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Cell swelling inhibits proteolysis in perfused rat liver

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Exposure of isolated single-pass-perfused rat liver to hypo-osmotic media resulted in liver cell swelling and an inhibition of release of branched-chain amino acids. Similarly, cell swelling inhibited [^{3}H]leucine release from perfused livers from rats in which liver proteins were prelabelled *in vivo* by intraperitoneal injection of L-[4,5-^{3}H]leucine 16–20 h before the experiment. The effects of cell swelling on [^{3}H]leucine release were fully reversible. [^{3}H]Leucine release was also inhibited when cell swelling was induced by addition of glutamine (0.5–2 mM). There was a close relationship between the inhibition of [^{3}H]leucine release and the degree of liver cell swelling, regardless of whether cell swelling was induced by hypo-osmotic perfusion or addition of glutamine. The data suggest that the known anti-proteolytic effect of glutamine is in large part due to glutamine-induced hepatocyte swelling.

INTRODUCTION

Amino acids such as glutamine are known to be potent inhibitors of proteolysis in rat liver; however, the underlying mechanism is not understood [1–7]. Recent studies have indicated that amino acids, even at physiological concentrations [8], elicit liver cell swelling and volume-regulatory ion-flux responses [8–11]. Furthermore, cell swelling was recognized as an important trigger of hepatic metabolism [10–14]. The present study was undertaken to examine the effect of cell swelling on proteolysis and to determine whether the known anti-proteolytic effect of glutamine can be ascribed to glutamine-induced alterations of liver cell volume.

MATERIALS AND METHODS

Livers from male Wistar rats (120–250 g body wt.), fed *ad libitum* on stock diet, were perfused as described previously [8,10,13] in a non-recirculating manner with bicarbonate-buffered Krebs–Henseleit saline plus L-lactate (2.1 mM) and pyruvate (0.3 mM), if not stated otherwise. In normo-osmotic perfusion, the osmolality was 305 mosmol·kg^{-1}. The perfusate was gassed with O_2/CO_2 (19:1); the temperature was 37 °C. Hypo- or hyper-osmotic exposure was performed by lowering or increasing the NaCl concentration in the perfusion medium, resulting in corresponding osmolality changes. Liver mass was recorded continuously with a specially constructed balance pan, as described recently [8]. The liver wet weight at the end of the perfusion experiment after restoration of normo-osmotic (305 mosmol·kg^{-1}) medium for at least 30 min was set to 100%, and the liver mass changes recorded during the experiment after hypo-osmotic exposure or glutamine addition were expressed as percentage liver mass increase. The rate of proteolysis was assessed either by measuring the release of branched amino acids during metabolic steady states or by measuring ^{3}H release from isolated perfused rat livers after prelabelling of liver proteins *in vivo* by intraperitoneal injection of 200 μCi of L-[4,5-^{3}H]leucine 16–20 h before the perfusion experiment. In these [^{3}H]leucine experiments the influent perfusate was supplemented with unlabelled leucine (0.1 mM) in order to prevent reutilization of [^{3}H]leucine for protein synthesis and to accelerate the wash-out of pre-existing free [^{3}H]leucine. Chromatographic analysis of the effluent perfusate revealed that ^{3}H released from the liver was more than 98% associated with effluent leucine. ^{3}H release into the perfusate amounted to about 10^3 c.p.m./min per g of liver during normo-osmotic perfusions and was determined by scintillation spectrometry. After a 70 min pre-perfusion period ^{3}H release from the liver had reached a steady state, which was maintained for at least a further 80 min. Radioactivity released under these conditions was seen to be derived from proteolysis. Lactate dehydrogenase release into effluent perfusate gradually increased in hypo-osmoticity experiments such as shown in Figs. 1 and 2 from 1.5±0.2 at the beginning to 12±6 m-units/min per g of liver at the end of the 3 h perfusion period. These values correspond to lactate dehydrogenase release rates of 0.024–0.20%/h per g and are much lower than the proteolytic rate in amino acid-free liver perfusion of about 4.5%/h per g of liver protein [2]. When indicated in the text, the influent perfusate contained a mixture of amino acids at concentrations found physiologically in the portal vein (for composition see [8]). The effluent K^+ concentration was continuously monitored with a K^+-sensitive electrode (Radiometer, Munich, Germany); volume-regulatory K^+ release was determined by planimetry of areas under curves [8,10,13–15]. Branched-chain amino acids were analysed with an automatic amino acid analyser (LKB 6151 alpha plus).

