Effect of depolarizing concentrations of potassium on calcium uptake and metabolism in rat liver

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Exposure of perfused livers of fed rats to 60 mM K^+ induces rapid responses in the Ca^{2+}-sensitive metabolic events, glycogenolysis, cytoplasmic and mitochondrial NADH/NAD ratios and octanoate oxidation. All increase within 45 s of K^+ addition. Metabolic responses were not observed following K^+ addition to livers perfused in the absence of added Ca^{2+}. Movements of Ca^{2+} into the liver were suggested from experiments in which $^{45}Ca^{2+}$ uptake was measured. The Ca^{2+} antagonists verapamil, diltiazem and Ni^{2+} essentially abolished changes to tissue metabolism and Ca^{2+} fluxes induced by K^+ addition. K^+-induced changes were consistent with Ca^{2+} channel activiation.

Liver *Potassium depolarization* *Glycogenolysis* *Calcium antagonist*

1. INTRODUCTION

Calcium channels in the plasma membrane play a crucial role in a number of different tissues by coupling membrane excitation to cellular responses such as hormone secretion, neurotransmitter release and muscle contraction (review [1]). While the activation of Ca^{2+} channels by membrane depolarization in tissues like smooth and cardiac muscles and brain has been known for a number of years, many important details remain to be established [1,2].

Although the possible operation of such Ca^{2+} channels in liver tissue has not been extensively investigated, in recent reports it has been suggested that Ca^{2+} channels or pores may play a role in the elevation of cytoplasmic Ca^{2+} concentrations in perfused rat liver and hepatocytes in response to the action of adrenergic agonists [3,4]. These compounds are known to stimulate unidirectional Ca^{2+} influx in rat hepatocytes [4–7] and in perfused rat liver [3].

One approach to the study of Ca^{2+} channels has relied on the ability of pharmacological agents known as Ca^{2+} antagonists to inhibit selectively Ca^{2+} channel operation [2,8]. In this regard a recent study which examined Ca^{2+} antagonist binding in a range of tissues indicated that Ca^{2+} channels may also be present in liver [2].

Here we have examined the effect of depolarizing concentrations of K^+ on aspects of Ca^{2+}-sensitive metabolism in perfused rat liver. The results described are consistent with the notion that Ca^{2+} channels are present in the liver plasma membrane.

2. EXPERIMENTAL

2.1. *Animals and perfusions*

Wistar-strain albino rats (200–280 g body wt) having free access to food were anaesthetized with sodium pentobarbitone (50 mg/kg). Livers were perfused with Krebs-Henseleit [9] bicarbonate medium equilibrated with O_2/CO_2 (19:1) in a flow-through mode as in [10]. The perfusion medium was modified to contain 1.3 mM $CaCl_2$ and a proportion of the final NaCl content (55 mM NaCl) was administered to the perfusate by an infusion syringe containing 4 M NaCl. During the perfusion the perfusate KCl concentration

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could be increased from 5 to 60 mM by replacing the NaCl infusion syringe with an identical syringe of 4 M KCl. In this manner the perfusate K^+ concentrations were routinely maintained at 60 mM from 16 min of perfusion till the conclusion of the experiment. When Ca^{2+} was omitted from the perfusion medium the Ca^{2+} concentration was reduced to approx. 5 μM as revealed by atomic absorption spectroscopy.

2.2. *Analytical procedures*

Perfusate oxygen concentrations were continuously monitored with a Clark-type oxygen electrode as detailed [10]. Effluent perfusate was assayed for glucose by the glucose oxidase/peroxidase method as described [11]. Perfusate lactate, pyruvate and ketone bodies as well as production of $^{14}CO_2$ from [1-^{14}C]octanoate was estimated as in [12]. Loss of Ca^{2+} from the perfusate was determined as described [3]. Lag times for oxygen responses and metabolite outputs (glucose, lactate, pyruvate, β-hydroxybutyrate, acetoacetate and $^{45}Ca^{2+}$ were determined as in [12] and the time courses of the responses corrected accordingly.

2.3. *Chemicals and materials*

The glucose assay kit (510-A) and nifedipine were obtained from Sigma, St Louis, MO. Lactate dehydrogenase and β-hydroxybutyrate dehydrogenase were from Boehringer, Mannheim. Hyamine hydroxide and [1-^{14}C]octanoate were supplied by New England Nuclear, Boston, MA. Verapamil was from Knoll, Munich, and diltiazem was a kind gift from Professor D. Marme, Goedecke Research Institute, Freiburg, FRG. All other chemicals were of analytical grade.

