Physiological effect of circulating glucagon on the hepatic membrane potential

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Lutz, Thomas A., Alois Estermann, Nori Geary, and Erwin Scharrer. Physiological effect of circulating glucagon on the hepatic membrane potential. Am J Physiol Regulatory Integrative Comp Physiol 281: R1540–R1544, 2001.—The pancreatic hormone glucagon hyperpolarizes the liver cell membrane under various conditions. Here we investigated the physiological relevance of this effect by testing the influence of infusions of glucagon antiserum on the liver cell membrane potential in vivo. Intracellular microelectrode recordings of liver cells (up to 60/rat over 2 h) were done in anesthetized male rats. Livers were fixed in place, and recordings were done 10-30 min after intraperitoneal injections of glucagon or hepatic portal vein infusions of glucagon or specific polyclonal glucagon antibodies raised in rabbits. The isotonic lactose vehicle was used as a control for glucagon antibodies. Intraperitoneal glucagon (400 μg/kg) hyperpolarized the liver cell membrane up to 12 mV, and intraportal glucagon (10 or 60 μg/kg) dose dependently hyperpolarized the liver cell membrane by 3–7 mV. Intraportal infusion of glucagon antiserum (in vitro binding capacity of 4 ng glucagon/rat) significantly depolarized the liver cell membrane by ~2.5 mV. The effects of both glucagon and glucagon antiserum reversed after 60–90 min. We conclude that glucagon is a physiologically important modulator of the liver cell membrane potential.

The liver appears to be the major site for glucagon’s satiating effect. First, glucagon infused in the portal vein produced a more potent satiating effect than glucagon administration via other routes (11, 28, 46). Second, hepatic branch vagotomy blocked the inhibitory effect of intraperitoneally or intraportally delivered glucagon (15, 17, 34). Third, lesions of the nucleus of the solitary tract, the initial central projection target of hepatic vagal afferents, blocked intraperitoneal glucagon’s inhibitory effect on feeding (45). Fourth, hepatic branch vagotomy blocked the stimulatory effect of intraportal infusion of glucagon antiserum on feeding (15). Fifth, brief infusions of glucagon decreased meal size when delivered intraportally but not when delivered in the vena cava near the junction of the hepatic vein (15). It is important to note, however, that some tests of hepatic vagotomy (2, 44) and of varied routes of glucagon administration (43) suggest that glucagon may act outside the liver to control feeding under some conditions.

The transduction mechanism initiating glucagon’s satiating action in the liver is not well understood. One interesting possibility arises from recent evidence that some of glucagon’s actions may be related to its actions on the liver cell membrane potential (22). According to Russel’s (41) potentiostatic hypothesis, the hepatocyte membrane potential regulates food intake by modulating the activity of hepatic afferent nerves, with hepatocyte hyperpolarization inhibiting feeding and hepatocyte depolarization increasing feeding. Consistent with this, glucagon hyperpolarizes the liver cell membrane in vitro (10, 38). Furthermore, this effect of glucagon can be counteracted by treatment with the antidiabetic drug metformin, which depolarizes the liver cell membrane (32) and increases food intake in rats (8). In view of these results, we wanted to test whether glucagon also hyperpolarizes the liver cell membrane in vivo and especially whether antagonism of endogenous glucagon with glucagon antibodies at doses that increase feeding (15, 25, 30) depolarizes the liver cell membrane in vivo.

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MATERIALS AND METHODS

Animals and maintenance. Adult male Sprague-Dawley rats (OFA; BRL, Füllinsdorf, Switzerland) with an approximate body weight of 250 g were used. Rats had ad libitum access to a diet containing 18% fat, 46% carbohydrate, 13% protein, 4% mineral mix, 3% vitamin mix, and 16% nondigestible components (33, 40) for at least 2 wk before experiments. Water was always available ad libitum.

