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EFFECTS OF GLUCAGON AND CYCLIC AMP ON ION FLUXES IN THE PERFUSED LIVER

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SUMMARY

In the isolated perfused rat liver administration of glucagon, or cyclic AMP, is followed by a biphasic response of K^+ and Na^+ movement. There is an initial uptake of these cations; during this period perfusate pH drops slightly. Subsequently an efflux of K^+ and Na^+ and a rise in pH of the perfusate occur. This alkalinization is associated with a decrease of intracellular pH. On the other hand, administration of tetracaine, a local anesthetic, is followed by a sharp drop of perfusate pH and intracellular alkalinization. This agent also interferes with the hormone of cyclic nucleotide-induced ion movements and metabolic effects. If the Na^+ in the perfusate is replaced with an equimolar concentration of choline, glucagon or cyclic AMP-induced glycogenolysis and gluconeogenesis are inhibited. On the other hand, substitution of perfusate K^+ by Na^+ does not interfere with the glucagon or cyclic AMP-induced increase in glycogenolysis. Omission of either Na^+ or K^+ from the perfusate does not interfere with the glucagon-induced increase in cyclic AMP levels.

INTRODUCTION

The hyperglycemic and hyperkalemic effects of glucagon and epinephrine have been known for some time. In the course of studies aimed at elucidating the mode of action of these hormones, cyclic AMP was discovered and its role as a mediator of their hyperglycemic effect demonstrated[1,2]. More recently the hormone-induced K^+ movement has also been attributed to the cyclic nucleotide[3–7]. Most recently cyclic AMP has been shown to effect Ca^{2+} and Na^+ fluxes in perfused liver[5,8]. The possibility that a functional connection might exist between the hyperglycemic and hyperkalemic effects was raised and investigated by several authors[9–11]. These studies focused on the relationship between the hormone-induced K^+ movement and increased glucose output. Hastings and co-workers[12], on the other hand, have emphasized the importance of the ionic environment on hepatic carbohydrate metabolism in general. As the pathways of gluconeogenesis and glycogenolysis have been clarified, it has been noted that certain cations either activate or inhibit key enzymes in these pathways[13–17]. This raised the possibility that ionic redistribution might play

Abbreviation: DMO, 5,5-dimethyloxazolidine-2,4-dione.

a direct role in the hormonal control of these pathways. This possibility is made even more likely in view of the fact that in many systems cyclic AMP has been found to change cellular Ca^{2+} distribution[18,19].

The observation that administration of glucagon or cyclic AMP is followed by a redistribution of ions in the perfused liver[5] led us to reexamine the possibility that the hormone-induced ion fluxes and the hyperglycemic effects are related. Subsequently, these hormone or cyclic nucleotide-induced ion fluxes were shown to be associated with significant change in membrance potential[20,21]. The notion that a connection might exist between the hormonal effects on ion movements and the carbonhydrate metabolism was supported by data obtained with local anesthetic tetracaine This agent which in other systems was proven to act by interfering with cation movement[22], blocked the effects of glucagon on gluconeogenesis, glycogenolysis, Ca^{2+} efflux, and membrane hyperpolarization[20,23]

The present studies were undertaken in order to accumulate further knowledge about the pattern of glucagon or cyclic nucleotide-induced ion movements and to gain further insight into the possible relationship between the 3',5'-AMP-induced ion movements and the metabolic effects evoked by these agents.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 100–120 g were used. When gluconeogenesis was measured, the rats were fasted 18–24 h prior to sacrifice, while for glycogenolysis fed animals served as liver donors.

The perfusion technique employed, a modification of Mortimore's method[24], has been described in detail[23]. The standard medium consisted of Krebs–Ringer bicarbonate buffer containing 4% bovine serum albumin (Fraction V.N.B.C.). It was gassed continuously with humidified O_2–CO_2 (95:5, by vol.) in a jacketed disc oxygenator.