3. RESULTS

3.1. *Effect of K^+ on various metabolic responses in the perfused rat liver*

The effects of increasing the perfusate K^+ concentration from 4 to 60 mM on a number of metabolic responses, previously shown to be stimulated following the administration of Ca^{2+}-mobilizing hormones to the perfused rat liver [10,12], are shown in fig.1. Increased glucose output (fig.1a) is evident between 30 and 40 s after K^+ addition. Maximal increases of approx. 150% are observed by 75 s. A sharp decline in glucose output follows to reach values only 35% above basal levels by 90–100 s. A further increase in glucose output is then observed which continues from 2 to 6 min of K^+ infusion, when the experiments were terminated.

Omission of Ca^{2+} from the perfusate completely prevents K^+-induced changes in glucose output. In addition the figure shows that maximally effective doses of the Ca^{2+} antagonists verapamil (4 μM), diltiazem (50 μM) and Ni^{2+} (1.3 mM) almost completely prevent K^+-induced changes in glucose output. Half-maximal inhibition by verapamil, diltiazem and Ni^{2+} was observed at approx. 0.5, 10 and 250 μM, respectively. In contrast, nifedipine, a potent Ca^{2+} antagonist in other tissues [2,8], had no effect on K^+-induced effects in the perfused liver when used at up to 2 μM (not shown).

As detailed in section 2, whenever perfusate K^+ concentrations were elevated to 60 mM, a corresponding decrease in Na^+ concentration was achieved. Changes in Na^+ concentration, however, did not appear to be involved in the metabolic effects described, since in control experiments where Na^+ was replaced by choline, no significant metabolic effects were observed.

Additionally, co-infusion of the α_1-adrenergic antagonist, prazosin, failed to influence the K^+-induced changes in glucose output observed in fig.1.

The effect of raising the external K^+ concentration to 60 mM on perfusate [lactate]/[pyruvate] and [β-hydroxybutyrate]/[acetoacetate] ratios, indicators of liver cytoplasmic and mitochondrial [NADH]/[NAD] ratios, respectively [13], are shown in fig.1b and c. Increases in the [lactate]/[pyruvate] and [β-hydroxybutyrate]/[acetoacetate] ratios were evident 30–40 and 70–75 s after K^+ addition, respectively. Whereas the enhanced [lactate]/[pyruvate] ratio rapidly decreased after 45 s, the increased [β-hydroxybutyrate]/[acetoacetate] ratio remained maximally stimulated from 2 min onwards. Similar to responses in glucose output, these K^+-induced effects were almost totally abolished by pretreatment of livers for 5 min with either 4 μM verapamil or 50 μM diltiazem. Likewise, K^+-induced effects were not observed if Ca^{2+} was omitted from the perfusion medium (not shown).

The rate of oxidation of tracer amounts of [1-^{14}C]octanoate to $^{14}CO_2$ has previously been

