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Leukotriene and purinergic receptors are involved in the hyperpolarizing effect of glucagon in liver cells

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Abstract

The pancreatic hormone glucagon hyperpolarizes the liver cell membrane. In the present study, we investigated the cellular signalling pathway of glucagon-induced hyperpolarization of liver cells by using the conventional microelectrode method. The membrane potential was recorded in superficial liver cells of superfused mouse liver slices. In the presence of the K^+ channel blockers tetraethylammonium (TEA, 1 mmol/l) and Ba^{2+} ($BaCl_2$, 5 mmol/l) and the blocker of the Na^+/K^+ ATPase, ouabain (1 mmol/l), no glucagon-induced hyperpolarization was observed confirming previous findings. The hyperpolarizing effect of glucagon was abolished by the leukotriene B_4 receptor antagonist CP 195543 (0.1 mmol/l) and the purinergic receptor antagonist PPADS (5 μmol/l). ATPγS (10 μmol/l), a non-hydrolyzable ATP analogue, induced a hyperpolarization of the liver cell membrane similar to glucagon. U 73122 (1 μmol/l), a blocker of phospholipase C, prevented both the glucagon- and ATPγS-induced hyperpolarization. These findings suggest that glucagon affects the hepatic membrane potential partly by inducing the formation and release of leukotrienes and release of ATP acting on purinergic receptors of the liver cell membrane.

Keywords: Glucagon; Hepatocyte; Hyperpolarization; Leukotriene receptor; Purinergic receptor

1. Introduction

Changes of the hepatic membrane potential seem to serve different functions in liver cells. The pancreatic hormone glucagon hyperpolarizes the liver cell membrane under in vitro [1] and in vivo conditions [2,3]. In contrast, glucagon antiserum depolarizes the liver cell membrane in vivo [3] suggesting that glucagon affects the hepatic membrane potential under physiological conditions.

The glucagon-induced hyperpolarization of the liver cell membrane stimulates the metabolism of amino acids [4]. The amino acid alanine, an important gluconeogenic substrate, moves into the liver cell by an electrogenic 1:1 cotransport with Na^+ [5]. The Na^+ electrochemical gradient is the driving force [4] that is increased by the glucagon-induced stimulation of the Na^+/K^+ ATPase and hyperpolarization of the liver cells.

Previous studies have shown that physiological concentrations of glucagon stimulate bile acid secretion in humans [6] and rats [7]. The transport of taurocholate through the hepatocyte membrane into the liver cell occurs via a Na^+-taurocholate cotransport mechanism of 2:1 or greater [8]. That suggests that both the glucagon-induced hyperpolarization of the liver cell membrane and the activation of Na^+/K^+ ATPase by glucagon stimulate the taurocholate uptake of hepatocytes [9].

These investigations suggest the physiological importance of the glucagon-induced hyperpolarization of the hepatic membrane potential. Despite these well defined functions of glucagon, the exact cellular pathway of the glucagon-induced hyperpolarization in liver cells is still unknown. It has already been shown, however, that an increase in K^+ permeability of the liver cell membrane [10] and the activation of the Na^+/K^+ ATPase are involved [1,11].

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The glucagon-induced hyperpolarization is associated with cell shrinkage because of the resulting efflux of K^+ and Na^+ ions in conjunction with enhanced Cl^- efflux [12]. Therefore, we hypothesize that the mechanisms mediating glucagon-induced hyperpolarization of liver cells may be related to the mechanisms of regulatory volume decrease (RVD) of liver cells as induced by exposure to hypoosmotic medium which is also associated with an hyperpolarization of the liver cell membrane due to the activation of the Na^+/K^+ ATPase and an opening of K^+ channels.

Previous studies proposed a model for the intracellular signalling during RVD in Ehrlich ascites tumor cells (EATC) in which intracellular Ca^{2+} ($[Ca^{2+}]_i$), phospholipase $A_{2\alpha}$ ($PLA_{2\alpha}$) and leukotrienes (LT) are involved [13]. Correspondingly, we investigated whether similar mechanisms may be activated by glucagon in liver cells.

Further, it is known that in EATC a hyperpolarization of the plasma cell membrane and an increase of $[Ca^{2+}]_i$ are triggered by extracellular ATP [14]. In addition, besides glucagon extracellular ATP also induces the shrinkage of hepatocytes [12,15]. Therefore, we finally tested whether nucleotides are involved in the cascade mediating the hyperpolarizing effect of glucagon on the liver cell membrane.

