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Control of gluconeogenesis: role of fatty acids in the α-adrenergic response

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Phenylephrine increases hepatic gluconeogenesis for as long as it is present in the extracellular medium. This effect is accompanied by a parallel increase in oxygen consumption. No apparent stoichiometric relationship exists between the phenylephrine-stimulated respiration and the energy required to meet the demands of gluconeogenesis. In the absence of extracellular calcium, no sustained stimulation of respiration was observed and phenylephrine failed to enhance gluconeogenesis; however, acute and transient effects of the α-adrenergic agonist were still observable. The following observations indicate that fatty acids are not involved in the α-adrenergic response: (1) the effects of phenylephrine and octanoate on respiration and gluconeogenesis were found to be additive; (2) unlike phenylephrine, octanoate is capable of stimulating gluconeogenesis in calcium-depleted liver; (3) in the absence of calcium, phenylephrine was incapable of further stimulating respiration or gluconeogenesis in the presence of octanoate. It is concluded that the conditions of increased lipid mobilization and/or oxidation are not sufficient to explain the metabolic response to α-adrenergic agonists. Fatty acids and α-adrenergic stimulation share a common role of stimulating gluconeogenesis in a manner dependent on their ability to stimulate respiration; however, the additive nature of their effects and distinct calcium requirements indicate that they act to trigger different mechanisms.

Introduction

Among other effects, α-adrenergic agents are known to stimulate the gluconeogenic flux in rat liver. This effect has been consistently reproduced under a wide variety of experimental conditions [1–4]; however, the mechanism mediating this action as well as its physiological significance, as far as we know, have not yet been clearly determined.

A characteristic feature accompanying the response to α-adrenergic agonists, as well as to other hormones, is an increased hepatic respiratory activity [5–7]. It has been proposed that the primary site of action in the hormonal response could be the stimulation of the respiratory chain [8,9]. The reducing equivalents for the respiratory chain would be provided by a simultaneous stimulation of fatty acid oxidation [10]. The increased energy production according to this proposal would, via a mechanism which has yet to be elucidated, lead to the enhancement of gluconeogenesis. This hypothesis, although supported by experimental evidence, is not consistent with current concepts on control of respiration, which point to increased energy demand as responsible for the increase in

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the electron flux and not vice versa [11]. In this regard, it should be noted that there is not a stoichiometric study available on the relationship between an α-agonist-mediated increase in respiration and the energy required to meet the enhanced biosynthetic processes.

The cellular depletion of calcium leads to unresponsiveness to α-agonists [2,4]. It has been shown that elevation of cytosolic free calcium is consistently found to accompany phenylephrine [12,13] and other hormones action [13–16]. These findings, taken together with the known stimulatory action of calcium on some key mitochondrial dehydrogenases [17], have led to the current view that calcium acts as a second messenger in the transduction of α-adrenergic stimulants [2–4]. Although this concept has not yet been seriously questioned, the role of fatty acids oxidation or calcium sensitive dehydrogenases in the hormonal response is a matter of debate [18–20].

The present investigation was undertaken as an attempt to elucidate the relationship between the α-agonist-induced stimulation of respiration and gluconeogenesis, and the possible role of fatty acid oxidation in these events. It is concluded that, in common with fatty acids, the α-agonist-induced energy production is not available for glucose synthesis. Octanoate and phenylephrine show distinct and additive effects on gluconeogenesis indicating that their action is mediated by different mechanisms.

Experimental procedures

Liver perfusion. Livers from starved male Wistar rats (180–200 g) were perfused with Krebs-Ringer bicarbonate buffer in a flow-through system. The technical details were similar to those previously described [21,22]. Substrates were administered diluted in the buffer. Linear increases in substrate supply were attained by placing the perfusate medium in a gradient former. Oxygen consumption was determined by measuring the p_{O_2} arterio-venous difference with a Clark-type oxygen electrode. The p_{O_2} in the effluent perfusate under basal conditions varied from 290 to 350 mmHg. Routinely, the perfusion flow rate was adjusted to 28 ± 2 ml/min. When required, the flow rate was increased in order to prevent limitation of the oxygen supply. All the experiments were carried out with continuous monitoring of perfusate p_{O_2}. Calcium depletion was achieved by perfusing the livers for 50 min before initiation of the experiments with calcium-free bicarbonate buffer. The same buffer was used throughout the experiment. Isolation of hepatocytes using similar technical protocol yields cells in which the total calcium content is more than one order of magnitude below that of control cells isolated and maintained in calcium-containing buffers (0.35 ± 0.02 and 4.1 ± 0.28 nmol · mg^{-1} dry weight).

