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Depolarization of the liver cell membrane by metformin

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Abstract

Metformin (1,1-dimethylbiguanide; MET) is used in the treatment of type 2 diabetes mellitus. MET's antihyperglycemic action depends at least in part on its inhibitory effect on hepatic gluconeogenesis. As to gluconeogenesis from amino acids (e.g. L-alanine), this is associated with an inhibition of L-alanine uptake into hepatocytes. Since this uptake is mediated by an electrogenic transport mechanism, the aim of the present study was to investigate whether MET has an influence on the liver cell membrane potential which might explain its inhibitory effect on L-alanine uptake. The experiments were performed in vivo in anesthetized rats and in vitro using superfused mouse liver slices with the conventional microelectrode technique. In vivo, MET (160 mg/kg intraperitoneally (i.p.)) significantly depolarized (dV) the liver cell membrane by 6 mV. MET (1 mmol/l) also depolarized the liver cell membrane in vitro (e.g. 15 min after start of superfusion: $dV = 8$ mV). MET's effect was at least partly reversible. Glucagon (10^{-7} mol/l), which hyperpolarized the liver cell membrane, abolished MET's effect. Further, the MET-induced depolarization was completely absent during superfusion with low Cl^- ($[Cl^-] = 27$ mmol/l) medium, and significantly attenuated by the Cl^- channel blocker NPPB (25 μmol/l). While MET's effect was only somewhat attenuated by blockade of the $Na^+/K^+/2Cl^-$ cotransporter or by superfusion with (HCO_3^--free) HEPES buffer, the carboanhydrase blocker acetazolamide (1 mmol/l) or blockade of the HCO_3^-/Cl^- exchanger by DIDS (100 μmol/l), which, however, also blocks Cl^- channels, abolished its effect. The depolarization of the liver cell membrane by MET was unaffected by a blockade of K^+ channels with Ba^{2+}, a blockade of the Na^+/K^+ pump or superfusion with low Na^+ medium ($[Na^+] = 26$ mmol/l). According to these results, the MET-induced depolarization of the liver cell membrane could be due to an activation of the Cl^-/HCO_3^- exchanger and thus depend on intracellular HCO_3^- formation. This activation could then lead to a disturbance of the equilibrium between intra- and extracellular Cl^- and therefore to an enhanced Cl^- efflux via Cl^- channels. It is plausible that the depolarizing effect induced by MET is associated with its inhibitory effect on gluconeogenesis by inhibiting uptake of L-alanine and other amino acids into hepatocytes.

Keywords: Metformin; Membrane potential; Liver; Antihyperglycemic effect; Amino acid uptake

1. Introduction

The biguanide metformin (1,1-dimethylbiguanide; MET) is used as an orally active antihyperglycemic drug in the treatment of human type 2 diabetes mellitus [1]. In contrast to another group of orally effective antidiabetics, the sulfonylureas, which act mainly by enhancing insulin secretion from pancreatic β cells, the mechanism of MET's antihyperglycemic action remains controversial [2]. While part of MET's antihyperglycemic effect may depend on reduced intestinal glucose absorption [3], its main action seems to

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be due to improving insulin sensitivity in peripheral insulin target tissues and suppressing hepatic glucose output [1,2].

Apart from MET inhibiting hepatic gluconeogenesis and hence hepatic glucose output by activation of pyruvate kinase [4,5], MET also appears to reduce the supply of gluconeogenic substrates, such as L-lactate or L-alanine. A recent study suggested that the inhibitory effect of MET on gluconeogenesis from L-lactate is secondary to an inhibition of hepatic L-lactate uptake [6], which is mainly mediated by a carrier-mediated transport system [7,8]. Further, numerous studies suggest that the inhibitory effect of MET and of other biguanides on hepatic gluconeogenesis is also secondary to an inhibition of L-alanine uptake into liver cells [9–11].