The experiments were performed employing superfused mouse liver slices in conjunction with the conventional microelectrode method.

2. Materials and methods

2.1. Animals and maintenance

Adult female mice (NMRI) with a body weight of 25–50 g were used. Mice were fed with a medium-fat standard diet containing 18% fat, 46% carbohydrate, and 13% protein and were adapted to this diet for at least 2 weeks. Water and food were provided ad libitum.

2.2. Liver slice preparation, maintenance, and temperature control

Mice were killed by cervical dislocation and the whole liver was removed quickly and put in a Petri dish filled with warm (37 °C) and oxygenated (95% O_2, 5% CO_2) modified Krebs-Henseleit (KH) bicarbonate buffer (medium: pH 7.4 and 300 mosml/l; containing (mmol/l): NaCl 118; KCl 4.7; $CaCl_2 \times 2H_2O$ 1.3; $MgCl_2 \times 6H_2O$ 0.6; $NaH_2PO_4 \times H_2O$ 1.2; $Na \times HCO_3$ 25). The liver was fixed by applying slight pressure with tweezers while preparing the liver slices (5×5 mm; thickness about 1 mm) with a scalpel blade. After preincubation in oxygenated control buffer, the liver slices were placed in an acrylic superfusion chamber with the encapsulated uncut surface of the liver slice upwards. All microelectrode impalements were of superficial cells on the uncut surface. The viability of this liver slice preparation has been evaluated previously by Wondergem and Castillo [16] who measured similar membrane potentials using superfused mouse liver slices or mouse hepatocytes in primary monolayer culture prepared from whole liver. The liver slices were superfused at a rate of about 28 ml/min with oxygenated buffer. Due to the presence of the glycocalix it is very unlikely that substances released by liver cells do not reach sufficiently high extracellular concentrations to exert autocrine or paracrine effects at this flow rate [17]. All buffer solutions were equiosmotic (300 mosml/l). In the experiment under Ca^{2+} free conditions, $MgCl_2$ concentration was increased (1.2 mmol/l) because of the added ethylenediaminetetraacetic acid disodium (EDTA-Na_2; 5 mmol/l), which was used to bind all remaining Ca^{2+} ions in the extracellular matrix, but which also binds Mg^{2+} ions. The NaCl concentration was reduced accordingly to maintain the osmolarity of 300 mosml/l (Ca^{2+} free medium: pH 7.4 and 300 mosml/l; containing (mmol/l): NaCl 111.5; KCl 4.7; $MgCl_2 \times 6H_2O$ 1.2; $NaH_2PO_4 \times H_2O$ 1.2; $NaHCO_3$ 25, EDTA-Na_2 5).

To investigate the cellular mechanism of the hyperpolarizing effect of glucagon on the liver cell membrane, various blockers, antagonists or agonists were added to buffer solutions. Blockers or antagonists were added to both the control and experimental solutions. NaCl was reduced if necessary to maintain osmolarity at 300 mosml/l. Water-insoluble substances were dissolved in ethanol or dimethyl sulfoxide (DMSO) before adding to the buffer solution (1 ml ethanol or DMSO/1 l KH buffer). In these cases, the control solution also contained the same amount of ethanol or DMSO. In all experiments, the buffer temperature was maintained at 37 °C and monitored continuously with a thermistor (Ebro CTA 1220; Ingolstadt, Germany).

2.3. Fabrication of open-tip microelectrodes

Open-tip microelectrodes were drawn in a horizontal puller (Sachs-Fleming Micropipette Puller PC-84; Sutter Instrument, San Raphael, CA, USA) from microfilament glass capillaries (1.5 mm o.d., 0.86 mm i.d.; A-M Systems, Everett, WA, USA). Pipettes were filled with KCl (0.5 mol/l).