Protocol. Rats were tested in the diurnal phase of the lighting cycle, without food deprivation. Each rat was tested in one condition. In the first experiment, rats were injected intraperitoneally with 100 or 400 µg/kg glucagon (Novo Nordisk, Kusnacht, Switzerland) dissolved in isotonic lactose solution (1 ml/kg) or with isotonic lactose alone (n = 6/group). Although intraperitoneal injection of 100 µg/kg glucagon inhibited feeding in some prior experiments (14, 16), the threshold for a satiating action in our rat strain appears nearer to 400 µg/kg (23). Rats were anesthetized with a mixture of ketamine (Ketavet; Parke-Davis; 66 mg/kg) and xylazine (8 mg/kg; Rompun; Bayer) ~20 min later, and recordings began 10 min later, i.e., 30 min postinjection. The liver cell membrane potential was measured in the left lateral liver lobe, as described previously (5, 31). Briefly, the rat was laparotomized, and the left lateral lobe of the liver was fixed in nictating membrane forceps. The abdominal cavity, including the liver, was covered in warmed (37°C) Krebs-Henseleit buffer (in mmol/l: 118.0 NaCl, 4.70 KCl, 1.25 CaCl2·2H2O, 0.60 MgCl2·6H2O, 1.20 NaH2PO4·H2O, and 25.0 NaHCO3), which was replaced every 5 min to maintain tissue euthermia. The membrane potential was measured using conventional open-tip microelectrodes in liver cells on the part of the parietal surface of the liver that was exposed in the open loop of the upper limb of the forceps. The reference electrode was placed in the abdominal cavity. Up to 60 consecutive impalements were made over 2 h in each rat.

In the second experiment, after laparotomy, the intestines were lifted momentarily to expose the hepatic portal vein, which was then cannulated with a 25-gauge butterfly needle. The intestines and portal vein were then returned to their normal positions, and 0, 10, or 60 µg/kg glucagon was infused intraperitoneally (0.5 ml/rat infused over 1–2 min). Previously, these doses inhibited feeding after intraperitoneal injection (28, 35, 46). The recording then proceeded as described above. The third experiment followed the same procedure as the second, except that intraperitoneal injections of polyclonal glucagon antiserum (0.5 ml/rat infused over 1–2 min; Peninsula Laboratories; Belmont, CA) were done. The in vitro binding capacity of the antiserum was 4 ng glucagon/rat, which presumably blocks all circulating glucagon. This dose of antiserum previously stimulated feeding when administered before meals (25, 30). Control infusions were saline containing an appropriate amount of normal rabbit serum.

Measurement of membrane potential. Open-tip microelectrodes were drawn in a horizontal puller (Sachs-Fleming Micropipette Puller PC-84; Sutter Instrument, San Rafael, CA) from microfilament glass capillaries (OD 1.5 mm; ID 0.86 mm; A-M Systems, Everett, WA). Pipettes were filled with 0.5 M KCl. The microelectrode was connected by an Ag-AgCl half cell to a high-input impedance preamplifier (1013 Ω, VF 180; Biologic, Echirolles, France). The reference electrode (Ag-AgCl) was placed in the abdominal cavity filled with Krebs-Henseleit solution (see above). Voltage was measured with a digital voltmeter and an oscilloscope (COS 5020; Kibusui, Kawasaki City, Japan) and was recorded on a two-channel recorder (B-281-L; Rikadenki, Kogyo, Japan). Criteria for valid micropipette impalements of liver cells were 1) a rapid deflection of the voltage trace on advancing the microelectrode in the liver; 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. Resistance of open-tip microelectrodes (20–50 MΩ) was measured one time before every impalement by passing alternating current pulses (1 nA; frequency 1,000 Hz).

At each time point, the membrane potential of five liver cells was measured consecutively within ~90–120 s. The mean of these five values was calculated. One such mean was obtained at each time point from each animal and used for further analysis. The mean coefficient of variation for the membrane potential measured in different cells of a liver lobe was 4.0 ± 0.5%. Because up to 12 time points were measured in an experiment, the liver of each animal was impaled up to 60 times.

Statistical evaluation. All values are presented as means ± SE (n = no. of animals/group; see RESULTS for details). Data were analyzed by repeated-measures two-way ANOVA (repeated for the time factor; treatment as the main effect). The Student-Newman-Keuls post hoc test was used for pairwise comparisons between individual means after significant ANOVA effects. In all cases, a P value <0.05 was considered significant.

