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THE EFFECTS OF VARYING IONIC COMPOSITION OF THE PERFU PERFUSATE ON LIVER MEMBRANE POTENTIAL, GLUCONEOGENESIS AND CYCLIC AMP RESPONSES

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SUMMARY

In the rat liver perfused in situ with Krebs–Ringer bicarbonate buffer, glucagon or cyclic AMP administration is followed by increased glucose production, hyperpolarization of the liver cells and a large efflux of intracellular Na. Total substitution of perfusate Na^+ with either Li^+ or choline lowered the membrane potential and resulted in an inhibition of the cyclic AMP-stimulated Na efflux, membrane hyperpolarization and glucose production, but did not block the cyclic AMP-stimulated Ca efflux. With partial replacement of Na^+ with either Li^+ or choline, the responses to the cyclic nucleotides were normal. Partial substitution of Na^+ by 100 mM K^+ caused a depolarization of the liver cells, and responses to cyclic AMP under this condition were greatly reduced. Substitution of the extracellular Cl^- with isethionate did not inhibit these effects of cyclic AMP.

INTRODUCTION

It has been demonstrated that the hyperglycemic effects of glucagon and cyclic AMP [1,2] are associated with a large scale ion redistribution [3–8]. Subsequently, it was shown that simultaneously with the ion movement a hyperpolarization of the liver cell occurs [9–11].

The possibility that the hormone induced ion movements and the increased glucose production are causatively related was raised and investigated by many authors [7,12–14]. The notion that changes in the ionic environment might influence metabolic parameters was supported by the studies of Hasting and co-workers [15] who demonstrated that changing the ionic composition of the medium does result in alteration of carbohydrate metabolism in the liver slices.

These earlier findings and suggestions that a redistribution of Ca^{2+} might serve as a switch mechanism between glycolysis and gluconeogenesis [16,17] led us to reexamine the question of whether the hormone induced ion movements and the metabolic effects are related. In subsequent studies we demonstrated that blocking the hormone induced ion movement by local anesthetics, agents which seem to act by interfering with ion movement [18–20], does inhibit the metabolic response

[5,9.21]. In a different approach in which we changed the ionic composition of the perfusate, we found an inhibition of the gluconeogenic effect of glucagon and cyclic AMP with Na^+-free perfusates [6].

The present studies were undertaken in order to investigate further the possible connection between the hormone induced transmembrane potential changes and the hyperglycemic effect and to gain some insight into the mechanism underlying the hormone induced hyperpolarization.

MATERIALS AND METHODS

Male, 90–120 gram Sprague–Dawley rats were used. Livers from overnight fasted animals were perfused in a hemoglobin-free in situ perfusion system which has been described in detail [5]. The basic perfusion solution was Krebs–Ringer bicarbonate buffer with 4% bovine serum albumin (Fraction V, NBC). The contents of the various perfusion solutions used is given in Table I.

If not otherwise noted, livers were perfused with a single recirculating solution of approx. 100 ml vol. throughout an experiment. Additions to the perfusate were administered to the perfusion reservoir to facilitate mixing and dilution to the final concentration before presentation to the liver. With this procedure, a delay of about 2 min occurred between the addition of material to the perfusate and its appearance in the liver. The perfusion rate was 24 ml/min and the temperature of the perfusate was 33 °C.

Glucose production was measured by the glucose oxidase method (Worthington glucostat reagent). Because glucose production is linear with time during control conditions each experiment was used to measure both a control and a cyclic AMP-stimulated glucose production [5].

Tissue Na and K content were determined by atomic absorption (Perkin Elmer model 303) from ashed samples (20–50 mg) taken both during control perfusion and during the peak membrane potential response to cyclic AMP (6–7 min following administration). Perfusate Na and K concentrations were determined by flame photometry (Beckman) of perfusate samples taken from the effluent of the liver simultaneously with excision of the tissue samples. Tissue Na and K content was calculated by subtraction of the extracellular space content from the total content of tissue samples and then dividing by the cell water content (total water minus extracellular water). The extracellular space content of Na and K was estimated from the perfusate samples and extracellular space measurements. The estimate of extracellular space was obtained with [^{14}C]-sucrose and previously published [6] as 28% of wet weight. Tissue water content, determined in all samples in the electrolyte experiments and in the space determination experiments were essentially identical, 76.3 ml/g and 75.5 ml/g respectively.