2.4. Measurement of membrane potential (Vm)

The microelectrode was connected by an Ag–AgCl half cell to a high input impedance preamplifier (10^{13} Ω; Biologic VF 180; Echirolles, France). The reference electrode was connected by an Ag–AgCl half cell to the tissue chamber by an agar bridge (4% in KH buffer). Voltage was measured with a digital voltmeter and an oscilloscope (Kikusui COS 5020; Kawasaki City, Japan), and recorded on a two-channel recorder (Rikadenki B-281-L; Kogyo, Japan). The criteria for valid micropipette impalements of the liver cells were: (1) a rapid deflection of the voltage trace on advancing the microelectrode into the

liver slice; (2) a stable voltage trace within 2 mV for at least 10 s; and (3) return of the voltage trace to within 2 mV of the baseline when the microelectrode was withdrawn. The resistance of open-tip microelectrodes (20–50 MΩ) was measured once before every impalement by passing AC pulses (I=1 nA; frequency 1000 Hz). The membrane potential was measured twice at each time point. The mean of these two values was used for the statistical evaluation of the results.

2.5. Standard experimental protocol

At least eight liver slices from at least two mice were used for each experimental condition. After the preparation of the liver slices, they were kept in warm oxygenated buffer until use. After transfer to the superfusion chamber, the liver cell membrane potential was measured after 5 and 10 min (Fig. 1: time −5 and 0 min). Then, the superfusion solutions were switched to the respective experimental solutions containing e.g. glucagon or ATPγS. Subsequently, the membrane potential was measured in 3 min intervals for 24 min, twice at each time point to use the mean of these two values for the statistical evaluation. This allowed a mean incubation time of 10 min (preincubation)+24 min (experimental solution)=34 min.

2.6. Chemicals used

Glucagon was from Novo Nordisk (Küsnacht, Switzerland). Tetraethylammonium (TEA), ouabain, gadolinium chloride ($GdCl_3$, Gd^{3+}), EDTA-Na_2, barium chloride ($BaCl_2$, Ba^{2+}), quinacrine, nordihydroguaiaretic acid (NDGA), α-pentyl-3-[2-quinolinylmethoxy]benzyl alcohol (REV 5901) indomethacin, pyridoxal phosphate-6-azo-benzene-2,4-disulfonic acid (PPADS), adenosine 5′-[γ-thio]triphosphate (ATPγS), uridine 5′-triphosphate (UTP), 1-(6-[([17β]-3-Methoxyestra-1,3,5 [10]-trien-17-yl)amino]-hexyl)-1H-pyrrole-2,5-dione (U 73122) and 1-(6-[([17β]-3-Methoxyestra-1,3,5 [10]-trien-17-yl)amino]hexyl)-2,5-pyrrolidinedione (U 73343), ethanol, and DMSO were all from Sigma (Buchs, Switzerland). CP 195543 was kindly provided by Pfizer Inc. (Groton, CT, USA).

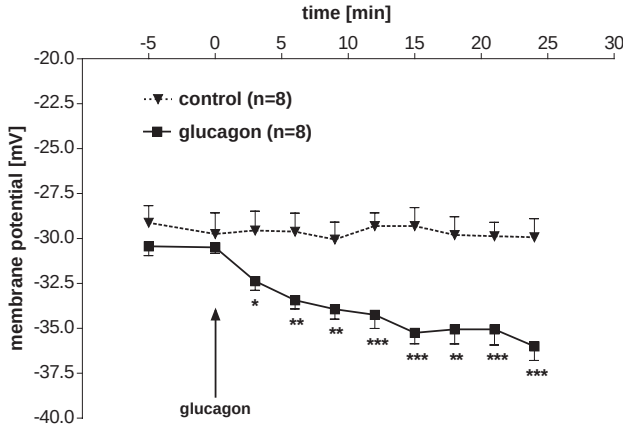

Fig. 1. Influence of glucagon (10^{-7} mmol/l) on the potential of liver cell membrane. Superfusion with Krebs–Henseleit buffer from t=−10 to t=0 min. Then, the glucagon superfusion was started. *P<0.05; **P<0.01; ***P<0.001 (unpaired t-test).

2.7. Statistical evaluation

All values are presented as mean±standard error (x±S.E.). As mentioned above, the membrane potential of two liver cells was measured at each individual time point. The mean of these two values was used for the statistical analysis.

When investigating the time course of the hyperpolarizing effect of glucagon, repeated measurement ANOVA was used. Differences in the hyperpolarizing effect observed under different conditions, i.e. differences in the membrane potential measured with the experimental solution compared with the respective control medium, were evaluated using the unpaired Student's t-test, or ANOVA with the Student–Newman–Keuls post hoc test if more than two groups were compared. In all cases, a P value <0.05 was considered significant.