Metabolite analysis. Perfusate samples were analyzed immediately after their collection. Metabolites were assayed spectrophotometrically or fluorimetrically, according to their expected concentrations, using previously described enzymatic procedures [2,3].

Materials. All the reagents were of the highest purity available, most were obtained from Sigma. The enzymes were obtained from Boehringer Mannheim.

Results

Fig. 1 shows the hepatic response to a linearly increasing supply of pyruvate up to a concentration of 0.7 mM. As previously reported [24], glucose production reaches a plateau at an arterial pyruvate concentration of 0.3 mM. Assuming a P/O ratio of 3, the increment in oxygen consumption is much larger than can be accounted for by the increases observed in the rate of gluconeogenesis. The administration of phenylephrine to livers isolated from starved rats, perfused without any substrate, was followed by characteristic biphasic stimulation of respiration (Fig. 2). The first spike-shaped phase lasted for about 1 min and was accompanied by an acute increase in glucose release. The second phase of sustained increased respiration was accompanied by a small increase in glucose production. The administration of progressively increasing concentrations of pyruvate (Fig. 2) brought about significant increases in the metabolic rates over those observed in control livers (Fig. 1) perfused without phenylephrine. It should be noted that the increased gluconeogenic rates were accompanied by parallel increases in oxygen uptake. The observed increase in energy

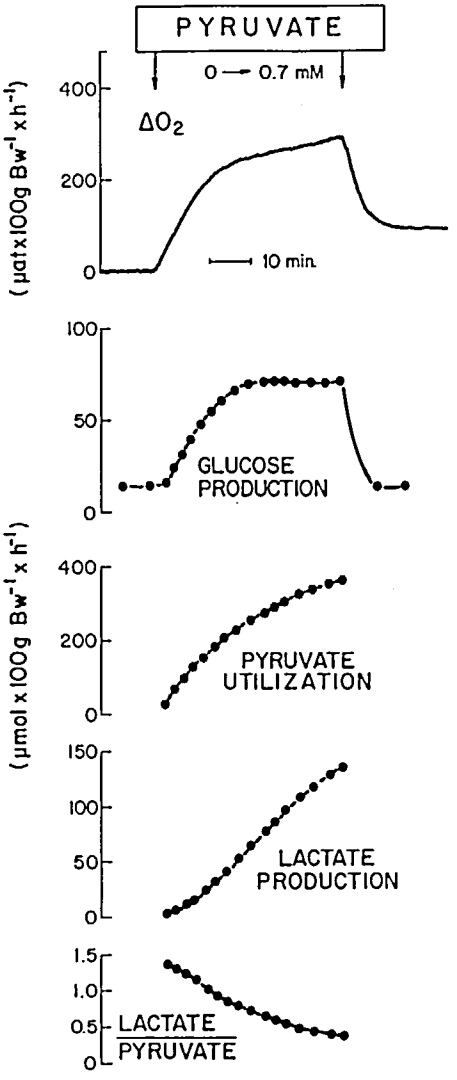

Fig. 1. Metabolic rates in livers perfused with progressively increasing concentrations of pyruvate. Livers isolated from rats starved for 48 h were perfused as described in the Experimental procedures. After a period of equilibration to attain steady p_{O_2} values in the effluent perfusate, pyruvate was administered at a constant, increasing rate of 0.016 μmol·min^{-1} using a gradient former, Perfusate samples were collected every 2 min and their metabolite content was assayed immediately after termination of the experiment. The experiment was repeated several times obtaining a high reproducibility. For the sake of clarity, standard error bars have been omitted.

production was far more than sufficient to account for the extra energy required for gluconeogenesis. Thus, the fate of the phenylephrine-induced energy production was not readily apparent. In agreement with previous observations [43] similar considerations were applicable to the stimulatory action of octanoate shown in Fig. 3. The higher rates of pyruvate utilization can be accounted for by increased rates of lactate production. This is an obvious consequence of the shifting of the NAD redox potential to a more reduced state that follows fatty acid oxidation.