Since this uptake is mediated by an electrogenic Na^+-coupled transport mechanism [12–16] and therefore depends on the membrane potential, the first aim of the present study was to investigate whether MET affects the hepatic membrane potential. As the membrane potential was reduced by MET, additional experiments aimed at clarifying the ionic mechanisms underlying this effect. Finally, we wanted to find out whether MET's effect on the liver cell membrane potential is counteracted by glucagon which has been shown to hyperpolarize liver cells [17,18]. The experiments were performed under in vivo conditions in anesthetized rats and under in vitro conditions using superfused mouse liver slices with the conventional microelectrode technique.

2. Research design and methods

2.1. In vivo experiments

2.1.1. Animals and maintenance

Adult male Sprague–Dawley rats (OFA; BRL, Füllinsdorf, Switzerland) with an approximate body weight of 250 g were used for the experiments. Rats were fed ad libitum with a diet containing 18% fat, 46% carbohydrate and 13% protein [19]. Rats were adapted to this diet for at least 2 weeks and had ad libitum access to food and water until the experiment.

2.1.2. Experimental protocol

Rats were injected intraperitoneally (i.p.) with MET (160 mg/kg (1 mmol/kg)) made up with distilled water to yield an isotonic solution. Injection of 0.9% NaCl served as control. Rats were anesthetized with a mixture of ketamine and xylazine approx. 20 min after the injection. Ten minutes later, i.e. 30 min after the injection of MET or NaCl, the liver cell membrane potential was measured in the left lateral liver lobe as described before [20,21]. Briefly, the liver was exposed by laparotomy and fixed with a nictating forceps after Demarres to prevent movements of the liver lobe due to respiration. The upper limb of this forceps is open, allowing puncturing of liver cells by the microelectrode.

Superficial liver cells on the parietal surface of the liver were punctured with the microelectrode to measure the liver cell membrane potential. The reference electrode was placed in the abdominal cavity filled with Krebs–Henseleit solution.

2.2. In vitro experiments

2.2.1. Animals and maintenance

Adult female mice (NMRI; BRL) with a body weight of 30–50 g were used for the experiments. Mice were fed the same diet and handled as described above for rats.

2.2.2. Liver slice preparation, maintenance and temperature control

Mice were killed by cervical dislocation and the median lobe of the liver was removed quickly and placed on gauze moistened with modified Krebs–Henseleit bicarbonate buffer (Table 1). The liver lobe was fixed by applying slight pressure with a glass microscope slide while preparing liver slices (5×5 mm; thickness about 1 mm) with a scalpel blade. After incubation in oxygenated (95% O_2, 5% CO_2) control buffer (Table 1; 37°C), the liver slices were placed in an acrylic superfusion chamber with the encapsulated uncut surface of the liver slice upward. All microelectrode impalements were of superficial cells on the uncut surface. Viability of this liver slice preparation has been evaluated previously by Wondergem and Castillo [22] 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 12 ml/min with oxygenated buffer (Table 1).

The composition of the buffer solutions used is shown in Table 1. All buffer solutions were equiosmotic. When MET, ouabain or $BaCl_2$ was added to the solutions, the NaCl concentration was reduced accordingly (by 0.5 mmol/l or 7.5 mmol/l, respectively). The measured osmolality of the buffer solutions was 288 ± 1 mosmol/l.

Part of the experiments were performed with superfusion solutions with a reduced Na^+ (approx. 26 mmol/l) concentration. In this case, NaCl was replaced equiosmotically with choline chloride. To investigate the influence of bicarbonate on the depolarizing effect of metformin, a bicarbonate-free superfusion solution buffered with *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES) was used. A similar bicarbonate-free buffer solution with a reduced Cl^- (approx. 27 mmol/l) concentration was also used. NaCl was replaced with sodium D-gluconate and the concentration of $CaCl_2$ was increased to 10 mmol/l to account for Ca^{2+} chelating by gluconate. In all experiments, the buffer temperature was maintained at 37°C and monitored continuously with a thermistor (Ebro CTA 1220; Ingoldstadt, Germany).

