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THE INFLUENCE OF THYROID STATE ON GLYCEROL-INDUCED HYPERPOLARIZATION OF THE CELL MEMBRANES IN ISOLATED, PERFUSED RAT LIVER

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Summary

Intracellular electric potentials were measured in isolated livers from starved rats of different thyroid states. The potentials were identical in hypo-, eu- and hyperthyroid livers being —28.9, —29.1, and —29.8 mV, respectively.

During a 15 min period with 2 mM glycerol in the perfusate, the intracellular negativity rose to approximately —36 mV in hypothyroid as well as in euthyroid livers, but no significant change occurred in hyperthyroid livers although these showed the highest rate of glycerol uptake.

In the same period, hypo- and euthyroid livers accumulated respectively 9.0 and 4.3 μmol/g of L-glycerol 3-phosphate. The accumulation of this substance was only 0.6 μmol/g in hyperthyroid livers. The P_i trapped in L-glycerol 3-phosphate balanced with the sum of P_i taken up from the medium and P_i made available from a decrease in the concentrations of P_i and ATP in biopsies. The uptake of P_i was closely accompanied by an uptake of K^+ which was 5.8 μequiv./g in hypothyroid livers, 3.3 μequiv./g in euthyroid livers, and 0.3 μequiv./g in hyperthyroid livers.

The results seem to exclude that the glycerol-induced hyperpolarization of liver cells is an effect related to the transport of glycerol across the cell membranes. A correlation with metabolic events distinguished by their small prominence or absence in the hyperthyroid state appears more likely. However, the exact mechanism remains to be clarified since in the present experiments only a minor part of the hyperpolarization could be accounted for by the uptake of K^+ connected with L-glycerol 3-phosphate accumulation.

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Introduction

Certain gluconeogenic substrates such as fructose, alanine, and pyruvate induce a sustained hyperpolarization of the cell membranes after 5—10 min when added to the perfusate of isolated rat liver [1—3]. This effect may at least partly explain why the basic value of the cell membrane potential in rat liver in vivo is more negative than that found in the isolated organ [4], but the mechanisms underlying the effect are largely unknown.

The fructose-induced hyperpolarization was accompanied by hepatic net uptake of K^+ [3] and fructose has also been shown to cause a large uptake of inorganic phosphate [5] with marked accumulation of fructose 1-phosphate in perfused rat liver [5]. Such observations suggest that substrate-induced hyperpolarization could be a consequence of ionic redistribution following the hepatic accumulation of organic phosphate esters.

To test this possibility we have examined the effects of glycerol on intracellular electric potentials, movements of K^+ and P_i and accumulation of L-glycerol 3-phosphate in livers from hypo-, eu- and hyperthyroid rats. This approach was chosen because the glycerol-induced accumulation of L-glycerol 3-phosphate is known to be strongly dependent on the thyroid state [6,7], i.e. hyperthyroid livers metabolize glycerol at a high rate without significant accumulation of L-glycerol 3-phosphate, whereas euthyroid and especially hypothyroid livers accumulate considerable amounts. On these grounds, hyperpolarization might be expected to be absent in hyperthyroid livers and this result was indeed obtained. Although a fairly close correlation between hepatic K^+ uptake and L-glycerol 3-phosphate accumulation was also established, the hypothesis mentioned above appeared to be too simple: other factors besides K^+ uptake are playing a role for the hyperpolarization observed in hypo- and euthyroid livers.

Materials and Methods

Analytical grade reagents were used when available. Glycerol and inorganic phosphate were supplied by E. Merck, Darmstadt, G.F.R., and bovine albumin by Armour Pharmaceutical Co., Ltd., Eastbourne, U.K. Enzymes and cofactors were purchased from Boehringer and Soehne, G.m.b.H., Mannheim, G.F.R., and 3,5,3′-triiodothyronine (Tertroxin®) was from Glaxo Laboratories, Greenford, U.K. Carrier free $Na^{131}I$ was from Radiochemical Centre, Amersham, U.K.

Animals were female Wistar rats of about 170 g of weight. They were starved for 48 h before the perfusion. To produce hyperthyroidism, rats were injected with a dose of 3,5,3′-triiodothyronine (50 μg/100 g body weight, intraperitoneally) on alternate days for a total of three doses, the last one being given 24 h before the perfusion. To produce hypothyroidism, rats were given one dose of 2.0 mCi ^{131}I intraperitoneally 21 days prior to the perfusion.