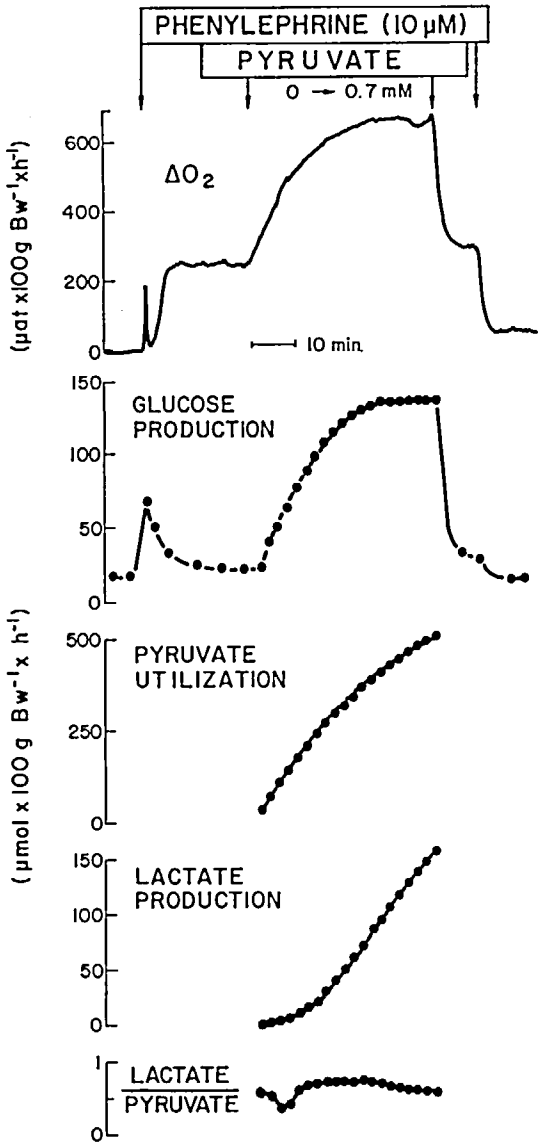

Fig. 2. Effect of phenylephrine on metabolic rates in livers perfused with progressively increasing concentrations of pyruvate. Experimental conditions were similar to those described in Fig. 1. Phenylephrine was administered diluted in the buffer.

The effect of the combined administration of octanoate and phenylephrine is shown in Fig. 4. It can be seen that the respiratory effects of both agents are additive. Despite the striking increase in the respiratory rate, the addition of pyruvate results in further stimulation of respiration which stoichiometrically exceeds the energy required for

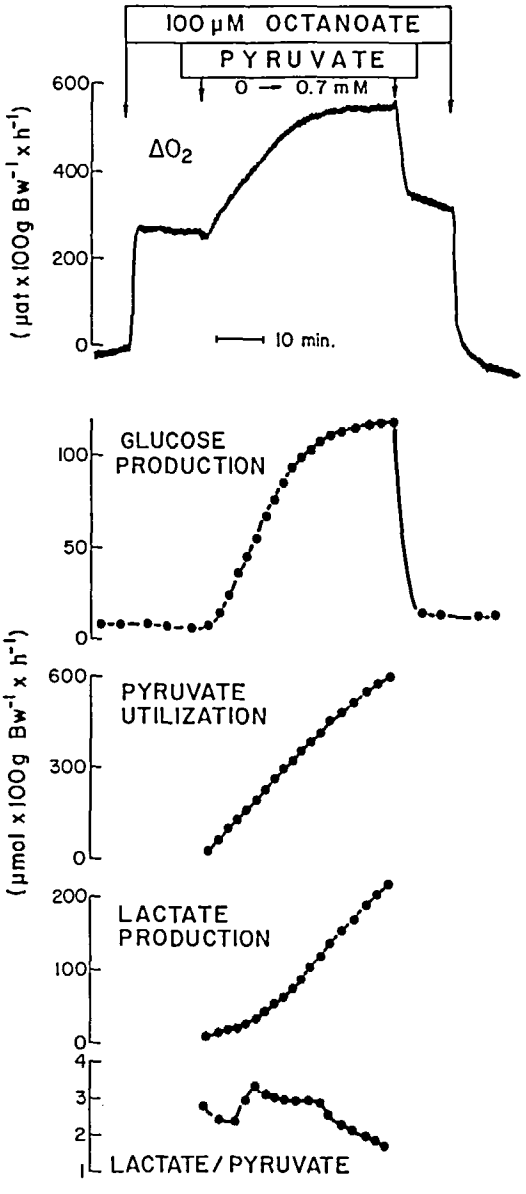

Fig. 3. Effect of octanoate on the rate of oxygen uptake and gluconeogenesis from increasing concentrations of pyruvate. Experimental conditions were similar to those described in Fig. 1. Octanoate (0.1 mM) was administered diluted in the buffer.