Liver cell membrane potentials were measured with drawn, pyrex glass capillary microelectrodes filled with 3 M KCl and connected to an electrometer (WP Inst. Model M4A) via an Ag–AgCl half cell. Potentials were monitored on an oscilloscope and also recorded on a moving paper recorder. Individual liver cell membrane potentials were sampled at a rate of 5–20 per min with each cell penetration sustained only long enough to ascertain a stable potential level. Membrane potentials were similar from all regions of the liver.

TABLE I

CONTENT OF PERFUSION SOLUTIONS

All solutions contained 4% bovine serum albumin (NBC fraction V) and were buffered at pH 7.40. The solutions were exposed to 95% O_2: 5% CO_2 (100% O_2 for the Li^+ perfusate) in a disc oxygenator. Values expressed as mM. KRB, Krebs–Ringer bicarbonate buffer.

Solution	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	SO_4^{2-}	Li^+	Choline	Isethionate	EGTA	Tris
KRB	143	5.9	2.5	1.2	128	24.9	1.2	1.2	—				—
Isethionate	143	5.9	2.5	1.2	9.8	24.9	1.2	1.2	—		118	—	—
Li^+	—	5.9	2.5	1.2	128	—	1.2	1.2	118	—	—	—	24.9
Li^+/EGTA*	—	5.9	—	1.2	120	—	1.2	1.2	118	—	—	2.3	29.5
Choline	—	5.9	2.5	1.2	128	24.9	1.2	1.2	—	143	—		—
Na^+/Li^+	43.4	5.9	2.5	1.2	128	24.9	1.2	1.2	100	—	—	—	—
Na^+/choline	43.4	5.9	2.5	1.2	128	24.9	1.2	1.2		100	—	—	—
Na^+/K^+	43.4	106	2.5	1.2	128	24.9	1.2	1.2	—	—	—	—	—

* EGTA (ethylene-glycol-bis-(β-aminoethyl ether)-*N,N'*-tetraacetic acid) was neutralized with Tris.

RESULTS

Glucose production

Glucose production in perfused livers from fasted rats is very low. Addition of pyruvate (20 mM) to the perfusate results in an increase in the rate of glucose production which is further stimulated following the administration of glucagon (not shown) or cyclic AMP (Fig. 1).

In order to evaluate the role of external Na^+ on the hormone- or cyclic AMP-induced responses and to insure that the observed effects are due to the lack of Na^+ rather than to a specific effect of the replacing cation, two kinds of perfusate were employed. In one, all the Na^+ was replaced with Li^+, in the second all the Na^+ was replaced with choline.

Alterations in the Na^+ content of the perfusion solutions had a significant effect on glucose production. With Na^+-free perfusate containing Li^+ as the Na^+ substitute, control glucose production (in the presence of pyruvate) was essentially abolished and the cyclic AMP effect was completely blocked. With 40 mM Na^+ in the perfusate plus 100 mM Li^+ substitution for the remaining Na^+ of the Krebs–Ringer bicarbonate buffer, both the control glucose production rate and cyclic AMP stimulation were normal (Fig. 1).

Total replacement of Na^+ by choline caused a smaller yet significant reduction in the control rate of glucose production and like the Li^+ substitution completely blocked the cyclic AMP stimulation. With partial replacement of Na^+ by choline