Experimental

The non-recirculating perfusion system has been described previously [8]. An Infusomat® infusion pump (Braun, Melsungen, G.F.R.) supplied a constant

flow of perfusate, which was adjusted to a value about 1.2 ml/min per g liver. The medium was a modified Krebs-Ringer bicarbonate buffer in which the concentration of inorganic phosphate (P_i) was increased to 2.2 mM by addition of the sodium salt at pH 7.4. The hematocrit was 30%.

The effects of glycerol in different thyroid states were investigated in experiments in which glycerol (2 mM) was added after 25 min of perfusion with only lactate (0.7 mM) and free fatty acids (about 1 mM) in the medium. The experiments were terminated by a liver biopsy performed 15 min after the addition of glycerol.

Analytical methods

Enzymatic assays were employed for ATP [9], lactate [10], glycerol [11] and L-glycerol 3-phosphate [11]. Inorganic phosphate was determined by the method of Wahler and Wollenberger [12]. Serum concentrations of Na^+ and K^+ were determined by flame photometry (Eppendorf®, Eppendorf-Gerätebau, Hamburg, G.F.R.). The hemoglobin concentration and the oxygen saturation were determined with automatic equipment (Hemoximeter type OSM 2, Radiometer, Copenhagen, Denmark), which analyzed the blood samples within 45 s.

Intracellular electric potentials

These were measured and recorded by conventional techniques: 10—30 MΩ glass microelectrodes filled with 3 M KCl, Ag/AgCl half cells, a FET-type electrometer (Model NF 1, Bioelectric Instruments) and a pen recorder. Using a hydraulic micro-drive (David Kopf Instruments), we advanced the electrodes slowly through the liver (2—5 μm/s) so that their tips penetrated new cells steadily. During 1 min, 3—12 successful cell punctures could be performed.

Results and Discussion

Oxygen consumption

Before the addition of glycerol, the rates of oxygen uptake (mean values ± S.E.) were 1.94 ± 0.07, 2.70 ± 0.10, and 3.44 ± 0.20 μmol/min per g liver in livers from hypo-, eu-, and hyperthyroid rats, respectively, thus reflecting the various thyroid states.

Glycerol uptake

After 15 min of perfusion with 2 mM glycerol, the rates of glycerol uptake (mean values ± S.E.) were 0.83 ± 0.05, 1.34 ± 0.13, and 1.62 ± 0.12 μmol/min per g liver in livers from hypo-, eu-, and hyperthyroid rats, respectively. These differences in the rate of glycerol uptake are partly due to competitive feedback inhibition by L-glycerol 3-phosphate as discussed previously [13] and further elucidated in present experiments [14].

Glycerol-induced changes in net movements of P_i and K^+

Before the addition of glycerol, there was no significant net movement of P_i and K^+ between livers and perfusate in any of the thyroid states (Fig. 1). After

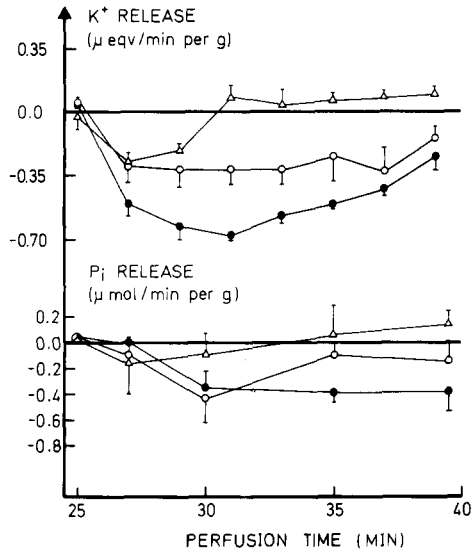

Fig. 1. The effect of glycerol on the rates of net movement of K^+ and P_i between livers and perfusate in different thyroid states. At 25 min, glycerol (2 mM) was added to the medium. •, hypothyroid (4); ○, euthyroid (3); △, hyperthyroid (3). The results are given as mean ± S.E.

the addition of glycerol, hepatic net uptake of these ions occurred to different degrees and in the case of hyperthyroid livers only initially (Fig. 1). No net movements of Na^+ could be detected.

The decrease in the concentrations of P_i and ATP in terminal biopsies together with the integral uptake of P_i from the medium could account for P_i accumulated in L-glycerol 3-phosphate (Table I). It may also be seen from Table I that the integral uptake of K^+ was roughly proportional to the accumulation of L-glycerol 3-phosphate.