L-[4,5-^{3}H]Leucine was from Amersham Buchler (Braunschweig, Germany). L-Lactic acid was from Roth (Karlsruhe, Germany). All other chemicals were from Merck (Darmstadt, Germany). Data are given as means±S.E.M. (n = number of perfusion experiments).

RESULTS

Hypo-osmotic exposure of perfused rat liver by lowering the perfusate osmolality from 305 to 225 mosmol·kg^{-1} led to a 23±1% (n = 6) decrease of leucine release into effluent perfusate (Fig. 1) and a decreased release of isoleucine and valine by 22±3% (n = 6) and 19±4% (n = 6) respectively. Accordingly, upon exposure of perfused liver to hypo-osmotic media branched-chain amino acid (leucine plus valine plus isoleucine) release decreased by 22±2% from 183±4 to 143±4 nmol/min per g (n = 6). These effects were fully reversible upon re-exposure to normo-osmotic perfusion media. The inhibition of branched-

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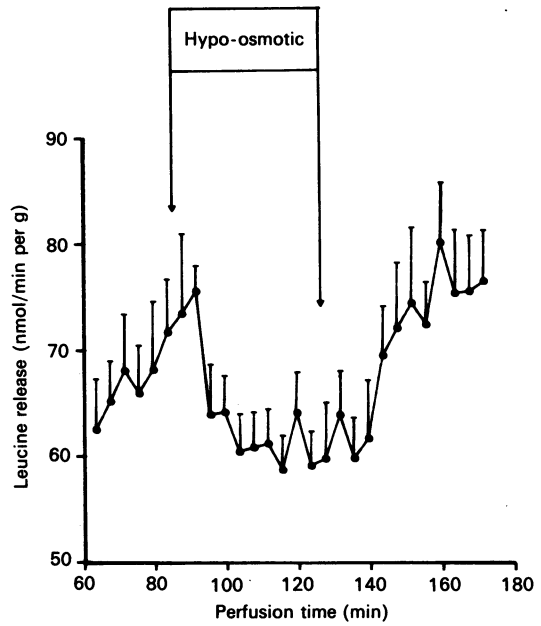

Fig. 1. Effect of hypo-osmotic perfusion on leucine release from perfused rat liver

Hypo-osmoticity was induced by lowering the influent NaCl concentration by 40 mM, resulting in a decrease of osmolality from 305 to 225 mosmol·kg^{-1}. Data are given as means±S.E.M. from 6 different experiments.

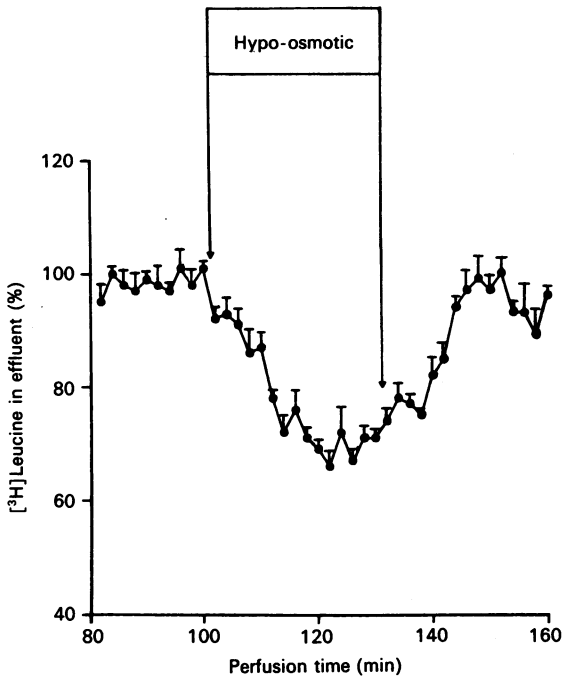

Fig. 2. Effect of hypo-osmotic perfusion on [^{3}H]leucine release from perfused rat liver

Liver proteins were prelabelled by injection *in vivo* of [^{3}H]leucine (see the Materials and methods section). The influent perfusate contained an amino acid mixture in physiological concentrations (for composition see [8]), but devoid of glutamine, and hypo-osmoticity was achieved by lowered the influent NaCl concentration by 40 mM. Data on [^{3}H]leucine release are given as percentages of steady-state ^{3}H release during the normo-osmotic (305 mosmol·kg^{-1}) perfusion period from 80 to 100 min in the individual perfusion experiment. Data are given as means±S.E.M. from 4 different perfusion experiments.