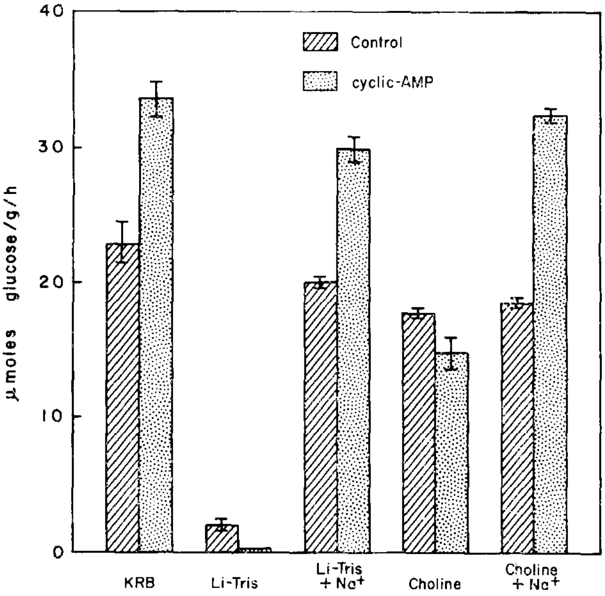

Fig. 1. The effects of varying perfusate Na^+ concentration on the rate of glucose production. Livers were perfused in a recirculating system without substrate. After 30 min Na^+ or K^+ pyruvate was added to the reservoir to give a final concentration of 20 mM. 45 min later cyclic AMP was added to give a final concentration of $5 \cdot 10^{-4}$ M. The experiment was terminated at 120 min. The period between 45–90 min served as control period, while the period between 90–120 min was taken as experimental period. KRB, Krebs–Ringer bicarbonate buffer.

(40 mM Na^+, 100 mM choline), control and cyclic AMP-stimulated glucose production were normal (Fig. 1).

In contrast to partial substitution of Na^+ by choline or by Li^+, substitution by K^+ (106 mM K^+, 40 mM Na^+) depressed the control rate of glucose production and blocked the cylic AMP stimulation (Table II).

TABLE II

THE EFFECTS OF CYCLIC AMP ON GLUCOSE PRODUCTION, ELECTROLYTE CONTENT, AND MEMBRANE POTENTIAL IN HIGH K^+ MEDIUM

Glucose determination was done as described in the legend for Fig. 1, electrolyte determinations as described in the legend of Table III, and membrane potentials (E_m) as described in the legend of Table III and Fig. 3. The Na^+/K^+ perfusion solution contained 106 mM K^+, 40 mM Na^+ (Table I). Values given are the mean ± S.E. from the number of experiments shown in parentheses. Statistical evaluation of cyclic AMP response by paired *t* tests (each experiment serving as its own control) showed that none of the cyclic AMP values are significantly different from the Na^+/K^+ perfusate controls at $P < 0.05$. KBR, Krebs–Ringer bicarbonate buffer; H_2O_i, intracellular water: total water (g wet wt − g dry wt) minus extracellular space.

	Perfusate		
	KRB	Na^+/K^+	Na^+/K^+ + cyclic AMP
Glucose production (μmoles gl/g per h)	23.4 ± 2.2 (20)	10.4 ± 2.3 (10)	16.3 ± 2.8 (10)
$[Na^+]_i$ (μmoles/g H_2O_i)	40.6 ± 5.5 (10)	33.6 ± 3.1 (6)	29.2 ± 4.4 (6)
$[K^+]_i$ (μmoles/g H_2O_i)	167 ± 9 (10)	196 ± 13 (6)	172 ± 14 (6)
E_m (mV)	35.0 ± 1.1 (10)	22.7 ± 1.0 (4)	27.5 ± 3.2 (4)

Substitution of isethionate for chloride had no effect on glucose production (Fig. 2).

Membrane potential

Agents which stimulate glucose production also elicit a transient cell membrane hyperpolarization [9–11]. A typical effect of cyclic AMP is shown in Fig. 3. This transient membrane hyperpolarization occurs both with and without substrate (pyruvate) in the perfusate. Pyruvate itself elicits membrane hyperpolarization which is sustained (10 mV with 20 mM pyruvate) [11]. Thus the control membrane potentials in the presence of pyruvate are larger. However, the net hyperpolarizations resulting from cyclic AMP administration (15 mV with $5 \cdot 10^{-4}$ M cyclic AMP) are not significantly different in the two experimental conditions [11].

Switching from Krebs–Ringer bicarbonate buffer perfusate to Li^+ Tris perfusate (Na^+-free) caused an immediate membrane depolarization, an effect that was rapidly reversed by returning the normal Krebs–Ringer bicarbonate buffer (Fig. 4). Membrane potentials were slightly but not significantly reduced in perfusions with the 100 mM Li^+, 40 mM Na^+ solution. Cyclic AMP elicited a transient hyperpolarization with the partially substituted (Li^+) perfusate (Fig. 5) whereas it had no effect on membrane potential with the Na^+-free, Li^+-Tris perfusate (Table III, Fig. 5). Na^+-free, Li^+-Tris, ethylene-glycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic

