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Glucagon-stimulated respiration and intracellular Ca^{2+}

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The effects of extra- and intracellular Ca^{2+} on glucagon-stimulated respiration were examined in perfused rat liver. Glucagon increased the uptake of O_2 to a significantly greater extent in Ca^{2+}-containing perfusate than in Ca^{2+}-free perfusate. If, however, the livers were perfused first with Ca^{2+}-containing perfusate for 60 min in order to load the hormone-sensitive Ca^{2+} pool(s) and subsequently with Ca^{2+}-free perfusate, glucagon was able to stimulate O_2 uptake to the same extent in Ca^{2+}-free, as in Ca^{2+}-containing perfusate. These experiments support previous observations of a connection between Ca^{2+} and the hormonal stimulation of respiration, but indicate a role for intracellular, rather than extracellular, Ca^{2+} in the process.

Ca^{2+} Respiration Glucagon (Liver)

1. INTRODUCTION

All hormones which stimulate gluconeogenesis were shown to stimulate O_2 uptake and mitochondrial metabolism [1,2]. The hormonal stimulation of the mitochondrial steps in the gluconeogenic pathway was first described by Adam and Haynes [3]. Since these initial studies, it has been recognized that hormonal treatment of the liver results in a multitude of changes in mitochondrial metabolism. These changes have been observed in crude liver homogenates [4] and in situ, in filipin-treated hepatocytes [5]. The stimulation of mitochondrial respiration was suggested to be a critical event in the gluconeogenic response [1,6,7].

The mechanism by which mitochondrial metabolism is altered is not agreed upon. It has been suggested by Halestrap et al. [8] that gluconeogenic hormones increase the activity of the respiratory chain through a Ca^{2+}- and pyrophosphate-dependent increase in mitochondrial volume [8]. Other suggestions include a dissociation of the intrinsic peptide inhibitor of the ATPase [9], an effect on adenine nucleotide translocase [10–12], the protection of functionally important thiol groups due to decreased peroxide generation [13], and the elevation of the intramitochondrial malate concentration [14].

In addition to the increased respiration, gluconeogenic hormones were shown to alter hepatic Ca^{2+} distribution (summarized in [7]). A connection between the hormonal stimulation of respiration and the Ca^{2+} fluxes was indicated by several reports [15–18]. The present investigation was undertaken to expand on those studies. It was found that the presence of Ca^{2+} in the intracellular hormone-sensitive pool rather than extracellular Ca^{2+} is essential for the hormonal stimulation of respiration.

2. MATERIALS AND METHODS

2.1. *Animals*

Male, fed (if not otherwise stated), Sprague-Dawley rats weighing around 130–200 g were used in all the experiments.

2.2. *Chemicals*

Glucagon was from Eli Lilly, Indianapolis, IN and albumin (bovine, Cohn fraction V, cat.no.A4503) from Sigma, St. Louis, MO.

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2.3. *Methods*

Livers were perfused in situ with haemoglobin-free Krebs-Ringer bicarbonate buffer (KRB) which, as indicated, either contained the usual 2.5 mM Ca^{2+} or was Ca^{2+}-free and contained 1 mM EGTA. Albumin, final concentration 4%, was included in the perfusate. The perfusion system has been described in detail [19]. The present experiments were carried out at room temperature in order to lower the metabolic rate and ascertain that there is sufficient O_2 supply available for maximal stimulation of respiration.

Oxygen uptake was monitored in the perfusate by a Clark-type oxygen electrode attached to a YSI model 53 oxygen monitor and a recorder. The electrode was calibrated with O_2-saturated water. Oxygen uptake was calculated as the difference between the influent and effluent O_2 content. Experimental details are listed in table 1.

Perfusate-free Ca^{2+} was measured with an Orion Ca^{2+}-electrode. The Ca^{2+}-free KRB had no measurable free Ca^{2+} in it.

Results are expressed as means ± SE. Statistical analysis of the data was done by Student's paired *t*-test.

3. RESULTS AND DISCUSSION

The effects of the presence and absence of Ca^{2+} in the perfusate on the glucagon stimulation of hepatic oxygen uptake was examined. Glucagon administration in regular, Ca^{2+}-containing KRB resulted in an increase in O_2 uptake which was apparent within 1–2 min (table 1[a]). The increase in O_2 uptake was about 22% above the basal rate of respiration and remained elevated at least for 15 min – the time when the measurement stopped. These results confirm the previously well demonstrated stimulation of respiration by glucagon.

