2-Mercaptoacetate, an inhibitor of fatty acid oxidation, decreases the membrane potential in rat liver in vivo

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Boutellier, Sandra, Thomas A. Lutz, Matthias Volkert, and Erwin Scharrer. 2-Mercaptoacetate, an inhibitor of fatty acid oxidation, decreases the membrane potential in rat liver in vivo. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R301–R305, 1999.—In former work, intraperitoneal injection of 2-mercaptoacetate (MA), an inhibitor of fatty acid oxidation, increased food intake in rats, which was attenuated by hepatic branch vagotomy, and intraportal injection of MA increased the discharge rate in hepatic vagal afferents. In the present study, we investigated, whether intraperitoneal injection or intraportal infusion of MA affects the hepatic membrane potential in rats in vivo. The liver cell membrane potential was measured in anesthetized Sprague-Dawley rats with the microelectrode technique. Intraportal injection of MA at a dose of 800 µmol/kg body wt significantly decreased the hepatocyte membrane potential by 3.8 mV, whereas at a dose of 400 µmol/kg, the depolarization (1.5 mV) of the membrane was not significant. In another strain of Sprague-Dawley rats, however, MA (400 µmol/kg) produced a significant depolarization of the hepatocyte membrane 50 min (2.6 mV) and 2 h (2.9 mV) after intraperitoneal injection. Intraportal infusion of MA (400 µmol/kg) significantly depolarized the membrane 20 and 50 min after infusion by 3.3 and 4.1 mV, respectively. MA at a dose of 800 µmol/kg also depolarized the membrane (4.8 mV after 50 min). These findings in principle are consistent with the “potentiostatic” hypothesis, postulating a link between the hepatic membrane potential, afferent vagal activity, and the control of food intake.

MATERIALS AND METHODS

Adult male rats of a Sprague-Dawley strain (ZUR:SD; Institut für Labortierkunde, University of Zurich, Zurich, Switzerland), with an approximate body weight of 250 g were used for the experiments. In some experiments, rats of another Sprague-Dawley strain with similar genetic background (OFA:SD; Biological Research Laboratories, Füllinsdorf, Switzerland) were used because ZUR:SD rats were no longer available. Rats were adapted to a fat-enriched diet (18% fat; Ref. 30) for at least 1 wk before the experiments and had ad libitum access to food and water until the experiment. The same diet had been used in previous experiments when the effects of MA on food intake (13, 15) and on the discharge rate in hepatic vagal afferents were investigated (17).

Preparation of the liver for in vivo measurement of the membrane potential of liver cells was performed as follows (see also Ref. 16). Rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (66 mg/kg body wt; Ketavet, Parke-Davis) and xylazine (8 mg/kg body wt; Rompun, Bayer). After laparotomy, the guts were transferred to the outside of the abdominal cavity and were covered with a moist tissue swab to avoid drying up. The liver was exposed by widening the opening of the abdominal cavity using two slings fixed in the abdominal skin. To prevent drying up of organs and to maintain body temperature, the abdominal cavity was filled with warmed (37°C) Krebs-Henseleit solution, which also covered the liver. The solution was replaced every 5 min to prevent a substantial decrease (∼2 K) in temperature. A special forceps (nicotating membrane forceps after Desmarres) was used to fix the left lateral liver lobe and to prevent movements of the liver lobe due to respiration. The upper limb of these forceps was open, enabling puncturing of liver cells by the microelectrode. The liver lobe was gently fixed for 4–5 min with the forceps mounted on a micromanipulator. This setting enabled in vivo measurement of the membrane potential in liver cells on the parietal surface of the liver.

There is growing evidence for a link between fatty acid oxidation and the control of food intake. Inhibitors of fatty acid oxidation, e.g., 2-mercaptoacetate (MA), methylpaloxirate (4, 9, 23, 30), or R-3-amino-4-trimethylaminobutyric acid (7), are the most important tools to examine this association.

The important pertinent role of fatty acid oxidation, especially in the liver, was underlined by the observation that the stimulatory effect of MA on feeding in rats fed a fat-enriched diet (30) was attenuated by hepatic branch vagotomy (4, 15) and eliminated by total subdiaphragmatic vagotomy (24) and capsaicin treatment, which destroys primary afferent fibers (23). Because intraportal infusion of MA increased the discharge rate in afferents of the common hepatic vagus branch (17), hepatic fatty acid oxidation appears to control food intake by modulating the discharge rate of vagal afferents. Furthermore, various inhibitors of fatty acid oxidation depolarized the cell membrane of liver cells in superfused mouse liver slices in the presence of pальмитее (25), and palmitate alone hyperpolarized it (6, 26). In principle these findings are in accordance with Russek’s “potentiostatic” hypothesis (27), postulating a reciprocal relationship between the membrane potential of liver cells and both the discharge rate of hepatic afferents and food intake.