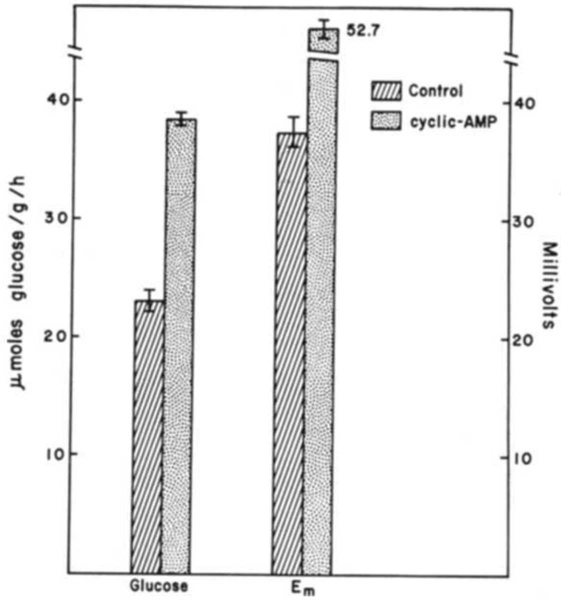

Fig. 2. The effects of a low $[Cl^-]$ perfusate on the rate of glucose production and transmembrane potential. Glucose determination was done as described in the legend for Fig. 1, cyclic AMP was present in a final concentration of $5 \cdot 10^{-4}$ M. Membrane potentials were averaged by the method described in the legend for Table III. The perfusion solution for these experiments was the isethionate Ringer.

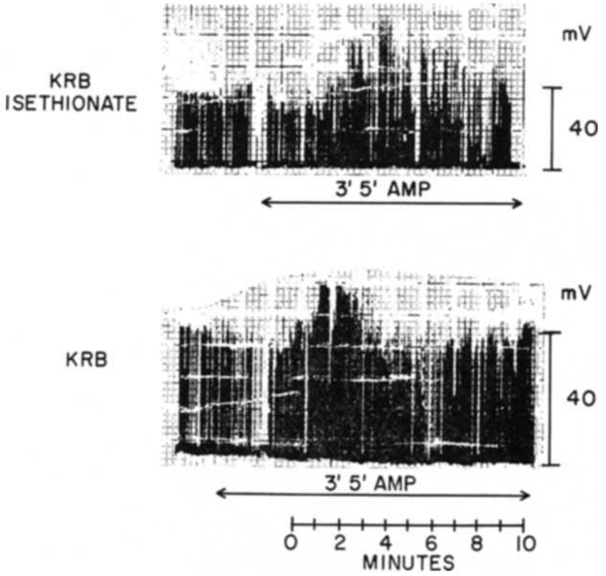

Fig. 3. Membrane potential records with KRB (Krebs–Ringer bicarbonate buffer) and isethionate Ringer perfusion solutions showing responses to cyclic AMP ($5 \cdot 10^{-1}$ M). The bottom of the records indicate the extracellular (perfusate) reference potential level. Upward deflections indicate cell penetrations and the intracellular potential is indicated by the top of the penetration record. (Upward is negative.) Cyclic AMP was present during the period enclosed by arrows in each record.

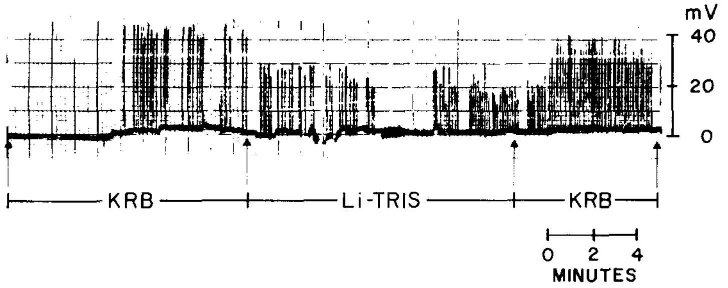

Fig. 4. The effect of Na^+-free, Li^+-Tris on membrane potentials. Livers were perfused with KRB in a recirculating system. At the times indicated the perfusate was changed to Na^+-free, Li^+-Tris buffer by switching to a different reservoir, and then back to the KRB perfusate. Membrane potentials are indicated by the upward deflections in the recording which represent individual cell penetrations. The base line is the perfusate reference potential and the upward direction is negative. KRB, Krebs–Ringer bicarbonate buffer.