3. Results

The superfusion of hepatocytes with glucagon (10^{-7} mol/l) [1] led to significant hyperpolarization (approx. 5–6 mV) of the liver cell membrane (Fig. 1). Since the time course of glucagon-induced hyperpolarization was very similar in all subsequent experiments, further analysis was performed at t=21 min after the administration of glucagon or ATPγS. The K^+ channel blockers TEA (1 mmol/l) and Ba^{2+} ($BaCl_2$, 5 mmol/l) [18] completely abolished this hyperpolarization (Fig. 2). Ouabain (1 mmol/l), an antagonist of the Na^+/K^+ ATPase, also prevented the glucagon-induced hyperpolarization (Fig. 2). TEA, Ba^{2+} and ouabain used alone showed no significant effect on the membrane potential (results not shown; see also [19]). Furthermore, in the presence of Gd^{3+} ($GdCl_3$; 100 μmol/l) [20], glucagon-induced hyperpolarization was not observed (Fig. 3).

In EATC, an involvement of phospholipases and LT in the cellular signalling pathway of RVD has already been shown [13]. This led us to investigate the involvement of phospholipase A_2 (PLA_2) being activated by Ca^{2+} in the effect of glucagon using the PLA_2 inhibitor quinacrine (200 μmol/l) [21]. In the presence of quinacrine the hyperpolarizing effect of glucagon was not observed (Fig. 3). In the presence of the lipoxygenase blocker NDGA (10 μmol/l) [22,23] inhibiting the first step of LT synthesis, the hyperpolarizing effect of glucagon was also not observed (Fig. 3). Unlike the LT D_4 receptor antagonist REV 5901, which did not influence glucagon-induced hyperpolarization (results not shown), the specific LT B_4 receptor antagonist

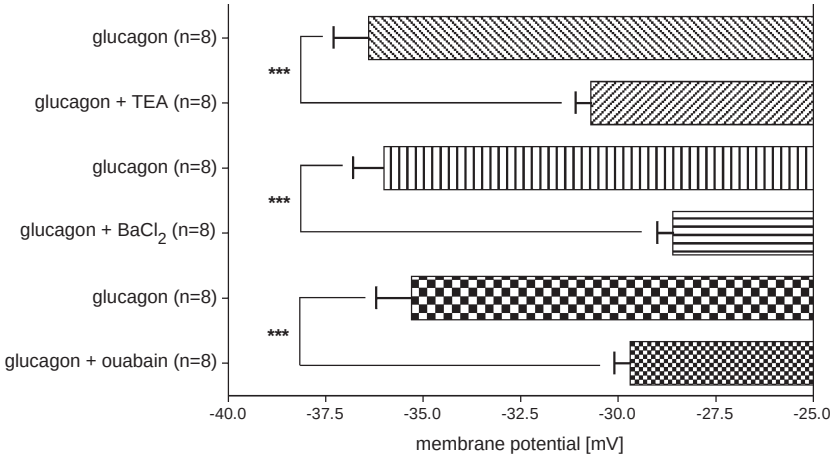

Fig. 2. Influence of the K^+ channel blockers TEA (1 mmol/l) and $BaCl_2$ (5 mmol/l) and the blocker of the Na^+/K^+ ATPase ouabain (1 mmol/l) on the hyperpolarization induced by the superfusion of mouse liver slices with glucagon (10^{-7} mmol/l) at time point 21 min after starting the glucagon superfusion. *P<0.05; **P<0.01; ***P<0.001 (unpaired t-test).

CP 195543 (0.1 mmol/l) [24] completely prevented the hyperpolarization induced by glucagon (Fig. 4).

Finally, the purinergic receptor antagonist PPADS (5 μmol/l; antagonist for $P2Y_{1,\ 4,\ 6}$ receptor) [25] completely abolished the hyperpolarizing effect of glucagon (Fig. 5). Further, similar to glucagon, ATPγS (10 μmol/l) [26], an ATP analogue resistant to hydrolysis by ecto-ATPase, induced an hyperpolarization of the liver cell membrane (Fig. 5). U 73122 (1 μmol/l) [27], a blocker of phospholipase C (PLC), which forms part of the second messenger system of purinergic and glucagon receptors, almost completely abolished the glucagon- and ATPγS-induced hyperpolarization of the liver cell membrane (Fig. 6).