2.2.3. Standard experimental protocol

Eight liver slices from at least two mice were used for each experimental condition. After preparation of the liver slices, slices were kept in warm oxygenated control buffer until use. After transfer to the superfusion chamber, the liver cell membrane potential was measured after 5 and 10 min. Then, the superfusion solutions were switched to the respective experimental solutions and subsequently, the membrane potential was measured in 3 min intervals. At each time point, the membrane potential was measured twice. The mean of these two values was used for the statistical evaluation of the results (see below). When the influence of various blockers (see below) on the depolarizing effect of metformin on the liver cell membrane was investigated, the respective blocker was added to both the control and the experimental solutions.

2.3. General procedure

2.3.1. 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).

2.3.2. Measurement of membrane potential (V_m)

The microelectrode was connected by an Ag-AgCl half-cell to a high input impedance preamplifier (10^{13} Ω; Biologic VF 180; Echirolles, France). For the in vivo experiments, the reference electrode (Ag-AgCl) was placed in the abdominal cavity filled with Krebs–Henseleit solution. In the in vitro setup, the reference electrode was connected by an Ag-AgCl half-cell to the tissue chamber by an agar (4% in Krebs–Henseleit buffer) bridge.

Voltage was measured with a digital voltmeter and an oscilloscope (Kikusui COS 5020 TM; Kawasaki

Table 1
Composition of the buffer solutions used for superfusion of liver slices in the in vitro experimental setup

	Krebs–Henseleit buffer	HCO_3^--free HEPES buffer	Low Na^+ buffer	Low Cl^- HEPES buffer
NaCl	118	123	–	–
KCl	4.7	4.7	4.7	4.7
$CaCl_2$	2.56	2.56	2.56	10
$MgCl_2$	1.3	1.3	1.3	1.3
NaH_2PO_4	1.15	1.15	1.15	1.15
$NaHCO_3$	25	–	25	–
Sodium D-gluconate	–	–	–	112.0
HEPES	–	10	–	10
Choline chloride	–	–	118	–

City, Japan) and recorded on a two-channel recorder (Rikadenki B-281-L; Kogyo, Japan). Criteria for valid micropipette impalements of liver cell 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; (3) return of the voltage trace to within 2 mV of the baseline when the microelectrode was withdrawn. Resistance of open tip microelectrodes (20–50 MΩ) was measured once before every impalement by passing AC pulses ($I = 1$ nA; frequency 1000 Hz).

In the in vivo setup, the membrane potential of five liver cells was measured, and the mean of these five values was calculated. Only one such mean was obtained from each animal. The mean coefficient of variation for the membrane potential measured in different cells of a liver lobe was $4.2 \pm 0.5\%$. In the in vitro experiments, 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 (see below).

2.3.3. Chemicals used

MET, acetazolamide, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), bumetanide, HEPES and ouabain were all from Sigma (Buchs, Switzerland). Glucagon was from Novo Nordisk (Küsnacht, Switzerland), and $BaCl_2$ from Siegfried (Zofingen, Switzerland).

2.3.4. Statistical evaluation

All values are presented as mean ± standard error of the mean ($x \pm$ S.E.). As mentioned, the membrane potential of two liver cells was measured at each individual time point in the in vitro setup. The mean of these two values was used for the statistical analysis. Similarly, the membrane potential of five liver cells was measured in the in vivo setup in each rat. The mean of these five values was then used for further analysis.

When investigating the time course of the hyperpolarizing effect of MET or glucagon, repeated measures 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.

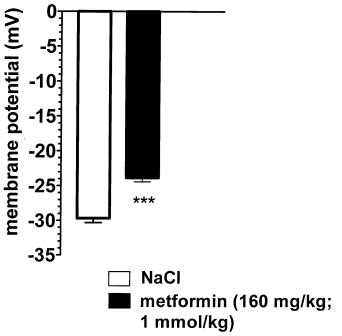

Fig. 1. Influence of MET (160 mg/kg body weight (1 mmol/kg)) injected i.p. on the liver cell membrane potential in anesthetized rats 30 min after injection. Injection of NaCl solution served as control. Values are means ± S.E.M. ***Significant difference between MET and NaCl ($P < 0.001$; unpaired Student's *t*-test; $n = 6$ for all groups).