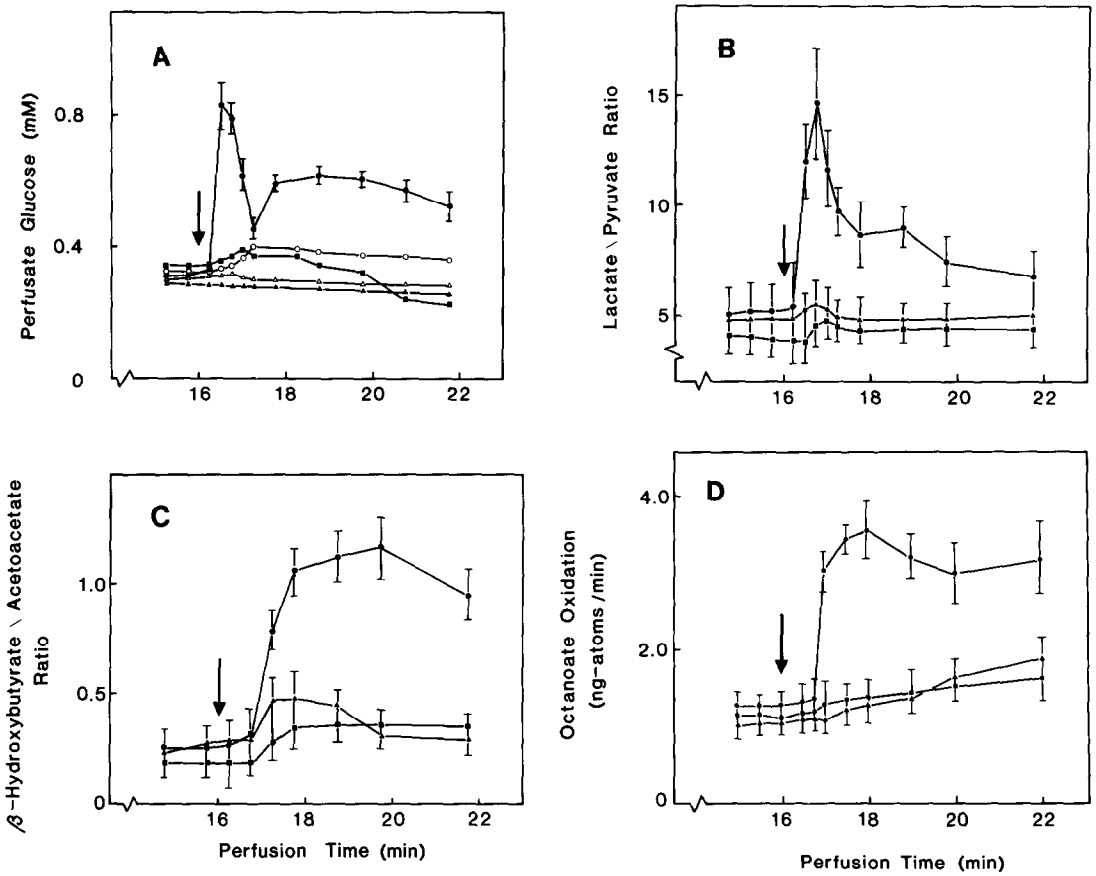

Fig.1. Effect of verapamil, diltiazem, Ni^{2+} and Ca^{2+} on K^+-induced changes to metabolic responses. Livers were perfused as described in section 2. The perfusate was sampled at the times indicated for glucose (A), for lactate and pyruvate (B), for β-hydroxybutyrate and acetoacetate (C), or for [1-^{14}C]octanoate oxidation to $^{14}CO_2$ (D). After 12 min of perfusion verapamil (4 μM, ■), diltiazem (50 μM, ▲) or Ni^{2+} (1.3 mM, ○) was continuously infused where indicated. The perfusate K^+ concentration was increased to 60 mM at 16 min (arrowed). In experiments where no added Ca^{2+} was present (△), livers were initially perfused for 10 min with buffer containing 1.3 mM Ca^{2+} and thereafter without added Ca^{2+}. In some experiments prazosin (2 μM) was infused at 12 min and exhibited K^+-induced responses identical to the control (●). In panel A data shown are the means ± SE of 8 experiments where K^+ alone was administered (●) and the means of 5 separate experiments for all other additions. For clarity the SE values (all of which were less than ± 10%) have been omitted in these latter experiments. In panels B and C, data shown are the means ± SE for 4 separate experiments in the absence (●) and 3 separate experiments in the presence of verapamil or diltiazem. In panel D livers were continuously infused with [1-^{14}C]octanoate (25.1 μCi/mol) to a final concentration of 0.2 μM. Samples of perfusate were assayed for $^{14}CO_2$ as described in section 2. Data shown are the means ± SE of 4 separate experiments in the absence (●) and 3 separate experiments in the presence of verapamil or diltiazem.

used to monitor tricarboxylic acid cycle flux in the perfused rat liver [12,14]. As shown in fig.1a, an increase in octanoate oxidation was evident 60 s after K^+ addition and maximal rates were observed by 90 s; thereafter the effect slowly declined. This K^+-induced effect was also abolished by pretreatment with verapamil or diltiazem or by the omission of Ca^{2+} from the perfusate (not shown).

3.2. *Evidence that increasing the perfusate K^+ concentration promotes Ca^{2+} inflow*

The change in Ca^{2+}-sensitive metabolism seen in fig.1 following K^+ addition to the perfusion medium suggested that such action led to Ca^{2+} influx. This was tested directly with experiments employing $^{45}Ca^{2+}$ [3], since the Ca^{2+}-selectrode [10] could not accurately monitor perfusate Ca^{2+}

concentration changes under these experimental conditions where K^+ concentrations were varied widely.

A tracer amount of $^{45}Ca^{2+}$ was continuously infused into the inflowing perfusion medium, from 5 min before K^+ addition. The $^{45}Ca^{2+}$ content of the effluent perfusion medium reached a steady level within 2–3 min, which corresponded to the continuous uptake of approx. 1.8% of the inflowing $^{45}Ca^{2+}$. The uptake of radioisotope apparently reflects the continuing exchange of extracellular $^{45}Ca^{2+}$ for $^{40}Ca^{2+}$ in both extracellular and intracellular compartments [3].

The effect of K^+ addition on the uptake of $^{45}Ca^{2+}$ from the perfusate is shown in fig.2. The effect is transient with an enhanced rate of uptake being observed some 20–30 s after K^+ addition. Maximal effects occur by 40–50 s and thereafter decline to reach basal values by 2–3 min. These Ca^{2+} movements are almost completely abolished by pretreatment with the Ca^{2+} channel blocker verapamil (fig.2).