4. Discussion

The present study shows that blockers of K^+ channels and non-selective cation channels inhibit the glucagon-induced hyperpolarization, suggesting that this hyperpolarization occurs in response to the activation of K^+ channels and non-selective cation channels. Further, Ca^{2+} ions and leukotriene and purinergic receptors also seem to be involved in the cellular signalling pathway of the glucagon-induced hyperpolarization of the liver cell membrane.

In the present study, we confirmed the involvement of K^+ channels and the Na^+/K^+ ATPase in the hyperpolarizing effect of glucagon under the current experimental conditions [1,10,11,28] because the glucagon-induced hyperpolarization was not observed in the presence of the K^+ channel blockers TEA and Ba^{2+} or the inhibitor of the Na^+/K^+ ATPase, ouabain. These observations show interesting parallels with previous data about the superfusion of liver cells with hypoosmotic medium, which also leads to hyperpolarization [29]. Lutz and colleagues have shown that the activation of K^+ channels and the Na^+/K^+ ATPase are involved in the latter effect [19] which is probably linked to a regulatory volume decrease (RVD) occurring by the opening of K^+ channels and additionally of anion

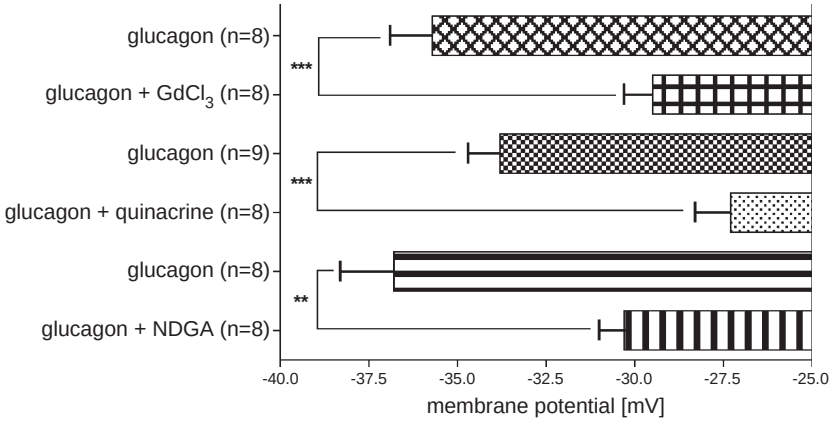

Fig. 3. Influence of the non-selective cation channel blocker $GdCl_3$ (100 μmol/l), of the inhibitor of phospholipase A_2 quinacrine (200 μmol/l) and of the inhibitor of lipoxygenase NDGA (10 μmol/l) on the hyperpolarization induced by the superfusion of mouse liver slices with glucagon (10^{-7} mmol/l) at time point 21 min after starting the glucagon superfusion. *P<0.05; **P<0.01; ***P<0.001 (unpaired t-test).

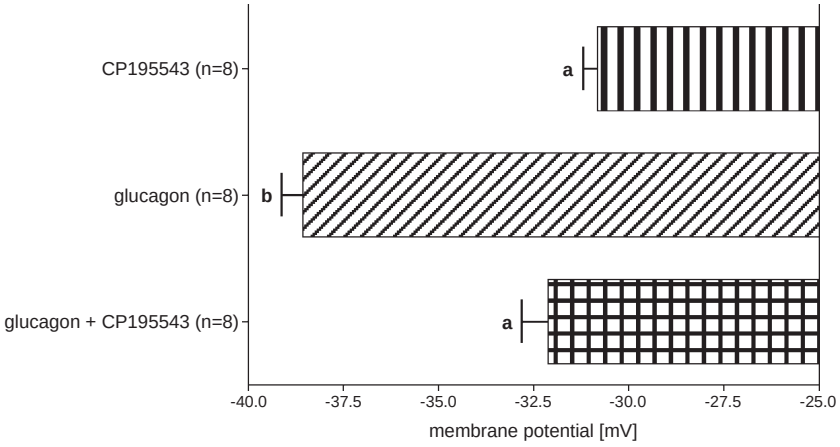

Fig. 4. Influence of the leukotriene B_4 receptor antagonist CP 195543 (0.1 mmol/l) on the hyperpolarization induced by the superfusion of mouse liver slices with glucagon (10^{-7} mmol/l) at time point 21 min after starting the glucagon superfusion. Significant differences are indicated by different letters ($P<0.05$).

channels [12]. K^+ efflux via K^+ channels and an activation of the Na^+/K^+ ATPase seem to be functionally coupled [30]. This may explain why both the blockade of K^+ channels and the inhibition of the Na^+/K^+ ATPase prevented the glucagon-induced hyperpolarization.