It was therefore the aim of the present study to investigate the effect of MA on the membrane potential of liver cells under in vivo conditions. In rats using MA doses that have been shown to increase feeding (Ref. 30; intraperitoneal injection of MA) and the discharge rate of vagal afferents (17; intraportal infusion of MA). The liver cell membrane potential was measured in anesthetized rats with the microelectrode technique. For comparison of the effects with previous feeding and electrophysiological studies, MA was injected either intraperitoneally (30) or into the portal vein (17).

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Measurement of membrane potential. Open-tip microelectrodes were drawn in a horizontal puller from microfilament glass capillaries (1.5 mm OD, 1.05 mm ID) and filled with 0.5 mol/ L KCl. The micropipette was connected to a high-input impedance (1013 Ω) preamplifier (biologic VF 180; Echirolies, France) by an Ag-AgCl half cell. The reference electrode (Ag-AgCl) was placed in the abdominal cavity filled with Krebs-Henseleit solution. Voltage was measured with a digital voltmeter and an oscilloscope (HM 303–4; Hameg, Frankfurt (Main), Germany) and recorded on a pen recorder (Graphtec Multicorder, MC 6625; Hugo Sachs Electronics, March, Germany). To measure the membrane potential, the micropipette was carefully advanced into the liver tissue (held by the forceps) by a micromanipulator. The advancement of the micropipette into the tissue was stopped as soon as a rapid deflection of the voltage trace could be seen. Criterion for a valid measurement was a stable voltage (±2 mV) during recording for at least 5 s and return of the voltage to baseline level (±2 mV) as soon as the micropipette was withdrawn from the tissue. The membrane potential of five liver cells was measured, and the mean of these five values was calculated. Only one such mean at one individual time point was obtained from each animal. The mean coefficient of variation for the membrane potential measured in different cells of a liver lobe was 9.5 ± 0.7%. Resistance (30–60 MΩ) of the open-tip micropipette was measured once before every impalement by passing a rectangular AC current (current = 1 nA; frequency = 1,000 Hz; Voltcraft function generator; Science Products).

Experimental protocol. MA (Fluka Chemie, Buchs, Switzerland) was freshly dissolved in distilled water and administered at doses of 400 and 800 µmol/kg body wt. All solutions were equiosmotic. Equiosmotic NaCl solution served as control.

In the first series of experiments, rats (ZUR:SD) were injected intraperitoneally with MA or control solution. Because the experiments were performed in two series, a separate control group was used for each MA dose (400 or 800 µmol/kg). After ~20 min, the rats were anesthetized with the ketamine-xylazine mixture. Twenty minutes later, when anesthesia was effective, the liver lobe was exposed by laparotomy as described above. Fifty minutes after the MA injection, the measurement of the membrane potential started and was normally completed within 3–4 min. If the micropipette had to be exchanged during measurement, the liver lobe was released from fixation to avoid damage to the liver due to a compromised blood flow until the new micropipette was in place and its resistance was measured. Unfortunately, recording of the time course of the hepatic membrane potential (e.g., before and after administration of substances) was not possible because the damage of liver tissue caused by repeated fixation with the forceps for several minutes was considered too severe.

A similar experiment was performed with OFA:SD rats. In a further experiment (OFA:SD rats), anesthesia was only induced after ~80–90 min, and the membrane potential was measured 2 h after MA injection.

In the second series of experiments, rats (ZUR:SD) were anesthetized with ketamine-xylazine, and the abdominal cavity was opened by laparotomy. The portal vein was catheterized using a 25-gauge butterfly catheter (Braun Melsungen, Melsungen, Germany). The catheter was fixed with cyanoacrylate glue or a sling. MA or control solution was infused intraportally with a maximum volume of 2 ml over ~2 min. Twenty or fifty minutes after the infusion, the membrane potential was measured as described above.

Statistical evaluation. All values are presented as means ± SE. Differences between treatments were assessed using the unpaired Student’s t-test. Two-factor ANOVA was used to investigate a dose × response and a time × response interaction of the effect of MA on hepatic membrane potential.