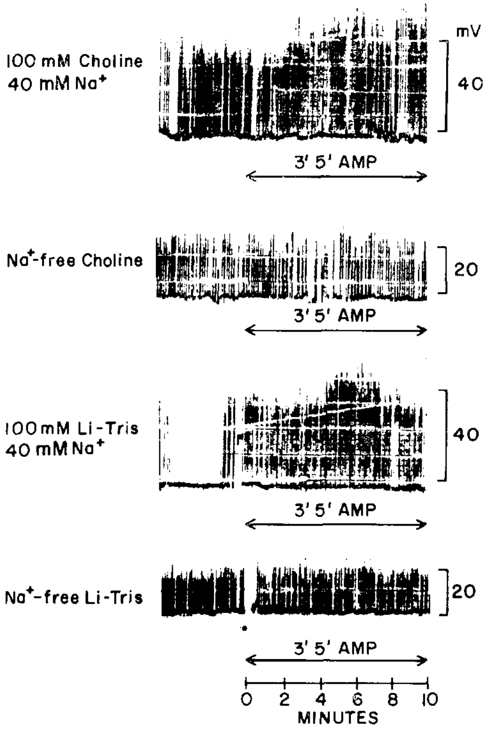

Fig. 5. The effects of varying the Na^+ concentration on the basal and the cyclic AMP-stimulated membrane potentials. Livers were perfused with the indicated perfusate in a recirculating system. Control membrane potentials were recorded for 10 min after a 30 min equilibration period in the perfusion solution indicated, then cyclic AMP was given ($5 \cdot 10^{-4}$ M) and membrane potentials were recorded for the next 10–15 min. The base line of each record is the perfusate reference potential and upward deflections indicate indivual cell penetrations. Cyclic AMP was present during the period enclosed by the arrows in each record.

TABLE III

THE EFFECTS OF VARYING PERFUSATE IONIC COMPOSITION ON ELECTROLYTE CONTENT AND MEMBRANE POTENTIAL

Livers were perfused in a recirculating system without added substrate. For electrolyte determination a small lobe was tied and removed after 30 min of perfusion. This served as control tissue. Cyclic AMP was then added to give a final concentration of $5 \cdot 10^{-4}$ M. 6 min later an additional lobe was removed. After determining water content by drying the tissue at 105 °C for 24 h, the samples were ashed (590 °C for 16 h)and the Na and K content determined using a Perkin-Elmer Atomic Absorption Flame Photometer. H_2O_i, intracellular water: total water (g wet wt − g. dry wt) minus extracellular space (estimating extracellular water space using a specific gravity of 1.0). E_m, transmembrane potentials are given as absolute value; intracellular potentials are negative with respect to the perfusion solution. In each experiment the control membrane potential was determined as the average of 20–50 cell penetrations and the cyclic AMP response was determined as the average value of at least 8 consecutive cell penetrations during the peak of the response. Entries are the mean ± S.E. from the number of experiments shown in parentheses below each entry. Statistical analysis was carried out by paired *t* tests (each experiment serving as its own control).

Perfusate	$[\mathrm{Na}]_i$ (μmoles/g H_2O_i)	$[K]_i$ (μmoles/g H_2O_i)	E_m (mV)
Krebs-Ringer bicarbonate buffer	40.6 ± 5.5	167 ± 9	35.0 ± 1.1
Krebs-Ringer bicarbonate buffer + cyclic AMP	$19.5 \pm 7.8^*$	167 ± 7	$46.2 \pm 2.2^*$
	(10)	(11)	(4)
Li^+	11.4 ± 0.9	86 ± 21	20.2 ± 0.8
Li^+ + cyclic AMP	10.3 ± 0.8	75 ± 19	19.1 ± 0.5
	(4)	(4)	(9)
Na^+/Li^+	45.4 ± 5.1	112 ± 14	30.0 ± 2.8
Na^+/Li^+	$25.0 \pm 14.0^*$	110 ± 11	$36.0 \pm 6.4^*$
	(3)	(3)	(3)
Choline	26.4 ± 4.9	146 ± 4	29.6 ± 2.9
Choline + cyclic AMP	21.5 ± 3.4	$111 \pm 1^*$	29.0 ± 2.8
	(3)	(3)	(3)
Na^+/Choline	33.4 ± 13.3	133 ± 22	37.9 ± 1.5
Na^+/Choline + cyclic AMP	$21.7 \pm 6.1^*$	130 ± 26	$48.0 \pm 3.4^*$
	(6)	(6)	(2)

* indicates significant difference from control at $P < 0.05$.

acid perfusate was used to test the possibility that the Na^+-free perfusate was blocking the cyclic AMP responses by an increased membrane stabilizing action of Ca^{2+}. Elimination of Ca^{2+} from the Na^+-free perfusate did not alter the complete block of the normal cyclic AMP effect on membrane potential.