3. Results

3.1. In vivo experiments

The membrane potential was measured 30 min after i.p. injection of MET (160 mg/kg body weight (1 mmol/kg)) or NaCl solution (control) in anesthetized rats. While the membrane potential measured under control conditions was similar to that in previous studies performed under similar conditions [20,21], MET significantly depolarized the liver cell membrane by about 6 mV (Fig. 1).

3.2. In vitro experiments

Under in vitro conditions using superfused mouse liver slices, MET (0.5 mmol/l, 1 mmol/l) caused a concentration-dependent depolarization of the liver cell membrane by 5 mV (dV; 0.5 mmol/l) and 8 mV (1 mmol/l; Fig. 2A). Superfusion with Krebs–Henseleit bicarbonate buffer which served as control did not affect the membrane potential over time (Fig. 2A). MET's effect became significant after 6–9 min from the start of superfusion and reached a plateau after 9 min (Fig. 2A). MET's effect was at least

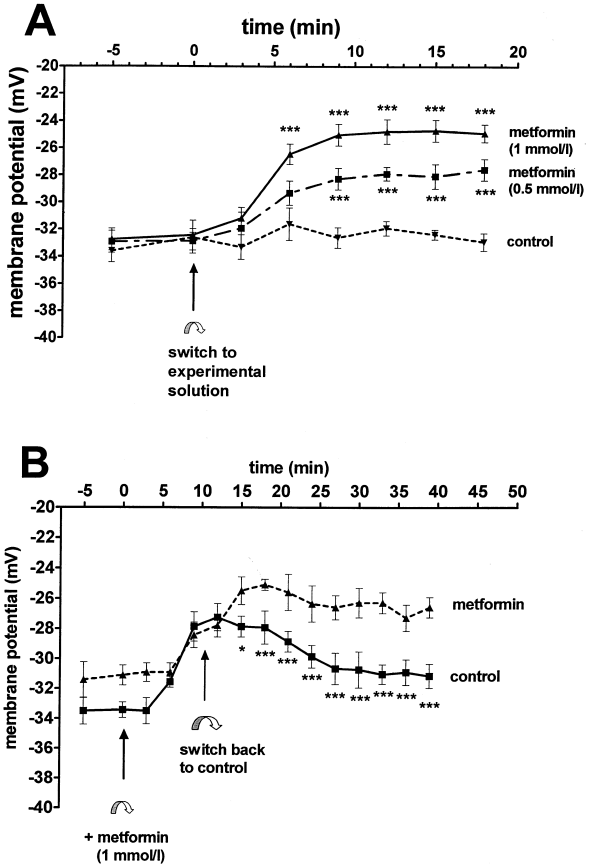

Fig. 2. (A) Influence of MET (0.5 mmol/l, 1 mmol/l) on the liver cell membrane potential of mouse liver slices. Superfusion with Krebs–Henseleit bicarbonate buffer (control) from $t=-10$ min to $t=0$ min. Then MET was added. (B) Reversibility of the depolarizing effect of MET (1 mmol/l). *,***Significant difference between metformin and control ($^{*}P<0.05$ or $^{***}P<0.001$, respectively; repeated measures ANOVA with the Student–Newman–Keuls post hoc test; $n=8$ for all groups).

partly reversible when switching back to superfusion with control solution (Fig. 2B).

The depolarizing effect of MET (1 mmol/l) was completely abolished under low Cl^- conditions ($[Cl^-]=27$ mmol/l; $dV=1$ mV) and significantly reduced when the Cl^- channel blocker NPPB (25 μmol/l; $dV=3$ mV) was added to the Krebs–Henseleit bicarbonate buffer (Fig. 3). The blocker of the $Na^+/K^+/2Cl^-$ cotransporter bumetanide (100 μmol/l in Krebs–Henseleit buffer) somewhat reduced the depolarizing effect of metformin ($dV=4$ mV; Fig. 3). As for low Cl^- conditions and NPPB, bumetanide also reduced the basal membrane potential compared to normal Cl^-, NPPB-free or bumetanide-free conditions (Fig. 3).