RESULTS

Effect of intraperitoneal injection of MA (400 or 800 µmol/kg) on the hepatic membrane potential in ZUR:SD and OFA:SD rats. The membrane potential was measured 50 min after intraperitoneal injection. MA at a dose of 800 µmol/kg body wt significantly depolarized the liver cell membrane in ZUR:SD rats by 3.8 mV compared with NaCl controls (Table 1), whereas at the dose of 400 µmol/kg body wt, the depolarization of the membrane (1.5 mV) was not significant. Despite this apparent dose-dependent depolarizing effect of MA after intraperitoneal application, the dose-response interaction did not reach the level of significance (2-factor ANOVA; effect of treatment: P < 0.01; drug × dose interaction: P ~0.11).

In OFA:SD rats, a significant depolarization of the liver cell membrane by 2.6 mV was observed 50 min after the intraperitoneal injection even at the low dose of MA (400 µmol/kg; NaCl −24.5 ± 1.0 mV vs. MA −21.9 ± 0.6 mV; n = 10; P < 0.05). The depolarizing effect (2.9 mV) was similar 2 h after intraperitoneal injection [NaCl (n = 18) −22.7 ± 0.9 vs. MA (n = 16) −19.8 ± 0.5 mV; P < 0.01].

Effect of intraportal infusion of MA (400 or 800 µmol/kg) on the hepatic membrane potential in ZUR:SD rats. The membrane potential was measured 20 or 50 min after intraportal infusion. MA at a dose of 400 µmol/kg body wt significantly decreased the membrane potential by 3.3 mV 20 min and by 4.1 mV 50 min after intraportal infusion (Table 2). The depolarizing effect 20 min after infusion was not different from that 50 min after infusion (time × response interaction in 2-factor ANOVA). MA at a dose of 800 µmol/kg also depolarized the liver cell membrane by 4.8 mV 50 min after intraportal infusion (Table 2). Two-factor ANOVA revealed a significant effect of treatment (P < 0.01), but no drug × dose interaction.

DISCUSSION

Using the microelectrode technique in anesthetized rats, we observed that MA, an inhibitor of fatty acid oxidation, depolarized the membrane potential of liver cells.

Table 1. Influence of an intraperitoneal injection of MA on hepatocyte membrane potential 50 min after injection in anesthetized ZUR:SD rats

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Membrane Potential, mV</th>
<th>Depolarization, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>16</td>
<td>−26.9 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>MA (400 µmol/kg)</td>
<td>16</td>
<td>−25.4 ± 0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>17</td>
<td>−27.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MA (800 µmol/kg)</td>
<td>17</td>
<td>−23.7 ± 0.8*</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Values for membrane potential are means ± SE (n = no. of animals). *Significant difference between 2-mercaptoacetate (MA) and respective control group (P < 0.001; unpaired Student’s t-test).
after infusion in anesthetized ZUR:SD rats.

Table 2. Influence of an infusion of MA into portal vein on hepatocyte membrane potential 20 or 50 min after infusion in anesthetized ZUR:SD rats

<table>
<thead>
<tr>
<th></th>
<th>Membrane Potential, mV</th>
<th>Depolarization, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min after infusion</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>12</td>
<td>-29.6 ± 0.8</td>
</tr>
<tr>
<td>MA (400 µmol/kg)</td>
<td>12</td>
<td>-26.3 ± 0.7*</td>
</tr>
<tr>
<td></td>
<td>50 min after infusion</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
<td>-30.4 ± 1.3</td>
</tr>
<tr>
<td>MA (400 µmol/kg)</td>
<td>10</td>
<td>-26.3 ± 1.3†</td>
</tr>
<tr>
<td>NaCl</td>
<td>7</td>
<td>-33.0 ± 1.0</td>
</tr>
<tr>
<td>MA (400 µmol/kg)</td>
<td>7</td>
<td>-28.2 ± 1.3†</td>
</tr>
<tr>
<td>NaCl (800 µmol/kg)</td>
<td>7</td>
<td>-28.2 ± 1.3†</td>
</tr>
</tbody>
</table>

Values for membrane potential are means ± SE (n = no. of animals). *Significant difference between MA and NaCl control group (P < 0.01; unpaired Student’s t-test); †significant difference between MA and respective control group (P < 0.05).

oxidation (1, 2), decreased the membrane potential of liver cells after intraperitoneal or intraportal administration. The doses used were similar to those that have been shown to increase food intake in rats after intraperitoneal injection (13, 15, 23, 30) and to increase the discharge rate in hepatic vagal afferents after intraportal administration (17). In particular, the effects of intraportal infusion of MA on the hepatic membrane potential (Table 2) reflected the effects of intraportal infusion of MA on the discharge rate of hepatic vagal afferents with regard to time course and dose response (17). The higher dose (800 µmol/kg) tended to have somewhat bigger effects 50 min after MA injection via either route of administration.

