coefficient
Lactate/pyruvate	0.5–0.7
Fatty acids	0.5–0.7
Vasopressin	1.0–1.2
Glucagon	≈ 1.0
Ca^{2+}-acting hormones generally	0.8–1.0
Ca^{2+} in heart muscle	1.0–1.1
Ca^{2+} in skeletal muscle	1.0–1.1

Because the method used is rough, only approximate values are presented.

(compare Eq. (4)). Rough calculations of proportional activation coefficients using changes in the respiration rate and ATP/ADP ratio during muscle contraction [34,35] gave 1.06 for heart muscle and 1.07 for skeletal muscle. The values obtained are very close to each other and very close to unity.

4.9. General conclusions

The 'proportional activation' approach described here allows the estimation of the relative stimulation (or inhibition) by an external effector of processes that produce and consume a common intermediate. The method is simple, quick, although crude, and needs very little experimental data if control elasticity values are already available. If these values are not available, or when more detailed information is required for the quantitative identification of the sites of action of external effectors, top-down elasticity analysis [36,37] is more appropriate. On the other hand, the proportional activation approach allows the estimation of the importance of blocks of reactions when the flux through the system is altered by an external effector and when the available experimental techniques preclude a full top-down elasticity analysis (e.g., identification of the sites of action of neural signals in muscle). It must be taken into account that the method is crude, as discussed above, and therefore the values of the P coefficient are only approximate ones. However, this does not change our general conclusions.

The results described in this paper arise from the application of the proportional activation approach to the oxidative phosphorylation system comprised of the Δp-producing (i.e., substrate oxidation) and Δp-consuming (i.e., proton leak and phosphorylation) subsystems. The external effectors considered included various substrates, hormones and inhibitors. The addition of lactate plus pyruvate and fatty acids results in similar effects. The Δp-producing subsystem is stimulated slightly more than the Δp-consuming subsystem. Ca^{2+}-acting hormones affect both the Δp-producing and Δp-consuming subsystems to a similar extent. The inhibitor, quinolinic acid, thought to be an inhibitor of gluconeogenesis at phospho*enol*pyruvate carboxykinase [20] was found to inhibit the Δp-producing subsystem as well as the Δp-consuming subsystem. The proportional activation approach is therefore a quick and simple way of determining the sites of action of physiological effectors such as substrates and hormones and in determining the specificity of inhibitors.

Table 1 presents briefly the results obtained in the present paper. The values of proportional activation coefficients for different respiratory substrates, Ca^{2+}-acting hormones and neural stimulation in muscle are shown. They are all close to unity. This is true especially for Ca^{2+}-dependent systems (e.g., calcium-acting hormones and neural stimulation of muscle contraction) and less true for the substrate mediated activation of oxidative phosphorylation (lactate/pyruvate and fatty acids). Thus, the oxidative phosphorylation subsystems are activated by the external effectors to a similar extent. It is perhaps not surprising that the balance of the activation of the subsystems is closer to unity for calcium-dependent effectors than it is for respiratory substrates such as lactate/pyruvate and fatty acids as changes in the respiration rate caused by physiological changes in substrate concentrations are lower than those caused by changes in Ca^{2+} concentration (especially in muscle). Calcium ion concentration seems to be a major physiological factor responsible for the bioenergetic behavior of the cell.

The advantage of such a situation can be constant intermediate metabolite M concentration. Many vital reactions and processes in the cell utilize ATP, Δp or NADH, important intermediate metabolites in bioenergetic systems. If concentrations of the intermediate metabolites were changed markedly, all these processes might be disturbed and internal homeostasis lost. It my be easier for the cell to keep the metabolite concentrations (especially Δp) as constant as possible rather than ensuring that each consuming pathway can cope with wide variations in [M].

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