RESULTS

Glucagon. Intraperitoneal injection of 400 µg/kg glucagon, but not of 100 µg/kg glucagon, significantly hyperpolarized the liver cell membrane potential (repeated-measures ANOVA: P < 0.01 for effect of treatment; Fig. 1). The hyperpolarization 60 min postinjection of ~12 mV

![Fig. 1. Influence of glucagon (100 and 400 µg/kg body wt) injected intraperitoneally (ip) on liver cell membrane potential in anesthetized rats. Injection of isotonic lactose solution served as control. Values are means ± SE. ***Significant difference between glucagon (400 µg/kg) and control, or glucagon (400 µg/kg) and glucagon (100 µg/kg), at respective time point (P < 0.001; repeated-measures ANOVA with the Student-Newman-Keuls post hoc test; n = 6 animals for all groups).](http://ajpregu.physiology.org/doi/10.1152/ajpregu.2001.281.11.1541)
appeared to be larger than that recorded 30 or 90 min postinjection, but this difference was not statistically significant. Intraportal infusion of 10 or 60 \( \mu \text{g/kg} \) glucagon elicited a dose-dependent hyperpolarization of the liver cell membrane (repeated-measures ANOVA: \( P < 0.001 \) for effect of treatment; Fig. 2). These doses produced relatively steady, long-lasting hyperpolarizations of \( \sim 3 \) and \( 7 \) mV, respectively, between 30 and 90 min postinfusion. The difference between the effects of the two doses was significant at every time point except one. These hyperpolarizing effects appeared to be fully reversible because the liver cell membrane potential returned to baseline within 100–110 min after glucagon infusion.

**Glucagon antiserum.** Intraportal infusion of glucagon antiserum significantly depolarized the liver cell membrane by \( \sim 2.5 \) mV 40–50 min postinfusion (repeated-measures ANOVA; \( P < 0.01 \) for the effect of treatment; Fig. 3). The depolarizing effect was reversible within \( \sim 60 \) min after the infusion of glucagon antiserum. The magnitude and time course of the effect of intraportal glucagon antiserum infusion was replicated in another group of rats (data not shown).

**DISCUSSION**

These experiments extend previous reports that glucagon administration hyperpolarizes the liver cell membrane (Refs. 9, 10, 38 and unpublished observations) by demonstrating 1) that exogenous glucagon has a hyperpolarizing effect in vivo under conditions where it decreases feeding, 2) that intraportal infusion of glucagon requires lower doses to hyperpolarize liver cells than intraperitoneal injection, and 3) that antagonism of endogenous glucagon with glucagon antiserum depolarizes the liver cell membrane in vivo. The latter result indicates that endogenous glucagon has a necessary role in the maintenance of the liver cell membrane potential. The results do not, however, reveal the physiological function of changes in the liver cell membrane potential.

The parallelism of our present findings with previous analyses of glucagon’s satiating action is consistent with the possibility that glucagon’s action on the liver cell membrane potential is related to its satiating action. That is, we observed a similar dose-response relationship for intraportal glucagon’s effect on the liver cell membrane as has been observed for glucagon’s satiating effect in rats (11, 14, 29). Also, as in the feeding experiments, higher doses were necessary for intraperitoneally than for intraportally injected glucagon to elicit an effect (11, 15, 17). Furthermore, a dose of glucagon antiserum that increased food intake in rats (15, 25, 30) also depolarized the liver cell membrane in the present study. Finally, the durations of the effects of glucagon and glucagon antiserum on the liver cell membrane potential match their respective effects on food intake in rats (17, 25). These parallels are in principle consistent with Russek’s hypothesis (41) that glucagon may affect feeding by virtue of its effect on the liver cell membrane potential, but do not prove it. Furthermore, one aspect of the data, the latency of the effect of glucagon antibodies on feeding and on the liver cell membrane potential, does not fit this interpretation well. Prandial glucagon administration or glucagon antagonism appears to have selective...
effects on meal size that occur within minutes (16, 28, 30, 46). In the present experiments, glucagon administration had similarly rapid effects on the liver cell membrane potential (i.e., within 10 min). Glucagon antibody administration, however, clearly required >30 min to elicit a significant effect. Further research is necessary to determine whether this is because glucagon’s effect on the liver cell membrane potential is not involved in its satiating action or whether the apparent discrepancy was caused by some procedural difference in the test situations. One such methodological factor could be our use of anesthetized rats here, since both ketamine and xylazine can increase glucagon release and induce hyperglycemia (6, 26), which may have reduced the functional potency of infused glucagon antibodies. It also may have been important that the animals were not simultaneously feeding in the present study because glucagon’s satiating action appears to involve an obligatory synergism with other food stimuli (27).