This close association is consistent with a causal relationship between the MA-induced depolarization of the liver cell membrane and the increase in vagal afferent activity as postulated originally in Russek’s (27) potentiostatic hypothesis but does not prove it. Russek proposed a theory of the hepatic control of food intake in which the membrane potential of liver cells was assumed to represent a major signal. In accordance with this concept, variation of the hepatic membrane potential reflecting hepatic metabolism of glucose would modulate the discharge rate of hepatic afferents (potentiostatic hypothesis). The discharge rate of afferents, being inversely related to hepatic membrane potential, was postulated to feed information into the central nervous system circuitry that controls food intake (27). In regard to hepatic glucose metabolism, however, this hypothesis seems no longer to be tenable, because it has been shown recently that glucose metabolism does not affect the hepatic membrane potential (25, 26, 31). Yet, in regard to the control of food intake by hepatic fatty acid oxidation, the potentiostatic hypothesis, postulating a reciprocal relationship between the hepatic membrane potential, (vagal) afferent activity, and feeding would fit.

There appeared to be some discrepancy, however, in the dose-response relationship between the action of intraperitoneally injected MA on the hepatic membrane potential (Table 1) and on food intake. MA (400 µmol/kg) injected intraperitoneally significantly stimulated food intake 30–60 min after injection (11, 13, 33), whereas the same dose administered via the same route did not significantly decrease the hepatic membrane potential 50 min after injection in anesthetized ZUR:SD rats (Table 1), although there was a pertinent tendency. Only at the higher dose (800 µmol/kg MA) was the feeding response to MA (23) associated with a highly significant decrease of the hepatic membrane potential ~1 h after injection in ZUR:SD rats (Table 1).

When the experiment was repeated, however, under the same conditions with OFA:SD rats (ZUR:SD rats were no longer available at this time), the lower dose of MA (400 µmol/kg) did in fact induce a significant depolarization of the liver cell membrane 50 min after intraperitoneal injection. The same holds true when the membrane potential was measured 2 h after injection, a time when the stimulatory effects of MA on food intake are still evident (13, 15).

Thus, even at a dose of 400 µmol/kg injected intraperitoneally, MA produced both a feeding response (11, 13) and a significant decrease of the hepatic membrane potential in anesthetized rats, at least in one of two experiments. Therefore, on the whole, the dose-response relationship of the effects of intraperitoneally injected MA on feeding and on the hepatic membrane potential appear to be similar and thus fits the potentiostatic hypothesis in regard to the control of food intake by fatty acid oxidation.

However, it needs to be noted in this context that recent findings concerning the effect of the fructose analog 2,5-anhydro-D-mannitol (2,5-AM), which depletes liver cells of ATP (21), on the hepatic membrane potential (16, 32) are not in accordance with the potentiostatic hypothesis. 2,5-AM, which elicits eating by acting on the liver (34), produced a hyperpolarization of liver cells (16, 32) and a simultaneous increase in hepatic vagal afferent activity (18). Thus if the hepatic membrane potential were a signal for the control of food intake, as suggested by Russek, 2,5-AM should rather suppress vagal afferent activity and feeding. It is possible, however, that 2,5-AM, as postulated for 2-deoxy-D-glucose (20), increased vagal afferent activity (18) by inhibiting the ATP formation in afferent terminals (16, 20). This could then produce an eating response despite the hyperpolarizing effect of 2,5-AM on the liver cell membrane (29).

The reason for the variation in baseline values of the hepatic membrane potential is unknown. We believe, however, that the variation in baseline values does not compromise the interpretation of the results because it is possible that relative changes rather than absolute values for the membrane potential are relevant to the regulation of food intake.

Russek originally assumed an electrical coupling between the hepatic “glucose receptors,” which he thought could be liver cells, and terminals of hepatic afferents via gap junctions. This, in his opinion, could explain the reciprocal relationship between the hepatic membrane potential and the discharge rate of hepatic afferents (27). However, such gap junctions have not yet been
identified. Moreover, at present there is no morphological evidence for an afferent innervation of the liver parenchyma in the rat (3). Only an afferent vagal innervation of intrahepatic and extrahepatic bile duct cells and of the portal vein was observed (3). Because, however, vagal afferents projecting to intrahepatic bile ducts are in close proximity to hepatocytes that are electrically coupled by gap junctions, a functional coupling between the hepatic membrane potential and vagal afferent activity cannot be definitely excluded. It is unlikely that MA increases vagal afferent activity by inhibiting fatty acid oxidation in vagal afferents, because fatty acid oxidation is not an important energy source for the peripheral nervous system (10). It is also possible that ketones, whose formation depends on hepatic fatty acid oxidation, are involved in the control of food intake by hepatic fatty acid oxidation (29, 31), because 3-hydroxybutyrate, used as fuel by peripheral nerve fibers (10), reduced food intake after parenteral administration in rats and this effect was eliminated by dissection of the common hepatic vagus branch (12). It remains to be investigated in this context, whether intraportal infusion of 3-hydroxybutyrate affects the discharge rate of hepatic vagal afferents without affecting the hepatic membrane potential. If the latter were the case, MA might also affect food intake in part independently of its effect on the liver cell membrane potential.