Perfusion with Na^+-free, choline–Ringer caused a significant reduction in membrane potentials and completely blocked the cyclic AMP-elicited hyperpolarization (Table III, Fig. 5). With the 100 mM choline, 40 mM Na^+ perfusate, control membrane potentials were normal and cyclic AMP elicited a significant hyperpolarization (Table III, Fig. 5).

In livers perfused with high K^+ medium (106 mM K^+, 40 mM Na^+) the control membrane potentials were significantly lower and the cyclic AMP effect was blocked (Table II).

In order to study the mechanism by which cyclic AMP hyperpolarizes the liver cell, experiments were done using a low Cl^- perfusate. The reason for doing so was that a decrease in chloride permeability could result in membrane hyperpolarization by a reduction of this high conductance current pathway [22,24]. According to the data we obtained this is not the case. The hyperpolarizing effect of cyclic AMP in low Cl^- medium was more pronounced than in regular Krebs–Ringer bicarbonate buffer (Fig. 3). The rate of glucose production was also similar to the values obtained in control experiments (Fig. 2).

Tissue Na and K content:

Previously published data has demonstrated shifts in electrolyte distribution following cyclic AMP administration [4–6]. An increase in perfusate K and Na were observed to occur shortly after cyclic AMP administration, a time period which appeared to coincide with the membrane hyperpolarization. To test the possibility of a correlation between these electrolyte movements and the hyperpolarization, tissue and perfusate (effluent from the liver) samples were taken just prior to cyclic AMP administration and again at the peak of the membrane hyperpolarization, about 6–8 min following administration. Membrane potentials were measured during the experiments to confirm the control and hyperpolarization values previously obtained.

The effects of total or partial substitution of Na^+ on liver Na and K content are presented in Table III. In the control experiments, when liver samples were taken at the peak of the hyperpolarization, electrolyte determinations showed a net loss of almost half of the liver Na. This amount of tissue loss (20 μmoles/g intracellular water) corresponds well with the estimate of Na loss previously reported on the basis of flux studies [6]. In contrast to the flux studies, where a release of K was observed, K determination in the liver did not show a change. Flux studies are more favorable for demonstrating small changes in K movement, whereas the demonstration of a loss of tissue K against the large K^+ concentration inside the liver is experimentally more difficult.

Complete substitution of Na^+ with Li^+ or choline significantly lowered tissue Na content. This effect was far more striking in the Li^+ substituted perfusate, than in the choline substituted one. Under these conditions (Na^+-free) addition of cyclic AMP was not followed by a further loss of tissue Na.

Tissue Na content was not significantly altered with partial substitution of Na^+ by choline or Li^+ and cyclic AMP administration elicited the Na efflux (net tissue loss) as in the normal perfusion experiments.

Tissue K was markedly decreased by perfusates in which Li^+ was substituted for Na^+, and decreased but by lesser amounts in the choline perfusates. The only significant changes in tissue K content elicited by cyclic AMP were with the complete choline substitution for Na^+. With partial substitution of Na^+ with either Li^+ or choline the intracellular levels of K were normal (Table III).

In order to investigate the possibility that membrane potential change by itself can influence the rate of glucose production, the potential was lowered by raising the perfusate K^+ concentration (Table II). Under this condition cyclic AMP administration was not followed by the usual Na efflux or hyperpolarization. This lack of Na efflux was not the consequence of a depletion of intracellular Na.

The level of intracellular K in these experiments was significantly higher than in the control experiments (Table II). It was observed that the control membrane potentials with 106 mM K^+ were about 5 mV more negative than the K equilibrium potential estimated with the Nernst equation and the tissue and perfusate K concentrations. Furthermore, in two of four experiments both pyruvate and cyclic AMP elicited small but significant hyperpolarizations (4–5 mV for each). The control membrane potentials were −24 and −25 mV in these experiments in contrast to −19 and −22 mV in the two experiments that gave no change with cyclic AMP.