When similar experiments were carried out using livers from fasted animals, glucagon also stimulated respiration, but to a smaller extent. The increase in O_2 uptake in this situation was 350 ± 16 ngatom O_2/min per g wet wt. Because in the fasted liver and without added substrate the rate of gluconeogenesis is negligible [20], these results indicate that the increase in O_2 uptake is not secondary to the stimulation of gluconeogenesis, but is a primary effect of the hormone. However, the effect of glucagon on O_2 uptake was less in fasted

Table 1

Effects of Ca^{2+} on glucagon stimulated respiration

Increase in respiration (ngatom O_2/min per g wet liver)				
Regular KRB[a]	Ca^{2+}-free KRB[b]	Ca^{2+}-free KRB[c]	Ca^{2+}-free KRB[d]	Ca^{2+}-free KRB[e]
600 ± 10(3)	200 ± 11(5)	190 ± 6(3)	320 ± 8(3)	550 ± 13(4)
$p < 0.025$	$p < 0.025$	$p < 0.01$	$p < 0.01$	$p < 0.01$

[a] Livers were perfused 60 min with regular KRB before glucagon addition

[b] As above, but Ca^{2+}-free, KRB containing 1 mM EGTA was used

[c] Livers were perfused 20 min before glucagon addition with Ca^{2+}-free KRB containing 1 mM EGTA

[d] Livers were perfused for 20 min with Ca^{2+}-free KRB containing 1 mM EGTA. Then Ca^{2+} was added to the reservoir to give a final perfusate Ca^{2+} content of 2.5 mM. 10 min later, glucagon was added (Ca^{2+} after-loaded)

[e] Livers were perfused first as in (a) then the perfusate was switched to Ca^{2+}-free KRB as in (c) (Ca^{2+} preloaded)

Glucagon was added to give a final concentration of 5×10^{-10} M. The table tabulates the increases in respiration elicited by glucagon above the rates of respiration observed before the addition of the hormone (basal rate). Basal rates of respiration varied between 2700 ± 90 and 2200 ± 20 ngatom O_2/min per g wet liver

than in fed livers, an observation which needs further studies.

Perfusion of livers with Ca^{2+}-free KRB for either 20 (table 1[c]) or 60 min (table 1[b]) resulted in drastically reduced responses to glucagon. To ascertain that this lack of response is due to the lack of Ca^{2+}, and not to some other factor, livers were perfused first with Ca^{2+}-free perfusate and then Ca^{2+} was added 10 min before the addition of glucagon. Under this condition, the response became considerably larger (table 1[d]), but it was less than the response obtained after 60 min perfusion with Ca^{2+}, though the same amount of Ca^{2+} was present in the perfusate in both experiments (table 1[a,d]).

It seemed possible, in light of these results, that the lack of response to glucagon in Ca^{2+}-free medium was not due to the lack of Ca^{2+} in the perfusate but rather to the depletion of intracellular Ca^{2+} from the hormone-sensitive Ca^{2+} pool. To test this hypothesis the following experiment was carried out. Livers were perfused with regular KRB for 60 min, to load the intracellular Ca^{2+} pools. Subsequently, the perfusion was continued with Ca^{2+}-free solution. After 20 min of perfusion with Ca^{2+}-free perfusate, glucagon was added. Under these conditions, the response to glucagon was identical to the response obtained in regular KRB (table 1[d]). Thus, the total absence of extracellular Ca^{2+} did not prevent the response to glucagon in these experiments. The plausible conclusion from these results is that it is the intracellularly stored Ca^{2+} which is important for the respiratory increase. These results also might explain the observation made by Siess and Wieland [21] who found decreased basal rates of respiration in hepatocytes suspended in Ca^{2+}-free medium, but still a stimulation of respiration by cyclic-AMP.

In conclusion, these data support earlier reports on a connection between Ca^{2+} and the increase in respiration following the administration of gluconeogenic hormones [15–18]. However, it indicates intracellular rather than extracellular Ca^{2+} as the important factor. As to the mechanism by which intracellular Ca^{2+} can influence respiration, I have recently suggested that an efflux of mitochondrial Ca^{2+} might result in such changes [22]. The efflux of Ca^{2+} might also be associated with calmodulin-mediated mitochondrial processes [23,24] though some calmodulin-dependent processes might not require Ca^{2+} [25]. It is interesting in this respect to note that the calmodulin antagonist W-7 was shown recently to inhibit the stimulation of respiration by retrograde perfusion in the pericentral regions of the liver [26]. These results support the notion that Ca^{2+} is involved in the hormonal regulation of respiration.

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