While MET's effect on the liver cell membrane potential was reduced ($dV=4$ mV vs. $dV=8$ mV ($P<0.05$) 18 min after the start of superfusion) when using (bicarbonate-free) HEPES buffer instead of Krebs–Henseleit bicarbonate buffer, inhibition of carboanhydrase by acetazolamide (1 mmol/l in Krebs–Henseleit bicarbonate buffer; $dV=1$ mV) or inhibition of the Cl^-/HCO_3^- exchanger by DIDS (100 μmol/l in Krebs–Henseleit bicarbonate buffer; $dV=0$ mV), which, however, also blocks Cl^- channels, abolished its effect (Fig. 4). In contrast, neither blockade of K^+ channels by Ba^{2+} (5 mmol/l), nor inhibition of the Na^+/K^+-ATPase by ouabain (1 mmol/l) or use of low Na^+ superfusion buffer

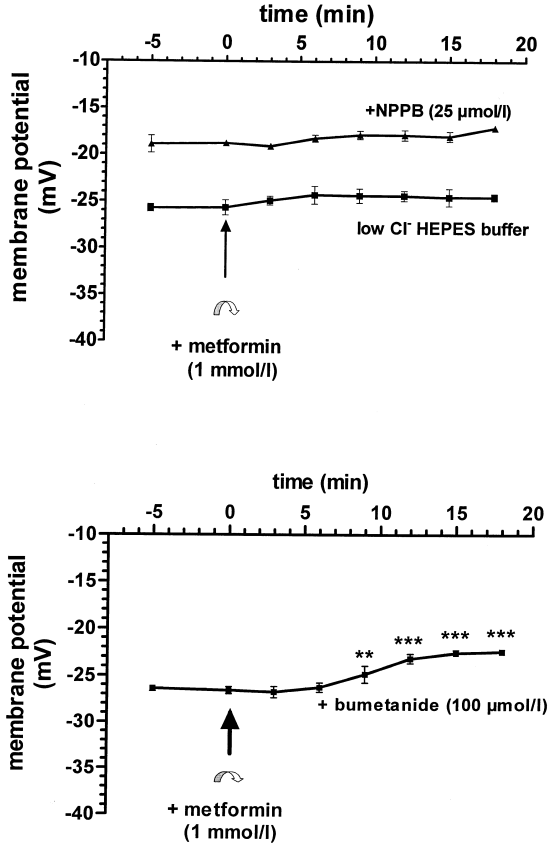

Fig. 3. Influence of superfusion with low Cl^- HEPES buffer ($[Cl^-]=27$ mmol/l), the Cl^- channel blocker NPPB (25 μmol/l; in Krebs–Henseleit bicarbonate buffer) or the blocker of the $Na^+/K^+/2Cl^-$ cotransporter bumetanide (100 μmol/l; in Krebs–Henseleit buffer) on the depolarizing effect of MET (1 mmol/l) in mouse liver slices. **,***Significant difference versus start of superfusion ($t=0$ min; $^{**}P<0.01$ or $^{***}P<0.001$, respectively; repeated measures ANOVA with the Student–Newman–Keuls post hoc test; $n=8$ for all groups).