Calcium efflux

It has been previously demonstrated that hormones which increase the liver cyclic AMP level or the cyclic nucleotide by itself elicit net efflux of Ca from the liver [4–6]. It was found in experiments where Na^+ was replaced with choline, that neither the uptake of Ca into the hormone-sensitive pool nor the release of Ca depends on the presence of extracellular Na^+ [6]. Because Li^+ substitution has a more marked effect on electrolyte movement and membrane potential than choline substitution (Fig. 5) the effects of cyclic AMP on ^{45}Ca release and K movement were measured in Na^+-free Li^+–Tris buffer. As illustrated in Fig. 6, cyclic AMP does stimulate Ca efflux under this condition. While the response appears to be similar to the responses obtained in control animals, the possibility that they might be quantitatively different cannot be excluded because no chemical

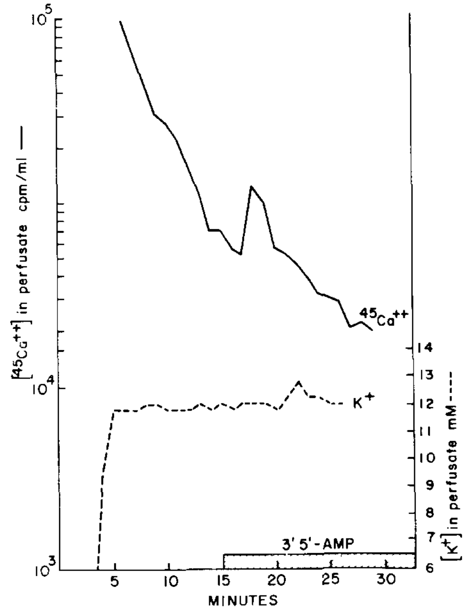

Fig. 6. The effects of cyclic AMP on ion fluxes in Na^+-free, Li^+–Tris buffer. Livers from fed rats were perfused for 60 min in a recirculating system with Krebs–Ringer bicarbonate buffer containing 0.5 μC/ml $^{45}Ca^{2+}$. The test period shown in the figure then began by switching to an isotope-free, Na^+-free, Li^+–Tris buffer in a non-recirculating (flow-through) system. The effluent was collected at 1 min intervals. Where indicated cyclic AMP was added to give a final concentration of $5 \cdot 10^{-4}$ M. The flow rate in these experiments was 12 ml/min. The figure represents the average of 3 experiments.

measurements were done. These data clearly demonstrate, however, that the efflux of Ca is not dependent on the presence of extracellular Na^+.

Switching from Krebs–Ringer bicarbonate buffer to Na^+-free, Li^+–Tris resulted in an immediate K release from the liver, as indicated by the elevated K level in the perfusate (Fig. 6). This data is complimentary to the results obtained in the K content determination of the liver which shows a loss of 50% of the liver K in Na^+-free, Li^+–Tris (Table II). It seems that the lack of Na^+ does not block the cyclic AMP elicited K movement although the response is smaller than in control rats. No release of Na was observed following cyclic AMP administration. However, because the very low Na^+ concentrations in the perfusate were close to the background level of the analysis, the absolute values obtained seemed to be questionable.

