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SUBSTRATE-INDUCED MEMBRANE POTENTIAL CHANGES IN THE PERFUSED RAT LIVER

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SUMMARY

In the hemoglobin-free perfused liver, administration of pyruvate, lactate, fructose, alanine and palmitate elicited a sustained hyperpolarization of the cell membrane. In contrast, glucose, galactose, lysine, acetate or α-aminoisobutyric acid had no effect on the membrane potential. The pattern of the substrate induced hyperpolarization was different from glucagon- or cyclic AMP-induced hyperpolarization in the onset and duration of the response and ouabain sensitivity. The effect of cyclic AMP ($5 \cdot 10^{-4}$ M) on membrane potential was additive to the effect of the hyperpolarizing substrates and seems to involve a mechanism different from the substrate-induced potential changes.

INTRODUCTION

In previous studies we found that administration of pyruvate to the perfused liver is followed by a sustained membrane hyperpolarization [1, 2]. The membrane potential was further increased upon the addition of glucagon or cyclic AMP [1, 2]. An increase in membrane potential following alanine administration has also been reported [3].

Earlier observations that glucagon or cyclic AMP administration is followed by a redistribution of ions and hyperpolarization of the liver cell membrane indicated that the changes in ion movement and membrane potential might be functionally related to the metabolic effects evoked by these agents [1, 2, 4–10]. This hypothesis is supported by the fact that inhibition of the hyperglycemic response is correlated with impairment of the ion movement, depolarization of the membrane and the lack of hyperpolarization responses in a variety of experimental conditions. Our observations that metabolites themselves affect membrane potential, lead us to examine these effects for comparison to the effects of cyclic AMP.

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METHODS AND MATERIALS

Overnight fasted male Sprague–Dawley rats weighing 100–120 g were used in the experiments. A hemoglobin-free in situ perfusion system was used as described previously [5]. The perfusate employed was Krebs–Ringer bicarbonate buffer, pH 7.4, with 4 % bovine albumin (Fraction V, Sigma).

Transmembrane potentials were measured and recorded by standard procedures: 10–40 MΩ microelectrodes (drawn pyrex capillaries filled with 3 M KCl), Ag : AgCl half cells, an electrometer (WP instruments, M4A), an oscilloscope, and a paper recorder. Averages from at least 15 individual cell penetrations were used for each data point in an experiment. A period of 1–2 min was sufficient for obtaining this number of potential measurements.

The standard experimental protocol included an initial perfusion period of 30 min without added substrates. The experimental substrate was added to the perfusion reservoir to give a final concentration of 20 mM, with the exception of palmitate. Palmitate was added as the Na salt bound to albumin to give a final concentration of 1 mM. Pyruvate, lactate and acetate were also added as Na salts. When the effect of added cyclic AMP was measured, the cyclic nucleotide was added to the reservoir (final concentration $5 \cdot 10^{-4}$ M) after a 30-min perfusion period with the substrate indicated. Glucose was measured by the glucose oxidase method (Worthington glucostat reagent).

RESULTS AND DISCUSSION

Addition of 20 mM pyruvate to the perfusate resulted in a progressive and sustained liver cell membrane hyperpolarization (Table I, Fig. 1). Pyruvate adminis-

TABLE I

SUBSTRATES- AND CYCLIC AMP-INDUCED MEMBRANE POTENTIAL CHANGES

Results are expressed as mean ±S.E.

	Substrate			Cyclic AMP		
Substrate	*n*	Control	30 min[a]	*n*	Control	Response[b]
Lactate	4	37±2	47±1	2	44±1	53±1[c]
Pyruvate	20	35±1	45±1	6	46±2	61±3
Alanine	6	36±3	59±6	1	49	56
α-Aminoisobutyric acid	3	39±3	38±1	3	38±1	51±1
Lysine	3	39±2	37±4			
Glucose	11	33±1	34±2	9	36±2	45±3
Galactose	4	35±2	37±1	3	34±1	42±0
Fructose	3	34±2	46±3			
Acetate	3	35±5	35±2	1	33	44
Palmitate	4	37±2	45±3	3	44±4	55±5

[a] Mean potential levels during a period 25–30 min after addition of the substrate.

[b] Mean values from the average potential of at least eight consecutive cell penetrations for each experiment. Measurements were taken from the peak response period, usually 4–6 min after addition of cyclic AMP.

[c] Glucagon 0.1 μg/ml was used instead of cyclic AMP.