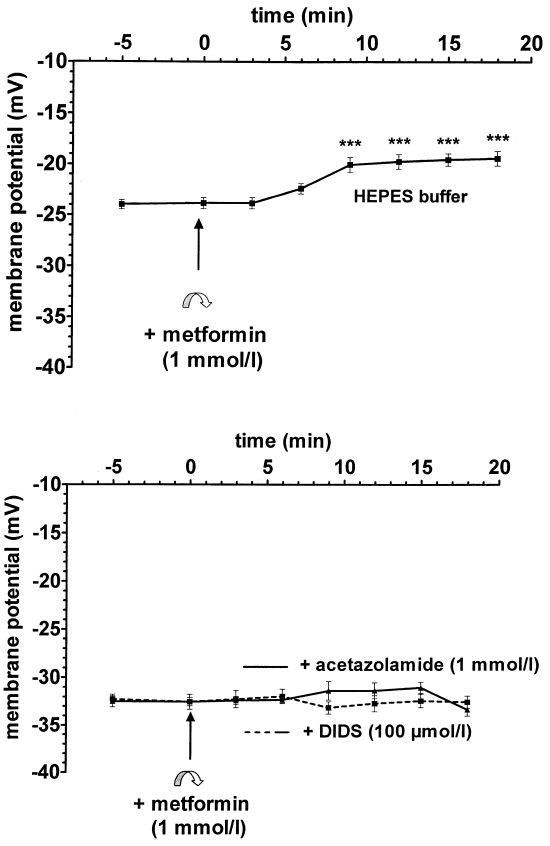

Fig. 4. Influence of superfusion with HEPES buffer, with the inhibitor of carboanhydrase acetazolamide (1 mmol/l; in Krebs–Henseleit bicarbonate buffer) or with DIDS (100 µmol/l; in Krebs–Henseleit bicarbonate buffer), an inhibitor of the Cl^-/HCO_3^- exchanger, on the depolarizing effect of MET (1 mmol/l) in mouse liver slices. ***Significant difference versus start of superfusion ($t=0$ min; $P<0.001$; repeated measures ANOVA with the Student–Newman–Keuls post hoc test; $n=8$ for all groups).

($[Na^+]=26$ mmol/l) affected the depolarization of the liver cell membrane induced by MET (1 mmol/l; Fig. 5).

Finally, while MET (1 mmol/l) depolarized the liver cell membrane and while glucagon (10^{-7} mol/l) hyperpolarized the liver cell membrane, co-superfusion of MET and glucagon left the membrane potential unchanged compared to control conditions (Fig. 6).

4. Discussion

The present study shows that metformin exerts a depolarizing effect on the liver cell membrane both under in vivo and in vitro conditions. Under in vitro conditions, the depolarizing effect of MET was observed at concentrations which are similar to therapeutic levels in portal blood (for review, see [23]). The in vitro experiments further show that MET's effect is at least partly reversible and that glucagon

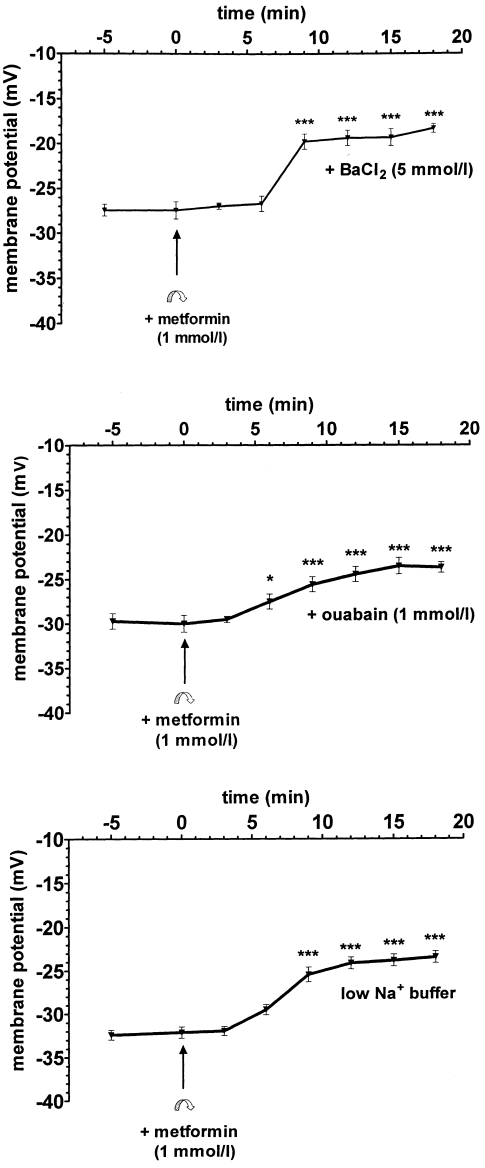

Fig. 5. Influence of blockade of K^+ channels by Ba^{2+} (5 mmol/l), inhibition of the Na^+/K^+-ATPase by ouabain (1 mmol/l) or use of low Na^+ superfusion buffer ($[Na^+]=26$ mmol/l) on the depolarizing effect of MET (1 mmol/l) in mouse liver slices. *,***Significant difference versus start of superfusion ($t=0$ min; $^*P<0.05$ or $^{***}P<0.001$, respectively; repeated measures ANOVA with the Student–Newman–Keuls post hoc test; $n=8$ for all groups).