Several lines of evidence suggest that Ca^{2+} may play an important role as intracellular messenger in the signalling pathway of the glucagon-induced hyperpolarization. First, previous studies have shown that glucagon increases the level of $[Ca^{2+}]_i$ in perfused liver [31]. Second, non-selective cation channels which are permeable to Na^+, K^+, and also to Ca^{2+} and which are activated by hypoosmotic-induced cell swelling are present in rat liver cells [32]. According to our observations, they also seem to be involved in the hyperpolarization induced by glucagon, because Gd^{3+}, an inhibitor of non-selective cation channels, abolished this hyperpolarization. Similarly, the hyperpolarization due to regulatory volume decrease in hypoosmotic cell swelling was also prevented by a blocker of non-selective cation channels, flufenamic acid [19].

We therefore hypothesize that Ca^{2+} influx occurs via non-selective cation channels, increases $[Ca^{2+}]_i$ and participates in the opening of K^+ channels which elicits an hyperpolarization of the liver cell membrane [33]. It seems that K^+ efflux via K^+ channels surmounts cation influx via non-selective cation channels and thus produces an hyperpolarization. Further, it is of interest in this context that the hyperpolarization of liver cells during regulatory volume decrease was significantly attenuated by apamin, a specific blocker of Ca^{2+} dependent K^+ channels with low conductance [19]. This hyperpolarization, in addition, appeared to be diminished (45%) by iberiotoxin, a blocker of Ca^{2+} dependent K^+ channels with large conductance, indicating that more than one K^+ channel was involved [19]. Possibly, this also applies to the hyperpolarization elicited by glucagon. Therefore, further studies are necessary to identify the various K^+ channels involved in the glucagon-induced hyperpolarization in mouse liver slices.

The non-selective cation channels in liver cells which are supposed to mediate Ca^{2+} influx and hence trigger the

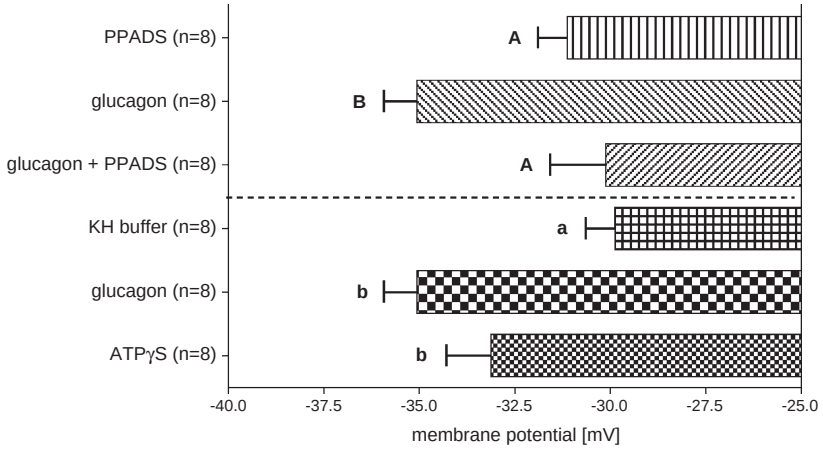

Fig. 5. Influence of the purinergic receptor antagonist PPADS (5 μmol/l; capital letters) on the hyperpolarization induced by the superfusion of mouse liver slices with glucagon (10^{-7} mmol/l) and of ATPγS (10 μmol/l; small letters) on the potential of liver cell membrane at time point 21 min after starting the glucagon superfusion. Significant differences are indicated by different letters ($P<0.05$).