Apart from obvious differences initially, Fig. 1 shows that the magnitude

TABLE I

BALANCE OF CHANGES IN P_i INDUCED BY GLYCEROL AND THE ACCOMPANYING UPTAKE OF K^+ IN DIFFERENT THYROID STATES

The approximate calculation was performed on the basis of measurements of adenine nucleotides, glycerol 3-phosphate and P_i in biopsies taken before and after 15 min of perfusion with glycerol. There was no decrease in the concentrations of ATP in eu- and hyperthyroid livers. The values for P_i and K^+ taken up from the medium were calculated as the integral uptake curve (Fig. 1).

	Change in 15 min (μmol/g liver)		
	Hypothyroid	Euthyroid	Hyperthyroid
P_i taken up from the medium	3.5	2.6	—
P_i from ATP	2.7	—	—
Decrease in $[P_i]$ in biopsies	2.3	1.3	0.6
Sum of P_i made available	8.5	3.9	0.6
P_i accumulated in glycerol 3-phosphate	9.0	4.3	0.6
K^+ taken up from the medium	5.8	3.3	0.3
Number of experiments	4	3	3

and the time-course of the rate of K^+ uptake followed a pattern similar to that displayed by the rate of P_i uptake, suggesting a certain degree of coupling between the net uptake of K^+ and the accumulation of P_i in L-glycerol 3-phosphate. In this context we may note that K^+ is probably taken up by the electrogenic action of membrane-bound (Na^+, K^+)-ATPase in an approximate 2 to 3 exchange for Na^+ [15,16]. On the other hand, uptake of the predominant extracellular cation, Na^+, is a passive process. It would, therefore, seem reasonable to suppose that the observed net uptake of P_i is coupled to an increase in the influx of Na ions; these ions could stimulate the (Na^+, K^+)-ATPase and thus again be extruded from the cells and partly exchanged with K ions. The integral uptake of K^+ (Table I) is largely compatible with such a scheme since the requirement for cotransport of cations during the observed integral uptake of P_i (Table I) is calculated at pH 7.2—7.4 to be about 6 μequiv./g in hypothyroid livers and 4 μequiv./g in euthyroid livers.

The mechanism of the initial uptake of K^+ occurring without significant uptake of P_i (Fig. 1) is less clear. A possibility which seems likely might be that K^+ is exchanged for H^+ liberated in the cells during the initial phosphorylation of glycerol with cellular ATP depletion, a process which is marked in the hypothyroid livers (Table I).

The intracellular potential

In accord with earlier observations [1,4,15,16], the intracellular potential in the present study was approximately −30 mV when isolated livers from euthyroid rats were perfused under resting control conditions. The basic value of the intracellular potential in rat liver is more negative in vivo, being about −45 mV [4,17]. The difference is not due to impairment of the isolated liver since the potential is rapidly restored when rat livers, even after several hours of perfusion in the isolated system, are connected to the vascular system of living rats [4]. The difference in membrane potential is rather due to a lack in the perfusate of certain hormones and substrates which are present, however, in the blood in vivo and modify the transport properties of the liver cell membrane [4].

Under control conditions in the present experiments (left set of data points in Fig. 2), there was no significant difference between hepatic intracellular potentials in the various thyroid states. Addition of 2 mM glycerol to the perfusate of hypo- and euthyroid livers was followed by a gradual rise in intracellular negativity (Fig. 2). The hyperpolarization reached 7 mV within 15 min and was preceded by a slight depolarization (not statistically significant). In contrast to the result in hypo- and euthyroid livers, the intracellular potential remained essentially unchanged after addition of glycerol to hyperthyroid livers. These observations may have some relevance to the situation in rat liver in vivo, where experimental hypothyroidism has been shown to lead to hyperpolarization while a tendency to depolarization was noted after treatment of the rats with thyroid hormone [18].

Complex interactions of several, mutually dependent factors are involved in the origin of the cell membrane potential. The potential is generally conceived to be governed by ionic concentration gradients and ionic permeabilities as quantified in the Goldman equation [19,20], but direct contributions,