On the whole, the results presented in this study and previous studies (15, 17) appear to support the assumption that the effects of MA on eating are largely confined to the hepatoportal area, because MA, which, as shown in the present study, depolarized the liver cell membrane, was markedly less potent in stimulating food intake after hepatic branch vagotomy (4, 15) and MA caused a dose-dependent increase in the hepatic vagal afferent discharge rate after intraportal infusion (17). However, the hepatoportal area may not be the only site of MA action, because the feeding response to MA was abolished by subdiaphragmatic vagotomy (24) or chemical ablation of sensory afferents with capsaicin (23) and was only attenuated by hepatic branch vagotomy (4, 15; but see Ref. 22). It is, however, unlikely that fatty acid oxidation affects the membrane potential in nonhepatic cells because cell swelling due to the formation of ketones, representing products of hepatic fatty acid oxidation seems to be involved in the modulation of the hepatic membrane potential by fatty acid oxidation (29).

The results of the present study show an influence of fatty acid oxidation and ketogenesis on the liver cell membrane potential under in vivo conditions and agree with previous in vitro studies. Various experiments showed that palmitate hyperpolarized the liver cell membrane in superfused mouse liver slices (25, 26) and the perfused rat liver (6), and this hyperpolarization was antagonized by various inhibitors of fatty acid oxidation (25). Both ouabain (1 mmol/l), an inhibitor of the Na⁺-K⁺-ATPase, and the K⁺-channel blockers TEA (1 mmol/l) and ceteidil (50 µmol/l) (5, 28) also inhibited the hyperpolarizing effects of palmitate (25, 26). These results indicate that the mechanism of the hyperpolarization induced by palmitate is associated with an activation of the Na⁺-K⁺-ATPase and of K⁺ channels, which might be related to cell swelling (19) due to the formation of ketones (25, 29).

To summarize, we observed in the present study that MA, an inhibitor of fatty acid oxidation, depolarized the liver cell membrane under in vivo conditions. These results, together with the stimulatory effects of MA on the hepatic afferent vagal discharge rate and food intake in rats, in principle are consistent with Russek’s hypothesis (27) postulating a link between the hepatic membrane potential, afferent vagal activity, and the control of food intake. Alternatively, hepatic release of ketones acting on terminals of vagal afferents may contribute to the control of food intake by fatty acid oxidation, possibly independently of a modulation of the hepatic membrane potential.

Perspectives

Metabolic sensors located in the hepatoportal area seem to be instrumental in the metabolic control of food intake, because hepatic branch or total subdiaphragmatic vagotomy eliminated or attenuated the feeding responses to various metabolic inhibitors, e.g., inhibitors of glucose metabolism (8), inhibitors of fatty acid oxidation (e.g., MA; 4, 15, 24), or ouabain, an inhibitor of the Na⁺-K⁺-ATPase (14). These inhibitors also increased the discharge rate of hepatic vagal afferents (17, 20). The metabolic sensors mediating these effects in principle may either be liver cells (27) or terminals of vagal afferents (20, 29, 31). The potentiostatic hypothesis, postulating a reciprocal relationship between the membrane potential of liver cells and the discharge rate in afferents (27), in principle is in accordance with the effects of MA on the hepatic membrane potential described in this study and on hepatic vagal afferent activity. However, this does not apply to the inhibitors of glucose metabolism (e.g., 2-deoxy-o-glucose) and 2,5-AM, an inhibitor of ATP formation and hepatic glucose release, which did not decrease the hepatic membrane potential (16, 25) but increased vagal afferent activity (18, 20) and food intake (8, 34). These inhibitors might increase vagal afferent activity and food intake by impairing the energetic metabolism in terminals of vagal afferents. Because ketones, products of hepatic fatty acid oxidation, are used as fuels by peripheral nerves (10), hepatic fatty acid oxidation might partly also modulate vagal afferent activity and eating by the release of ketones (17). The definite coding mechanism of hepatic metabolic sensors responding to fatty acid oxidation thus remains to be elucidated.

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