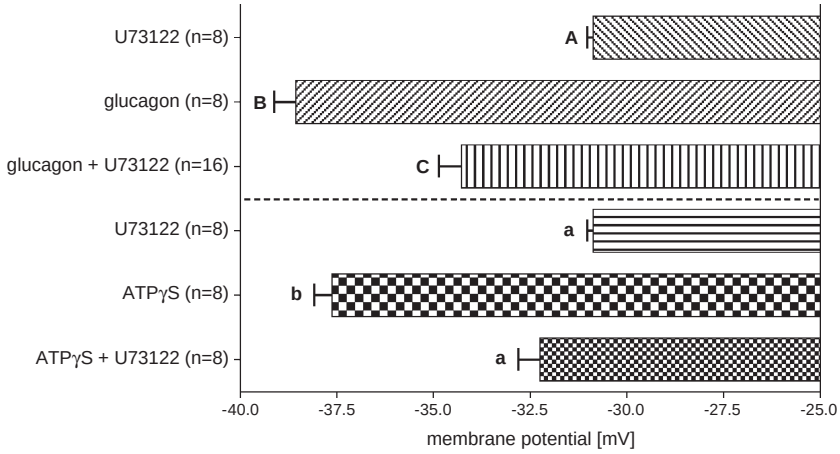

Fig. 6. Influence of U 73122 (1 μmol/l), an inhibitor of phospholipase C, on the hyperpolarization induced by the superfusion of mouse liver slices with glucagon (10^{-7} mmol/l; capital letters) or with ATPγS (10 μmol/l; small letters) at time point 21 min after starting the superfusion. Significant differences are indicated by different letters ($P<0.05$).

signalling cascade leading to glucagon-induced hyperpolarization may be activated by cAMP resulting from the binding of glucagon to its G protein-coupled glucagon receptor [34].

Besides the formation of cAMP after glucagon binding to its receptor, an increase in the production of inositol triphosphate (IP_3) and diacylglycerol occurs. The increase in intracellular IP_3 causes Ca^{2+} release from endoplasmatic reticulum and $[Ca^{2+}]_i$ consequently increases further [35,36] which may contribute to the above affects. The activation of protein kinase C by diacylglycerol leads to a Ca^{2+} dependent increase in the activity of the Na^+/K^+ ATPase [37] and this may also contribute to the glucagon-induced hyperpolarization since the latter effect was blocked by ouabain (see above).

The present experiments also support the hypothesis that arachidonic acid metabolites are involved in glucagon-induced hyperpolarization of the liver cell membrane, because both quinacrine, an inhibitor of PLA_2, and NDGA, a blocker of lipoxygenase, inhibiting leukotriene synthesis, abolished the hyperpolarizing effect of glucagon. Further, the glucagon-induced hyperpolarization was eliminated by the LT B_4 receptor antagonist CP 195543, suggesting that LT B_4 is involved in the hyperpolarization. Indomethacin, a blocker of cyclooxygenase, did not effect the glucagon-induced hyperpolarization under our experimental conditions (data not shown). Therefore, prostaglandines or thromboxanes do not appear to be involved.

LT B_4 is moved via LT B_4 transporter from the intra- to extracellular space [38]. Two LT B_4 receptor subtypes have been described: B-LT_1 with high and B-LT_2 with low affinity for LT B_4 [38]. Since CP 195543 is a B-LT_1 and B-LT_2 antagonist [24] it is not clear which type of these receptors is involved in the glucagon-induced hyperpolarization. However, the signalling pathway of the B-LT_2 subtype involves Ca^{2+} mobilization either via G protein using IP_3 as second messenger system or via the opening of Ca^{2+} permeable channels such as non-selective cation channels [39]. The LT B_4 induced Ca^{2+} mobilization may therefore amplify the increase in $[Ca^{2+}]_i$ that finally may directly and/or indirectly activate K^+ channels with the K^+ efflux inducing the hyperpolarization. Whether LT B_4 is derived from hepatocytes or the non-parenchymal cells of the liver is still unknown. It is however possible that glucagon, which also affects Kupffer cells in liver [40], may stimulate LT B_4 release from Kupffer cells which then may act on hepatocytes to bring about the described effects. However, the hyperpolarizing effect of glucagon occurring in isolated hepatocytes [41] is not in accordance with this proposal. Therefore, it remains to be investigated whether hepatocytes produce leukotrienes in response to glucagon.