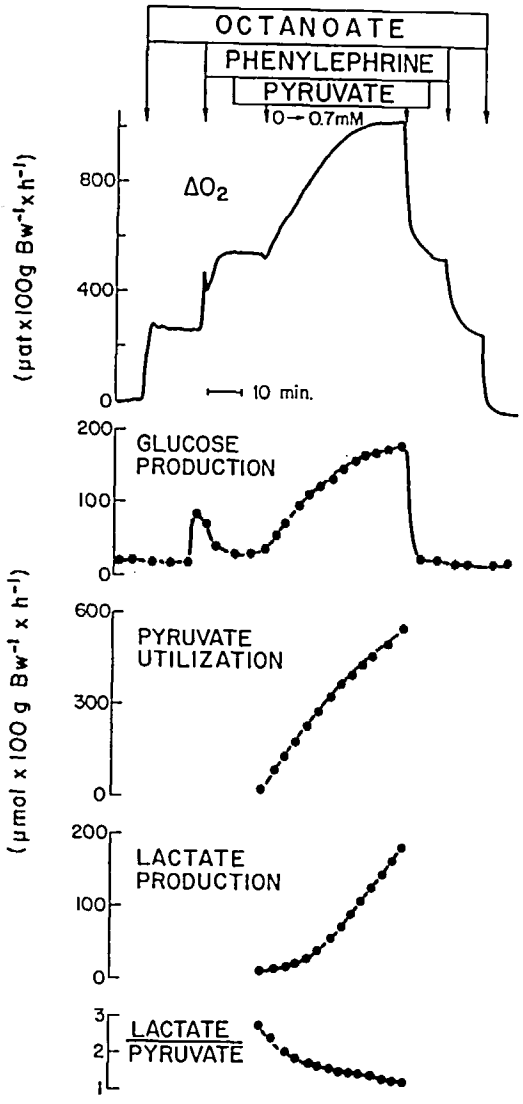

Fig. 4. Effect of the combined administration of octanoate and phenylephrine on oxygen uptake and gluconeogenic rates from increasing pyruvate concentrations. Experimental conditions were similar to those described in Fig. 1.

glucose synthesis. The observed rates of glucose production from pyruvate indicates the additive nature of the stimulatory effects of octanoate and phenylephrine.

Role of extracellular calcium in the metabolic response to phenylephrine

The data in Fig. 5 show how hepatic calcium depletion, induced by perfusing with calcium free