DISCUSSION

We have presented data demonstrating that the hyperglycemic effects of glucagon and cyclic AMP are associated with a redistribution of ions and membrane hyperpolarization. The net efflux of Na which involves half of the total intracellular Na is quantitatively the largest ion shift observed. The Na efflux occurs against a concentration and electrical gradient and thus is an energy requiring process. The most likely mechanism for the active extrusion of Na would be the stimulation of a Na^+ pump [23], such as the Na^+–K^+ ATPase. Because, the liver Na^+–K^+ ATPase has been shown to be activated by intracellular Na^+, and to pump K^+ in exchange for Na^+ with a ratio of 2:3 [24] an activation of this enzyme would be expected to be associated with (1) an increased influx of K^+ and (2) membrane hyperpolarization from the net outward current. However, administration of epinephrine, glucagon or cyclic AMP is followed by hyperkalemia [3,4,7,8]. In our studies the efflux of Na and K from the liver (as determined by measuring the concentration of these ions in the perfusate) seems to occur simultaneously [6]. Therefore, if the extrusion of Na^+ is due to the activity of this enzyme, one has to assume that the increase in K concentration in the liver is masked. One possible mechanism for the masking effect could be an increase in K^+ permeability occurring simultaneously with the Na efflux. Our data supports such a hypothesis. In our experiments a loss of K was noticed in the experiments where the level of intracellular Na had been lowered by perfusing the liver with Na^+-free perfusate (Table III). The efflux of K observed in the flux studies and in Na^+-free perfusate occurs in the direction of the K gradient and could therefore, be the consequence of a transient increase in K^+ permeability. An increase in K^+ permeability following norepinephrine administration has been reported [25]. The increased efflux of Na could be also the consequence of a transient decrease in Na^+ permeability, thus a reduced influx with the outwardly directed pump functioning at its usual rate. However, the observation that 50% of the tissue Na is lost in 5–6 min suggests that the normal cellular Na turnover would have a half time in the range of 5–6 minutes which is several fold faster than reported values [24].

The observed efflux of cations occurs following a release of intracellular Ca [4–6]. This Ca movement is probably associated with a transient increase in the cytoplasmatic Ca^{2+} level. An increase in Ca^{2+} concentration has been shown to affect the permeability of the cell membrane to monovalent cations in a variety of systems [26,27]. Thus it is possible, that the efflux of K^+ and the concomitant hyperpolarization is the result of the mobilization of intracellular Ca^{2+}.

The observation that one of the earliest effects of cyclic AMP is on Ca efflux supports the suggestion of Rasmussen [28, 29] that many effects of cyclic AMP could be related to an effect on Ca^{2+} movement.

Another mechanism which might contribute to the observed changes in ion distribution could be a glucagon or cyclic AMP-stimulated phosphorylation of a membrane component, which could result in changes in the permeability characteristics of the membrane [30–32].

The transient cell membrane hyperpolarization was not dependent on Cl^- or a change in Cl^- permeability. Several other mechanisms appear to be reasonable explanations of the hyperpolarization in view of the evidence. An increase in potassium permeability would be hyperpolarizing because the control membrane potential level is about 55 mV below the potassium equilibrium potential. A decrease in the Na^+ permeability could be hyperpolarizing in view of a calculated control level of P_{Na}/P_K of 0.23 using our membrane potential and electrolyte data and the Goldman equation for membrane potential [22]. An electrogenic Na^+ pump could account for the transient hyperpolarization [23]. The large net tissue loss of Na^+, measured during the period of the membrane hyperpolarization, indicates that a membrane Na^+ pump is active during the hyperpolarization. The blocking effect of the Na^+-free perfusates (on the membrane hyperpolarization induced by cyclic AMP) is apparently not the result of an increased membrane stabilization by Ca^{2+} in the absence of Na^+.

In our studies, a block in glucagon- or cyclic AMP-induced gluconeogenesis, Na efflux and hyperpolarization was observed in Na^+-free perfusate. Initially, cyclic AMP causes a small uptake of Na, thus, it might be that this influx is necessary to trigger further events [6]. It is possible also that the inhibition observed in Na^+-free perfusate is secondary to the depletion of the intracellular Na pool.

The suppressed effects obtained in the K^+ depolarized liver raises the possibility that the membrane potential by itself might have some influence on the hormone- or cyclic AMP-evoked responses. It has been proposed that the polar groups in liver cell membranes may be exposed to the changes in the field strength [21]. This might result in alterated function of the molecules or membranes as demonstrated in a variety of systems [33–38]. The increase in the intracellular level of K (Table II) or the altered Na^+/K^+ ratio with the high K^+ perfusate may also account for the inhibition of the cyclic AMP-stimulated events. However, under the four different experimental conditions tested so far where inhibition of gluconeogenesis was observed, namely Na^+-free, Li^+-Tris buffer; Na^+-free, choline buffer; high K^+ buffer; and tetracaine administration [21], the levels of intracellular K and Na varied widely. In all the four conditions a depolarization was observed. The possible direct influence of membrane potential on metabolic parameters deserves therefore, further consideration.

The present data are consistent with our previous studies in indicating that a functional connection might exist between the hormone- or cyclic AMP-induced ion fluxes, cell membrane hyperpolarization and the metabolic effects evoked by these agents.

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