It is interesting to note that, despite the sensitivity of the liver cell membrane potential to infusions of glucagon antiserum, only large, supraphysiological (23) intraperitoneal glucagon doses affected it. This also parallels previous feeding studies (14, 17, 23). Thus, according to the usual endocrine criteria, the antagonist data are strong evidence for a physiological effect of glucagon on the liver cell membrane potential, whereas the agonist data mitigate against such a role. There are several possible explanations for this (12). For example, under the conditions in the present experiments, circulating endogenous glucagon may have nearly saturated the signaling mechanisms for glucagon’s effect on the liver cell membrane potential (perhaps because of anesthesia-induced glucagon secretion), thus necessitating unusually large amounts of exogenous glucagon to elicit an additional effect. Another possible explanation for intraperitoneal glucagon’s poor potency is that the rate of increase of plasma glucagon concentration may affect the biological response (12). This would explain the greater potency of intraportal than intraperitoneal glucagon. In either case, however, the fact that blocking endogenous glucagon with glucagon antibodies produced a depolarizating effect on the liver cell membrane indicates that the signaling system is very sensitive in the opposite direction. This favors the idea of a physiologically important role of glucagon in the regulation of the liver cell membrane potential.

The hyperpolarization of the liver cell membrane by glucagon also fits with Niijima’s (37) observation that intraportal glucagon administration reduced the discharge rate of vagal afferents. This is in principle consistent with a hepatic vagal afferent glucagon signaling system. It is as yet unclear, however, whether and how liver cells and hepatic afferent nerve endings communicate, especially in rats (for review, see Ref. 42). In contrast to many other species, the liver parenchyma in rats appears to be only poorly innervated, and the innervation seems to be confined largely to intra- and extrahepatic bile duct cells, hepatic para-ganglia, and the portal vein (4). Nevertheless, the hepatocytes are extensively coupled by gap junctions and are in close proximity to vagal afferents in the intrahepatic bile ducts (4, 39). Thus a functional coupling of the hepatocyte membrane potential and the activity in hepatic vagal afferents, whether direct or indirect, might be possible (also see Ref. 42). Potentially, this coupling could be chemical in nature, i.e., it might involve the potential-dependent release of some signaling substance from the liver cells acting on vagal afferents.

Glucagon’s influence on the hepatic membrane potential may also be related to its other physiological and metabolic effects. This may apply especially to the control of gluconeogenesis from amino acids because the intracellular uptake of amino acids, e.g., alanine and glutamine, occurring by Na+ cotransport, depends on the membrane potential (21, 36, 47). Glucagon’s effects on the uptake of bile salts by liver cells and on bile secretion also seem to be mediated by its effect on the membrane potential (36).

Finally, the influence of glucagon on the hepatic membrane potential may be related to cell volume regulation. The opening of nonselective cation channels, which are present in liver cells (1), by glucagon’s intracellular second messenger cAMP (20) could trigger a Ca2+ influx in the liver cells, leading to an opening of K+ channels and subsequently hyperpolarization of the liver cell membrane (33). Because of a coupling of K+ channels, Na+-K+-ATPase, and Cl− efflux, this could result in net Na+ and Cl− efflux, osmotic water loss, and cell shrinking, which may contribute to increased glycoconlysis and proteolysis (18, 19).

In summary, we have shown that glucagon hyperpolarizes and that glucagon antiserum depolarizes the liver cell membrane under in vivo conditions. This points to a physiologically important role of glucagon as a modulator of the liver cell membrane potential. It is possible that glucagon’s satiating or metabolic effects may depend in part on this action of the hormone on liver cell membrane potential.

REFERENCES