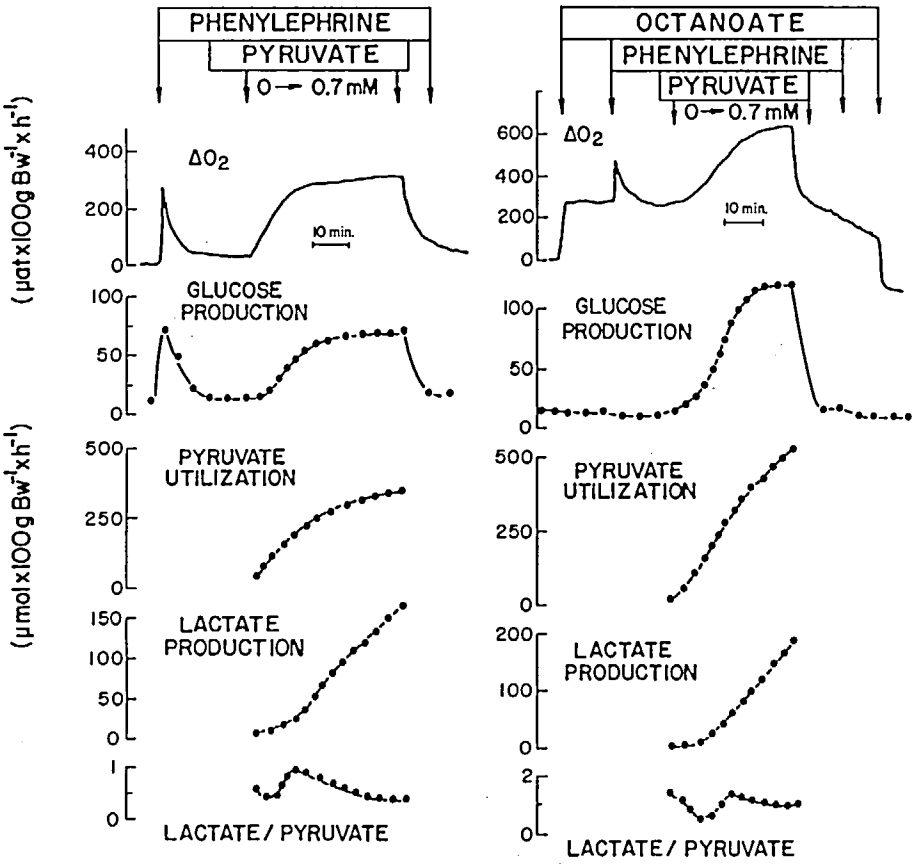

Fig. 5. Effect of hepatic calcium depletion on the metabolic response to phenylephrine alone or combined with octanoate. Calcium depletion was induced by perfusing the livers for 50 min with calcium-free bicarbonate buffer before initiation of the experiments. Substrates administration and rates of sampling were similar to those described in Fig. 1. The concentrations of octanoate and phenylephrine were 100 and 10 μM, respectively.

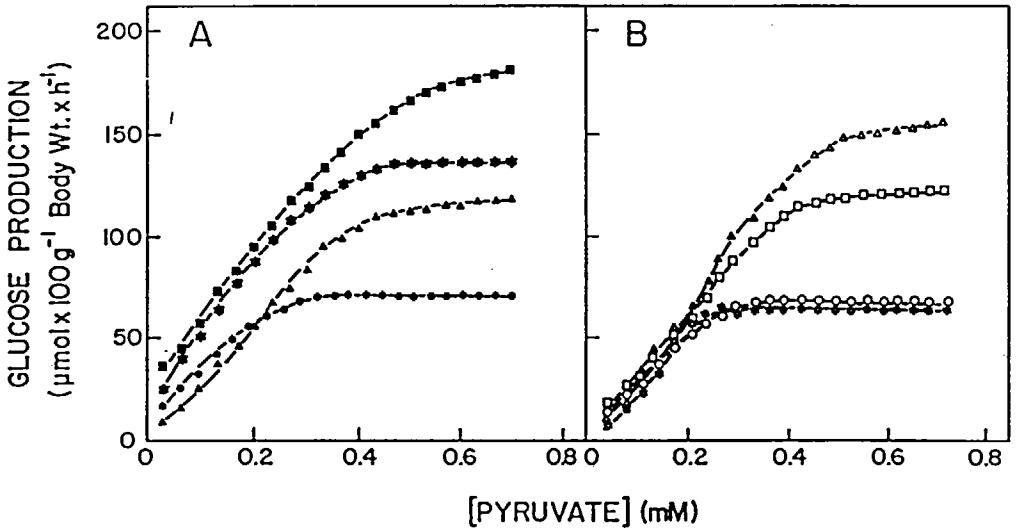

Fig. 6. Effect of hepatic calcium depletion on the stimulatory action of phenylephrine and/or octanoate on gluconeogenesis from increasing concentrations of pyruvate. The filled symbols in panel A refer to control and the empty symbols in panel B refer to calcium-depleted livers. Symbols: ●------●, control; ★------★, phenylephrine; ▲------▲, octanoate; ■------■, octanoate plus phenylephrine. The values are means of at least four observations. Intermediate points and standard error bars have been omitted for the sake of clarity.

bicarbonate buffer, result in a loss of its ability to respond to phenylephrine with a sustained increase of respiration (Fig. 5, upper left corner). The absence of a stimulatory response in respiration was accompanied by a lack of effect in stimulating glucose production, either from endogenous substrates or from added pyruvate (Fig. 5, second row). In contrast to phenylephrine, octanoate is capable of stimulating gluconeogenesis from pyruvate even in the absence of extracellular calcium (Fig. 6).

Fig. 6 summarizes the gluconeogenic responses under the different experimental conditions. The slopes of the linear section of the saturation curves are all similar when corrected for the basal rates of glucose production. This observation indicates that the major effect exerted by these agents is to increase the apparent V_{max} of the pathway. The displacement to the right of the saturation curve obtained in the presence of octanoate (Fig. 6A) is caused by a diminished pyruvate availability secondary to the increased NAD redox potential following fatty acid oxidation.