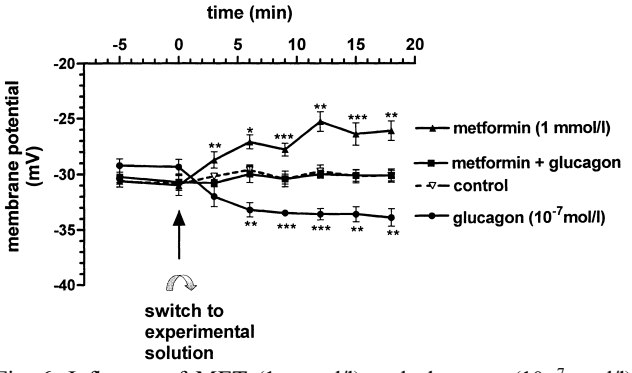

Fig. 6. Influence of MET (1 mmol/l) and glucagon (10^{-7} mol/l) on the liver cell membrane potential in superfused mouse liver slices. *,**,***Significant difference versus MET+glucagon or versus control ($P<0.05$, $P<0.01$ or $P<0.001$, respectively; ANOVA with the Student–Newman–Keuls post hoc test; $n=8$ for all groups).

antagonized its effect. MET's depolarizing effect was absent when liver slices were superfused with low Cl^- buffer, and was also absent in the presence of the Cl^- channel blocker NPPB while the blocker of the $Na^+/K^+/2Cl^-$ cotransporter bumetanide had only a minor influence on MET's depolarizing effect. Further, MET's effect was also abolished by blockade of the carboanhydrase by acetazolamide and of the Cl^-/HCO_3^- exchanger by DIDS. Since DIDS also blocks hepatic Cl^- channels [24], its effect might also partly be due to this action. Finally, MET's effect was not influenced by $BaCl_2$, by superfusion with low Na^+ buffer or by the inhibitor of the Na^+/K^+-ATPase, ouabain.

The results provide evidence for the depolarizing effect of MET on the liver cell membrane being mediated by an enhanced Cl^- efflux from liver cells via Cl^- channels. Further, MET's effect depends on intracellular bicarbonate which, probably through the Cl^-/HCO_3^- exchanger importing Cl^- in exchange for HCO_3^-, is functionally coupled to Cl^- efflux. The results also provide evidence that MET's depolarizing effect on the liver cell membrane is independent of an influence on K^+ or Na^+ fluxes, the $Na^+/K^+/2Cl^-$ cotransporter or the function of the Na^+/K^+-ATPase.

Interestingly, the depolarizing effect of MET was only somewhat reduced when liver slices were superfused with nominally bicarbonate-free HEPES buffer. While at first sight this indicates that HCO_3^- is only of minor importance for this effect, MET's effect was completely abolished during superfusion with Krebs–Henseleit bicarbonate buffer containing the carboanhydrase blocker acetazolamide. This clearly indicates that MET's depolarizing effect depends on the intracellular formation of bicarbonate while extracellular bicarbonate seems to play only a minor role.

While intracellular and extracellular Cl^- is generally supposed to distribute passively under steady state conditions depending on the liver cell membrane potential [25,26], an influence on Cl^- fluxes, e.g. by MET, may affect the liver cell membrane potential when the steady state equilibrium is disturbed. It is thus plausible that MET, by activating the Cl^-/HCO_3^- exchanger, disturbs the Cl^- distribution across the plasma membrane and thus enhances Cl^- efflux, leading to a depolarization of liver cells. A functional coupling between Cl^- channels and the Cl^-/HCO_3^- exchanger has already been described in other cell types such as pancreatic duct cells [27]. The exact mechanism how MET affects the Cl^-/HCO_3^- exchanger and Cl^- channels is unknown. Since MET seems to exert its metabolic effects at least in part at the cell membrane level by interfering with membrane fluidity and protein configuration [23], it is plausible that its effects on the liver cell membrane potential are also caused by influencing the Cl^-/HCO_3^- exchanger and/or Cl^- channel function at the cell membrane level. Whether other agents having an effect on Cl^- channels also influence glucose production and/or gluconeogenesis is unknown to the authors.