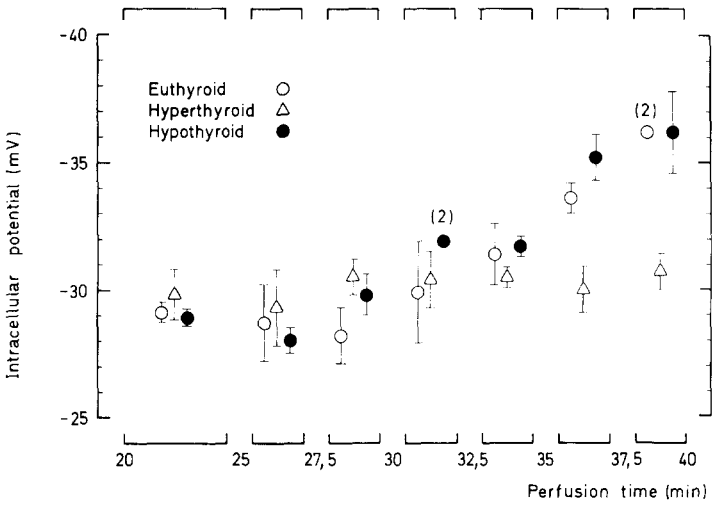

Fig. 2. The effect of glycerol on intracellular potentials in perfused rat livers of different thyroid states. At 25 min, glycerol (2 mM) was added to the medium. In each liver 3—12 successful cell punctures per min were performed. In each of the time periods shown, the mean value of potentials obtained from each liver was regarded as one observation. The results are given as the mean of such observations ±S.D. •, hypothyroid (3); ○, euthyroid (3); △, hyperthyroid (3). Two of the points represent the results from 2 livers (indicated in the figure).

although small under ordinary conditions, from the non-neutral (electrogenic) Na^+—K^+ pump have also been demonstrated [15,16,21].

The lack of glycerol-induced hyperpolarization in hyperthyroid livers is consistent with their insignificant accumulation of K^+ (Table I). It would, however, be hard to explain the hyperpolarization in euthyroid and in hypothyroid livers as due to the accumulation of K^+ alone. Most of the K^+ is taken up within the first 10 min of perfusion with glycerol, whereas the major part of the hyperpolarization occurs after this time. Furthermore, the hyperpolarization observed in euthyroid and in hypothyroid livers is about the same, whereas the accumulation of K^+ is far greater in hypothyroid than in euthyroid livers. An estimate of the minimum accumulation of K^+ which is necessary as a single factor to change the intracellular potential from —29 mV to —36 mV would be about 25 μequiv./g liver. Conversely, the observed accumulation of K^+ in hypothyroid livers (5.8 μequiv./g) and in euthyroid livers (3.3 μequiv./g) would lead to hyperpolarizations of only 1.8 mV and 1.0 mV, respectively, if K^+ uptake were the only factor involved.

The above estimates are based on the simplified Goldman equation for Na^+ and K^+ when Cl^- is distributed at equilibrium as appears to be the case in perfused rat liver [15]. Accordingly, the intracellular potential (E_m) is supposed to be determined as

$$E_m = \frac{RT}{F} \ln \frac{[K^+]_o + p[Na^+]_o}{[K^+]_i + p[Na^+]_i} ,$$

where p denotes the permeability ratio P_{Na}/P_K, and R, T and F have their usual meanings. In the above calculations of K^+ accumulation and of changes in E_m, control values for $[K^+]_i$ and $[Na^+]_i$ were taken to be respectively 165 and 20

μequiv. per ml of intracellular water [15,22] and the intracellular space was set to 0.5 ml/g liver. Even marked changes in the distribution of Na^+ would also have had only small effects on E_m in such calculations. As already noted, no net movements of Na^+ after the addition of glycerol could be detected.

Thus, to explain the major part of the hyperpolarization evoked during the metabolism of glycerol in hypo- and euthyroid livers, an effect on membrane permeability reducing the ratio p or an effect on the electrogenic Na^+—K^+ pump are the possibilities left open within the conventional framework. Further studies involving the use of ouabain and radioisotopes of Na^+ and K^+ are needed to evaluate these possibilities. It may be noted that direct evidence of a substantial increase in P_K during alanine-induced hyperpolarization has been obtained in recent experiments with perfused rat liver (Folke, M. and Paloheimo, M., unpublished), but the underlying metabolic events were not identified.

The relationship of the intracellular potential to the pattern of hepatic glycerol metabolism in the various thyroid states cannot be understood in terms of a simple hypothesis, such as proposed in the Introduction. Apparently, the basic problem would be to locate metabolic events, distinguished by their absence in the hyperthyroid state, which in some manner might induce changes in ionic permeability or in electrogenic ion transport and lead to hyperpolarization. The reported depolarizing action of H^+ on hepatic cells thought to be mediated by an increase in P_{Na} [17] may also have to be taken into account as a factor which might modify the hyperpolarizing response to glycerol in the hypothyroid livers. This consideration is relevant because in the present study on 48 h starved rats, hypothyroid livers as opposed to eu- and hyperthyroid livers produced large amounts of acid equivalents, partly as lactate and partly from the depletion of cellular ATP (Table I). During 15 min of perfusion with glycerol, hypothyroid livers released 2.1 μmol/g of lactate whereas eu- and hyperthyroid livers consumed this metabolite in amounts of 5.2 and 7.4 μmol/g, respectively.

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