Previous studies indicate that extracellular nucleotides are also involved in the mobilization of intracellular Ca^{2+} ions in hepatocytes [26,42]. Purinergic receptors are surface receptors for extracellular nucleotides and can be subdivided into two groups: the P2X receptors that are ligand-gated ion channels and the P2Y receptors being G-protein coupled [43]. Interestingly, similar to glucagon, extracellular ATP induced cell shrinkage in isolated perfused rat liver [15]. Further, in Ehrlich ascites tumor cells, an increase of $[Ca^{2+}]_i$ and an hyperpolarization of the plasma cell membrane occurred after the addition of extracellular ATP [14]. Extracellular ATP and UTP increased $[Ca^{2+}]_i$ by activation of P2Y receptors coupled to the IP_3 cellular pathway in single primary human hepatocytes [44]. According to our findings, similar mechanisms may be instrumental in glucagon-induced hyperpolarization of liver cells because the purinergic receptor antagonist PPADS, antagonizing $P2Y_{1,\ 4,\ 6}$ receptors [43], abolished the glucagon-induced hyperpolarization. Therefore, purinergic receptors seem to be involved.

As mentioned, Ca^{2+} seems to play an important role as intracellular messenger in the signalling pathway of the glucagon-induced hyperpolarization. In isolated rat hepatocytes the glycogen phosphorylase is regulated by ATP acting on subtypes of P2Y receptors that leads to an increase of $[Ca^{2+}]_i$ [45]. Further, the superfusion of liver cells with

ATPγS, a non-hydrolyzable ATP analogue, also produced a hyperpolarization of liver cells. All cloned and functionally defined P2Y receptors are able to activate PLC which leads to an increase in IP_3 [43,46]. IP_3 then triggers Ca^{2+} release from the endoplasmatic reticulum [47,48]. Our studies have shown that both the glucagon- and the ATPγS-induced hyperpolarizations were abolished by using the PLC inhibitor U 73122 but not its inactive analogue U 73343. These results can be regarded as further evidence for the involvement of purinergic receptors in glucagon-induced hyperpolarization and suggest the activation of the IP_3 pathway with subsequent Ca^{2+} release after binding of glucagon to its receptor [36,48].

In various cells, ATP is secreted via anion channels and activates purinergic receptors at the outside of the cell membrane [49]. Moreover, hypoosmotic-induced cell swelling leading to regulatory volume decrease stimulates ATP release via anion channels [50] or separate ATP channels [51]. It thus seems plausible that glucagon also activates ATP-permeable anion channels. ATP may then amplify the activation of K^+ channels via the pathway outlined above. It therefore seems that purinergic receptors may represent a physiological signalling mechanism in hepatocytes in regard to glucagon-induced hyperpolarization. Extracellular nucleotides may be important autocrine and paracrine signalling molecules influencing liver function. The hyperpolarizing effect of glucagon may enhance the driving force for electrogenic Na^+-coupled cotransport of various substrates into hepatocytes.

In summary, our studies indicate that the hyperpolarizing effect of glucagon on the liver cell membrane involves multiple mechanisms. The following hypothesis about the cellular mechanism cascade is proposed: Ca^{2+} influx via non-selective cation channels and Ca^{2+} mobilization from intracellular stores are stimulated by glucagon via cAMP and IP_3, respectively. Increasing $[Ca^{2+}]_i$ in addition leads to an activation of PLA_2. PLA_2 activation through various steps leads to the formation of LT B_4 which leaves the cell via a LT B_4 transporter and binds to its membrane-bound LT B_4 receptor. The activation of this receptor elicits a further increase in $[Ca^{2+}]_i$ via the IP_3 pathway and/or an activation of Ca^{2+} permeable channels of the cell membrane. Finally, the release of nucleotides through cAMP activated anion channels may further amplify the glucagon-induced increase in $[Ca^{2+}]_i$. The hyperpolarization of the cell membrane finally results from the activation of Ca^{2+} dependent K^+ channels and maybe other K^+ channels in conjunction with the activation of the Na^+/K^+ ATPase.

It is not yet definitely known how the different components depend on each other. Interestingly, the hyperpolarizing effect of glucagon was blocked completely even though only one of the components, the ion channel pathway, the LT or extracellular nucleotide pathway, was inhibited. This suggests that the blockade of one of these various components may be sufficient to reduce $[Ca^{2+}]_i$ beyond the threshold level being necessary for the glucagon-induced hyperpolarization. Therefore, it seems plausible that a common and simultaneous activation of all components of the cellular signalling pathway is necessary to produce the glucagon-induced hyperpolarization of the liver cell membrane.

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