We observed a lower baseline membrane potential when liver slices were superfused with HEPES buffer or with low Cl^- HEPES buffer than with Krebs–Henseleit bicarbonate buffer. This is in accordance with previous observations and may perhaps be related to a decrease in K^+ conductance due to a decrease in intracellular pH [28,29].

The baseline membrane potential was also reduced when liver slices were superfused with Krebs–Henseleit bicarbonate buffer containing NPPB or bumetanide. While in the case of NPPB this may have been due to an inhibitory effect of NPPB on K^+ conductance [30], the mechanism underlying bumetanide's effect on the baseline membrane potential is not clear.

The depolarizing effect of MET on the liver cell

membrane may explain its inhibitory effect on L-alanine uptake in liver cells which is thought to be at least partly responsible for the MET-induced inhibition of gluconeogenesis and hepatic glucose output [9–11]. Uptake of L-alanine into liver cells is mediated by an electrogenic Na^+-coupled transport mechanism [12–16] and depolarization of the liver cell membrane has been shown to reduce amino acid uptake [22]. Depolarization of the liver cell membrane through MET-stimulated Cl^- efflux would reduce the driving force for electrogenic Na^+-coupled uptake of L-alanine and other gluconeogenic amino acids, e.g. glutamine [33]. Therefore, the MET-induced depolarization of the liver cell membrane could be directly responsible for at least part of the inhibition of gluconeogenesis from L-alanine through reduced L-alanine uptake into liver cells [9–11].

In this context, it was interesting to note that the depolarizing effect of metformin, an agent which inhibits gluconeogenesis [1,2], was antagonized by glucagon, a pancreatic hormone which stimulates gluconeogenesis [31] and hyperpolarizes the liver cell membrane ([17,32], and present study). This antagonism is most likely due to superposition of the effects of MET and glucagon on the liver cell membrane potential.

In line with our hypothesis of an inhibition of L-alanine uptake into hepatocytes due to the depolarizing effect of MET on liver cells, the glucagon induced hyperpolarization of liver cells seems to account for at least part of the increased rate of L-alanine uptake into liver cells and hence the glucagon stimulated gluconeogenesis from amino acids [10]. Apart from the antagonistic effects of MET and glucagon on the uptake of gluconeogenic amino acids into liver cells and on gluconeogenesis, MET and glucagon also seem to exert opposite effects on fat metabolism [1].

Finally, while the control of the hepatic membrane potential by glucagon has been supposed to be related to cell volume regulation and cell metabolism (cell shrinking contributing to increased glycogenolysis, gluconeogenesis and proteolysis; see [18]), it remains to be investigated whether the same also applies to MET, though with reversed premises.

The clinical relevance of the effect of MET on the membrane voltage is underlined by the fact that the depolarizing effect of MET was observed at concentrations which are similar to therapeutic levels in portal blood (for review, see [23]). Because L-alanine transport is enhanced in diabetes mellitus [34] and because increased gluconeogenesis is a metabolic hallmark in diabetes contributing to fasting and post-prandial hyperglycemia [35], MET could antagonize these changes at their very base through its depolarizing effect on the liver cell membrane, leading to reduced availability of L-alanine and other amino acids and thus a reduced rate of gluconeogenesis.

Since the uptake of conjugated bile acids into hepatocytes is mediated by an electrogenic Na^+-coupled transport mechanism and thus depends on the membrane voltage [36], it remains to be investigated whether MET, by depolarizing the liver cell membrane, also affects bile acid secretion and the electrogenic transport of other substances.

In summary, we have shown that MET depolarizes the liver cell membrane under in vivo and in vitro conditions. This effect appears to be due to Cl^- efflux from liver cells being coupled to intracellular bicarbonate and an activation of the Cl^-/HCO_3^- exchanger by MET. MET's depolarizing effect on the liver cell membrane could directly contribute to reduced electrogenic uptake of L-alanine and other amino acids in liver cells and thus MET's inhibitory effect on gluconeogenesis.

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