chain amino acid release persisted throughout hypo-osmotic exposure even though the volume-regulatory K^+ efflux restores partially the initial liver cell volume within the first 8 min of hypo-osmotic exposure [10,12–15]. On the other hand, exposure of perfused liver to hyperosmotic media (from 305 to 385 mosmol·kg^{-1}) resulted in a 22±2% ($n = 6$) stimulation of branched-chain amino acid (leucine plus valine plus isoleucine) release from 168±5 to 203±7 nmol/min per g ($n = 6$), owing to a 15–26% increase in the release of the individual amino acids. Because branched-chain amino acids are neither synthesized nor catabolized in rat liver [16], their release into effluent perfusate of isolated perfused rat liver can be used as an estimate of the proteolytic rate [2–4,6,7]. The possibility that the decreased leucine release is explained by an inhibition of leucine transport out of the cell during hypo-osmotic cell swelling is very unlikely, because steady-state release rates are reached, and restoring normo-osmotic perfusion conditions is not followed by an overshoot in leucine release, as one might expect when leucine would accumulate intracellularly during hypo-osmotic cell swelling. Thus these data suggest that liver cell swelling after hypo-osmotic exposure inhibits proteolysis in amino acid-free single-pass-perfused rat liver, whereas proteolysis is stimulated after cell shrinkage during hyper-osmotic exposure.

The findings in Fig. 1 were also confirmed in perfusion experiments taking the release of [^{3}H]leucine into effluent as a measure of proteolysis, when liver proteins had been prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine: [^{3}H]leucine release decreased by 25±3% ($n = 5$) after hypo-osmotic exposure (from 305 to 225 mosmol·kg^{-1}) and returned to baseline levels after restoration of normo-osmotic media. A 28±2% inhibition of [^{3}H]leucine release was also observed when the influent perfusate contained an amino acid mixture in concentrations found physiologically in portal venous blood; this value was 29±3% ($n = 4$) when it contained such an amino acid mixture which was devoid of glutamine (Fig. 2).

During the first 30 s of hypo-osmotic exposure there was a marked increase of liver mass (up to about 20%). Owing to a volume-regulatory K^+ efflux during the first 8 min of hypo-osmotic exposure (for details see [10,12,15]), liver mass then returned to a new steady state which, however, was significantly above the starting value. As shown recently [10], this steady-state liver mass increase is due to cell swelling, because it is not accompanied by an increase of the [^{3}H]inulin space in perfused rat liver. Both the steady-state mass increase and the volume-regulatory K^+ efflux were strongly dependent on the extent of the hypo-osmotic challenge (Fig. 3). There was a close relationship between the hypo-osmoticity-induced inhibition of [^{3}H]leucine release and the hypo-osmoticity-induced steady-state increase in liver mass (Fig. 4), suggesting that cell swelling and/or volume-regulatory K^+ fluxes trigger the inhibition of proteolysis.

As shown recently, glutamine addition to perfused rat liver leads to cell swelling owing to the concentrative uptake of this amino acid [8,10], with cell swelling being maintained throughout exposure to glutamine. Glutamine addition to iso-osmotic perfusion media led to a concentration-dependent inhibition of [^{3}H]leucine release from perfused rat liver, with a time course similar to that observed after hypo-osmotic exposure (compare Fig. 2), demonstrating an inhibition of hepatic proteolysis by glutamine, in line with previous data [2,4]. The extent of the glutamine-induced inhibition of [^{3}H]leucine release was strongly dependent on the glutamine (0.5–2 mM)-induced degree of cell swelling (Fig. 4). As also shown in Fig. 4, the inhibition of [^{3}H]leucine release progressively rose with the degree of liver cell swelling regardless of whether cell swelling was induced by hypo-osmotic exposure or by addition of glutamine during iso-osmotic perfusion.

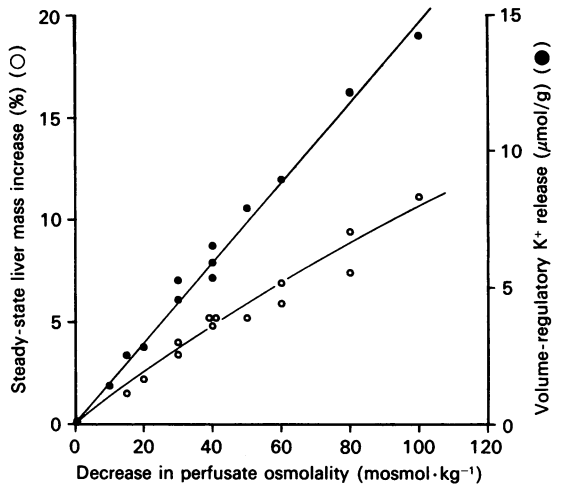

Fig. 3. Effect of hypo-osmotic perfusion on volume-regulatory K^+ efflux and the consecutive steady-state liver mass increase

The influent perfusate contained 0.1 mM-leucine. Hypo-osmoticity was induced by lowering the influent NaCl concentration by 0–50 mM, resulting in the respective decreases in perfusate osmolality. Volume-regulatory K^+ efflux was completed during the first 8 min of hypo-osmotic challenge, and the liver mass reached a steady state which persisted throughout the hypo-osmotic perfusion. Data are from 14 different perfusion experiments.