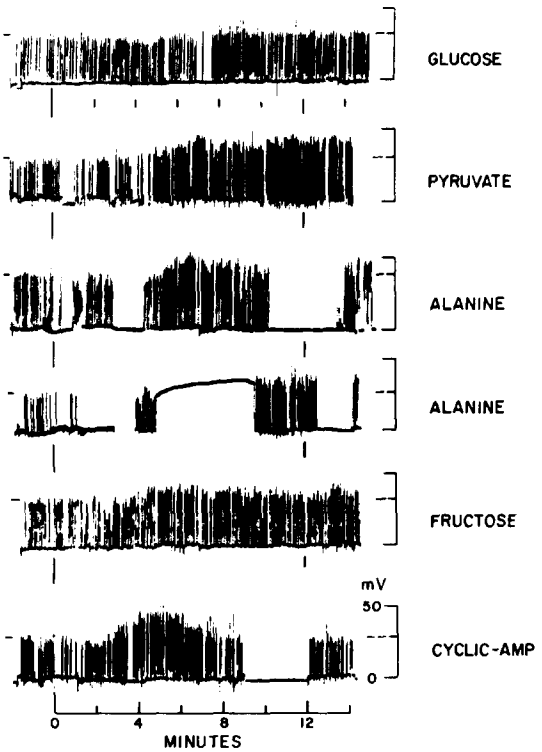

Fig. 1. Records from experiments which show the membrane potentials of individual cell penetrations as the height of the vertical deflections from the base line which represents the reference (perfusate) potential level. Substrates were added at time 0 ; the single hash mark on the left and dashed lines on the right indicated the mean potential level during the control period. The brackets on the right indicate 50 mV for each record. The concentrations of glucose, pyruvate, alanine and fructose were 20 mM and cyclic AMP was $5 \cdot 10^{-4}$ M (final concentrations in the perfusate). The lower of the two alanine records shows the potential recording of a single cell from 5 to 10 min for comparison to the multiple cell samples shown in the other records.

tered as either the Na or the K salt was equally effective, whereas the addition of NaCl or KCl (20 mM) produced depolarizations of 2 mV and 6 mV, respectively.

Lactate, which is metabolized via pyruvate, had an effect similar to pyruvate on membrane potential (Table I). Fructose a substrate which is partially converted to glucose, but also yields pyruvate [11–13], hyperpolarized the liver cells to the same degree as pyruvate or lactate (Table I, Fig. 1). Alanine, a major physiological precursor of glucose in the fasting state via its conversion in the liver to pyruvate [14, 15] also evoked membrane hyperpolarization (Table I, Fig. 1). In contrast, administration of lysine, a non-glucogenic amino acid, had no effect on the membrane potential. In order to check the influence of additional substrates, which are not converted to glucose, the effects of palmitate and acetate on membrane potential were measured. While addition of 1 mM sodium palmitate was followed by an increase in the membrane potential, addition of 20 mM sodium acetate had no effect (Table I).

All of the substrates which were effective in hyperpolarizing the membrane (pyruvate, lactate, alanine, fructose, and palmitate) elicited this response 5–10 min after their addition to the perfusate. The effects were sustained throughout the experimental recording period lasting at least 30 min. This contrasts with controls which showed no significant membrane potential changes over the same period.

We measured the effect of added glucose on membrane potential because of its reported feedback effect on glucose production, its alteration of cyclic AMP-elicited responses [16–22] and its structural similarity to fructose. The addition of either glucose or galactose had no significant effect on the membrane potential (Table I, Fig. 1).

Cyclic-AMP added to the perfusate 30 min after the addition of one of the substrates elicited the characteristic transient hyperpolarization in all cases. The hyperpolarization was additive to the membrane potential changes induced by the substrates (Table I, Fig. 1).

In order to gain some insight into the mechanism underlying the substrate-induced hyperpolarization we studied the effects of α-aminoisobutyric acid and ouabain. The possibility, that the substrate-induced hyperpolarization is related to a Na^+-coupled transport system mediating the substrate entry into the liver cells, was considered. The uptake of the non-metabolized amino acid (α-aminoisobutyric acid) is coupled to Na^+ transport [23]. It was reasonable to assume that if Na^+ coupled transport processes leads to hyperpolarization, then α-aminoisobutyric acid administration would evoke such a response. However, addition of α-aminosiobutyric acid to the perfusate showed no change in membrane potential, suggesting that substrate-induced hyperpolarization may be a result of substrate metabolism rather than an effect correlated with substrate transport.

Administration of high concentration of ouabain (10^{-3} M) either prior to or following pyruvate administration interfered with the hyperpolarization induced by pyruvate (Fig. 2). However, ouabain did not inhibit the increased rate of glucose production stimulated by pyruvate (puryvate, 27±0.5 μmoles/per h; pyruvate+ouabain, 27±0.9 μmoles/g per h). These data might be interpreted as an indication that the substrate-induced membrane hyperpolarization might involve an activation of the electrogenic (Na^+–K^+)-ATPase [24–26].

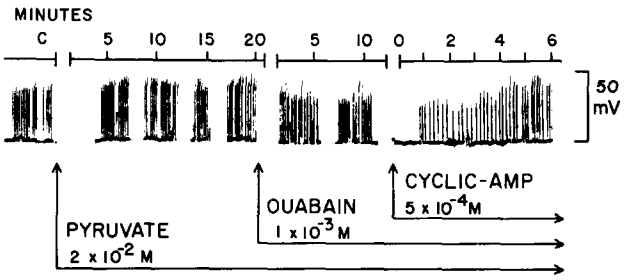

Fig. 2. The effect of ouabain on the membrane potential responses to pyruvate and cyclic AMP are shown in this record. Segments of a continuous record of membrane potential samplings are shown for the times indicated. In continuous time, pyruvate was added at 0, ouabain at 23 and cyclic AMP at 35 min. The base line represents the extracellular reference potential level, upward deflections show individual cell penetrations with the potential negative with respect to the reference level.

While ouabain administration did interfere with the hyperpolarization induced by pyruvate, it did not block the effect of added cyclic AMP on membrane potential (Fig. 2). The differential effect of ouabain on substrate- and cyclic AMP-induced hyperpolarization, and the fact that the hyperpolarizing effect of cyclic AMP is additive and qualitatively different from the effects of the substrates suggest that the cyclic nucleotide-induced hyperpolarization involves a different mechanism from the substrate-induced potential changes. The elucidation of the differential response to the various substrates and the exact mechanism underlying the substrate-induced membrane potential changes needs further studies.

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