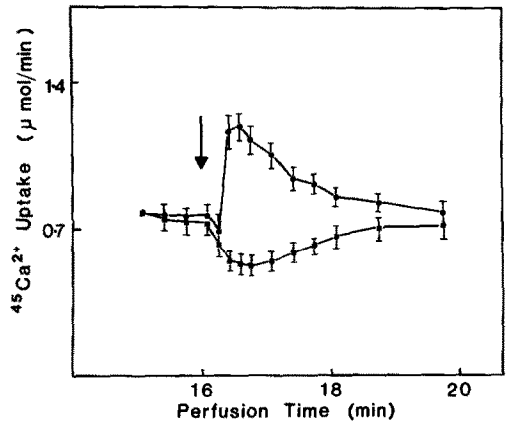

Fig.2. Effect of verapamil on K^+-induced uptake of perfusate $^{45}Ca^{2+}$. Livers of fed rats were perfused with Krebs-Henseleit buffer containing 1.3 mM Ca^{2+} as described in section 2. A tracer amount of $^{45}Ca^{2+}$ (final spec. act. ~50 nCi/ml) was continuously infused into the inflowing buffer after 11 min of perfusion. In some experiments verapamil (final concentration, 4 μM) was continuously infused after 12 min of perfusion. At 16 min the perfusate K^+ concentration was increased to 60 mM (arrowed) as detailed in section 2. Effluent samples were taken for determination of radioactivity. Data shown are the means ± SE of 4 experiments in the absence (●) and 3 experiments in the presence of verapamil (■).

4. DISCUSSION

Our data show that a number of metabolic responses known to be enhanced by Ca^{2+}-mobilizing hormones in the perfused rat liver (review [15]) are similarly stimulated by a rapid increase in the perfusate K^+ concentration. The K^+-induced metabolic responses occurred in the presence of the α_1-adrenergic blocker prazosin, clearly suggesting the non-involvement of the α_1-receptor in any mobilization of the Ca^{2+} that may have resulted here.

The conclusion that an increased inflow of Ca^{2+} into the perfused rat liver did take place in the present work consequent to the action of depolarizing concentrations of K^+ is supported by the following observations.

(i) Changes observed in perfusate $^{45}Ca^{2+}$ content were consistent with a transient stimulation of Ca^{2+} uptake by the tissue. (ii) All metabolic responses to K^+ addition were obligatorily dependent on the presence of extracellular Ca^{2+}. (iii) The responses were almost completely inhibited by the Ca^{2+} channel blockers verapamil, diltiazem and Ni^{2+}. The concentration of these agents required to inhibit the effects of K^+ addition were in the range used to inhibit Ca^{2+} channels in other tissues and were well below the range where these agents may interact with calmodulin [16]. Thus the inhibitory effects of Ca^{2+} antagonists on metabolic responses were unlikely to result indirectly from an inhibition of calmodulin-sensitive steps in the various metabolic pathways. Further evidence suggesting enhanced Ca^{2+} influx was provided in experiments in which the metabolic effects of K^+ were examined. These effects mimicked those induced by α-adrenergic agonists and the Ca^{2+} ionophore A23187, agents previously shown to alter Ca^{2+} distribution in liver cells and to elevate cytoplasmic Ca^{2+} concentrations [17–20]. Thus increases in glucose output in response to α-agonists, A23187 and K^+ addition may all arise from an enhanced cytoplasmic Ca^{2+} concentration and subsequent activation of phosphorylase kinase. Other similar metabolic effects induced by these agents may also result from an altered distribution of cellular Ca^{2+}.

The question arises as to whether the inflow of Ca^{2+} suggested by the above-mentioned observations occurs via specific Ca^{2+} channels located in the plasma membrane. Certainly concentrations of

K^+ ranging from 50 to 110 mM previously have been used to induce membrane depolarization and activation of Ca^{2+} channels in a number of different tissue preparations [21–23]. Although the operation of such channels in liver tissue has been suggested by previous workers [7], it is only recently that more substantive evidence for their presence has appeared [3,4].

However, the present data also do not necessarily indicate the presence of voltage-dependent Ca^{2+} channels in rat liver. Whilst K^+-induced effects were inhibited by agents which effectively block these channels in other tissues, the Ca^{2+} antagonists used may also inhibit receptor-operated Ca^{2+} channels [24] and may have other non-specific effects [25].

The K^+-induced metabolic responses described probably do not result from the operation of Ca^{2+}-activated K^+ channels since some evidence suggests that these channels are not operative in rat liver [26,27] and apamin, an agent which inhibits Ca^{2+}-activated K^+ channels in other tissues did not affect the K^+-induced responses (not shown).

Finally, work in the present paper has revealed a further useful means whereby the interplay of extracellular and cytoplasmic Ca^{2+} and the ensuing induced physiological responses (review [15]), can be explored in the intact perfused rat liver independent of α_1-receptor occupancy.

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