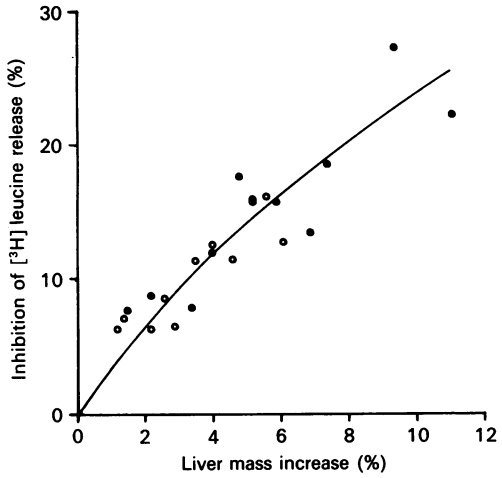

Fig. 4. Relationship between liver cell swelling and the inhibition of [^{3}H]leucine release

Different degrees of liver cell swelling were induced either by hypo-osmotic (205–290 mosmol·kg^{-1}) perfusion (●; data from the experiments shown in Fig. 3) or by addition of glutamine (0.5–2 mM) during normo-osmotic perfusions (○). In all experiments the perfusate contained unlabelled leucine (0.1 mM). Livers were pre-labelled *in vivo* by injection of [^{3}H]leucine (see the Materials and methods section). ^{3}H release and liver mass during normo-osmotic perfusion and in the absence of glutamine were set to 100%. ^{3}H release and liver mass were determined when steady states were obtained, i.e. 30–40 min after hypo-osmoticity- or glutamine-induced cell swelling. Data are from 23 different perfusion experiments.

DISCUSSION

The data in this paper indicate that cell volume changes following aniso-osmotic exposure affect the rate of proteolysis in perfused rat liver. This is observed regardless of whether or not amino acids were present in the perfusion fluid (compare Figs. 1 and 2). These effects persist even after completion of volume-regulatory ion fluxes, indicating that the liver is left in an altered metabolic state. This is in line with the previous suggestion that alterations of liver cell volume act as an important trigger of liver cell function [10–14]. Also, glutamine leads to cell swelling [8,10] and inhibits proteolysis [2,4]. Our present data (Fig. 4) suggest that the anti-proteolytic effect of glutamine [2,4] can largely be explained by a glutamine-induced cell swelling. In line with this is also the observation that a maximal inhibition of proteolysis, which was directly related to glutamine (and not to glutamine-derived ammonia), was obtained at concentrations of 3 mM [4], i.e. at a concentration which also gives a maximal cell swelling response to glutamine [8]. Half-maximal liver cell swelling by glutamine is observed at a concentration of 0.6–0.8 mM [8]. Thus physiological fluctuations of the portal glutamine concentration are expected effectively to modify cell volume and proteolysis. In view of our data and the known concentrative uptake of glutamine into skeletal muscle, one might also speculate that the inhibition of muscle protein breakdown by glutamine [17] might also be due to cell swelling. It should be noted that the anti-proteolytic effect of glutamine in our experiments cannot be ascribed to an increased ammonia formation [18], because glutaminase is largely inactive under the conditions of our study [19].

Other amino acids, such as alanine, serine and glycine, have similarly been shown to elicit liver cell swelling and volume-regulatory responses [8,9] and also to be potent inhibitors of proteolysis [1–7]. It remains to be established to what extent their anti-proteolytic effect is also mediated by cell volume changes. Whereas our data (Fig. 4) strongly suggest that the anti-proteolytic effect of glutamine is due to cell volume changes, the known anti-proteolytic effect of a complete amino acid mixture cannot be explained exclusively by cell volume changes: addition of an amino acid mixture in physiological concentrations inhibited [^{3}H]leucine release from perfused rat liver more than 3-fold as strongly (not shown) as would be predicted from the relationship given in Fig. 4. Furthermore, the known high anti-proteolytic potency of leucine [2] contrasts with its minimal effects on cell volume [8]. This suggests that liver cell volume may only be one factor determining the rate of proteolysis, in addition to other mechanisms involved in proteolysis control by amino acids, ammonia, hormones and the energy state.

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