Discussion

An increased electron flux along the respiratory chain should be expected when the energy demand is increased [11]. Nevertheless, the opposite appears to be suggested and to occur. This seems to be the case, for example, for the proposed action of fatty acids [25,26] or α-adrenergic agonists [10,18,27] in increasing gluconeogenesis by their ability to increase the energy supply by stimulating respiration. We have presented evidence [43] indicating that fatty acid-induced respiration can be accounted for by the production of ketones, and this process does not seem to be influenced by variations in the energy demand imposed by rate changes in the gluconeogenic flux. Furthermore, stimulation of gluconeogenesis is followed by stoichiometrical increases in oxygen uptake (Fig. 3) despite the high rates of fatty acid-induced energy production. The phenylephrine stimulation of gluconeogenesis was also accompanied by parallel increases in oxygen uptake, despite the stoichiometric availability of energy production induced by the α-agonist (Figs. 1 and 2). From this observation it is evident that energy support is not the mechanism by which gluconeogenesis from three carbon substrates is enhanced. From this finding, the question of the bioenergetic significance of the energy production induced by these agents arises. It has been suggested that fatty acid-induced stimulation of respiration is due to extramitochondrial utilization of ATP, perhaps related to the functioning of ion pumps [29].

If energy support is not responsible for the fatty acid or phenylephrine stimulation of gluconeogenesis, and the effect is observable at pyruvate concentrations (above 0.3 mM) which are not limiting for its mitochondrial transport [30,31], it seems plausible to conclude that flux through pyruvate carboxylase has to be activated. Knowledge of the regulation of pyruvate carboxylase flux is incomplete, largely due to uncertainties concerning the actual concentrations of free metabolites prevailing in vivo [32]. Our data support the concept that increased respiratory rates, regardless of the inducing agent, might lead to increased maximal rates of gluconeogenesis by increasing the intramitochondrial free ATP-to-ADP ratio [33,34] resulting in enhanced flux through pyruvate carboxylase [35].

Role of fatty acids and/or calcium in the α-adrenergic metabolic response

It has been proposed that increased lipid mobilization and/or β-oxidation are essential features in the α-adrenergic and other hormone responses. We recently presented rather conclusive evidence indicating that phenylephrine can stimulate respiration in the absence of fatty acid oxidation [36]. The following observations support the conclusion that fatty acid oxidation and α-adrenergic agonists act to stimulate gluconeogenesis via different mechanisms: (1) the effects of both are additive (Figs. 2–4); (2) they show distinct sensitivities to calcium depletion. Calcium is essential in the α-adrenergic response (Fig. 5), but not for octanoate to display its stimulatory action (Fig. 6). The latter finding is an invitation to evaluate the regulatory role of the calcium-sensitive tricarboxylic acid cycle dehydrogenases [17]. If normal stimulatory responses to octanoate are obtained in calcium-depleted livers, it seems plausible to conclude that it is not calcium deficiency which prevents the α-adrenergic response. A positive corre-

lation has been observed between the increment in respiratory activity over the basal rates, regardless of what the inducing agent was, and maximal gluconeogenic rates (Figs. 4 and 5). According to these observations and based on previous reports in which metabolic inhibitors were utilized [37], we would like to present the proposal that variations in the intramitochondrial phosphorylation potential accompanying rate changes in respiration may play a major regulatory role in controlling maximal gluconeogenic flux rates from substrates yielding pyruvate. This proposal agrees with the idea of respiratory chain activation as a primary event in the hormonal response [5–7,27], although the role assigned to this activation is different. From what has been evidenced it follows that elucidation of the mechanism of α-adrenergic action in stimulating gluconeogenesis is synonymous with studying the mechanism(s) involved in the respiratory response. In this respect, we would like to make some additional comments. In calcium-depleted livers, acute and transient responses on respiration, glycogenolysis and NAD redox potential are observed (Fig. 5). This finding implies that the phenylephrine transducing mechanism(s) is operative under our experimental conditions. The observation that phenylephrine increases cAMP levels in liver cells depleted of calcium [38,39] further supports this interpretation. This latter finding also rules out β-stimulation being involved in the metabolic response to α-adrenergic agents, in agreement with other studies [38,40]. Concerning the role of calcium, it has been observed that phenylephrine increases the cytosolic free calcium concentration in the absence of extracellular calcium [12]. As mentioned above, mitochondrial activity can also be enhanced by octanoate in calcium-depleted livers. All these observations, although not conclusive, strongly suggest that extracellular calcium is essential for a complete transduction of the α-adrenergic signal(s). A recent observation also suggests the involvement of extracellular Ca^{2+} in the action of α-adrenergic agents [41]. Calcium could act, perhaps through conformational changes, by allowing interaction of α-agonists with a particular type of receptor in charge of transducing the message to activate respiration. This idea finds support in those reports indicating the possible hetereogenicity of the α-receptor-mediated metabolic response [42,28].

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