In the experiment in which the ionic composition of the medium was changed (Tables II, III, IV; Figs 3 and 4) the following perfusates were used. (1) Na^+-free Krebs–Ringer bicarbonate buffer, in which all the Na^+ was replaced with equimolar concentration of choline; (2) Krebs–Ringer bicarbonate buffer–80 mM choline, 80 mM Na^+ was replaced with choline; (3) K^+-free Krebs–Ringer bicarbonate buffer, all the K^+ was replaced with Na^+. In the experiments in which glucose production was measured, a recirculatory system was used in which the effluent from the liver returned to the oxygenator. Because glucose production was linear under control conditions[23], every liver served as its own control. For determination of perfusate glucose concentration, the glucose oxidase method was employed (Worthington Glucostal reagent). In the experiments in which $^{45}Ca^{2+}$ fluxes were measured, the livers were prelabelled with the isotope for a period of 60 min in a recirculating system. The system was then switched to a flow-through system and the effluent collected in timed aliquots, usually once each minute. To measure $^{45}Ca^{2+}$, samples of the perfusate were deproteinized and counted in a Packard liquid scintillation counter. Chemical estimates of Na^+ and K^+ were made with diluted perfusate, using a Beckman flamephotometer. Ca^{2+} determinations were done with a Perkin-Elmer atomic absorption spectrophotometer. Calculations of net Ca^{2+}, K^+, and Na^+ fluxes were done as previously described[5]. To monitor change in effluent pH, a Radiometer pH meter, type PHM22 equipped with a recorder was used.

Measurement of intracellular pH was done by a modification of the DMO (5,5-dimethyloxazolodine-2,4-dione) method of Waddel and Butler[25] as described by Bianchi *et al.*[26]. [^{14}C]DMO was a product of New Englander Nuclear. In preliminary experiments the equilibration time was determined by perfusing livers with [^{14}C]DMO in a recirculating system. At 20, 40 and 60 min liver samples were taken for [^{14}C]DMO space determinations. The [^{14}C]DMO spaces were respectively 0.460 $\pm$ 0.021, 0.784 $\pm$ 0.091 and 0.803 $\pm$ 0.021 ml/g.

Because between 40–60 min the [^{14}C]DMO space was unchanged, the period between 40–50 min was chosen as the experimental period. From the values obtained for [^{14}C]DMO space, the intracellular pH was calculated according to the formula,

$$pH_i = pK_a + \log\left\{\left[\frac{C_t}{C_e}\left(+\frac{V_e}{V_i}\right) - \frac{V_e}{V_i}\right][1 + 10^{(pH_e - pK_a)}] - 1\right\};$$

where pH_i = intracellular pH; pK_a = $-$log dissociation constant for DMO; pH_e = extracellular pH; C_t = concentration of DMO in total tissue water; C_e = concentration of DMO in extracellular water; V_e = extracellular water; V_i = intracellular water.

Total water content was calculated from wet and dry weights. In separate experiments [^{14}C]sucrose space was measured and used as a marker for V_e.

For cyclic AMP determinations, livers were frozen in liquid N_2 at the end of the experiment and a modification of the method of Goldberg *et al.*[27] was employed.

RESULTS

Effect of cyclic AMP on ion movements

Livers previously labelled with $^{45}Ca^{2+}$ were perfused in a flow-through system. The radioactivity, Na^+ and K^+ concentration, and the pH of the effluent perfusate were measured (Fig 1A). Initially, the radioactivity of the effluent declined sharply. After approximately 10 min $^{45}Ca^{2+}$ was released at a slow and rather steady rate. The concentrations of Na^+ and K^+ were not appreciably changed, while perfusate pH rose slightly. These parameters did not change on addition of AMP. In contrast, administration of cyclic AMP (Fig. 1B,C) or glucagon (not shown) was followed by an immediate redistribution of ions as indicated by changes in perfusate K^+ and Na^+ concentrations, $^{45}Ca^{2+}$ efflux, and changes in perfusate pH. Within the time resolution of our system (1 min) no lag was observed between the arrival of the test substance at the liver and the onset of ion redistribution. The delay plotted in Fig. 1 represents the time lapse between the addition of the test substance to the reservoir and its arrival at the liver. As determined in separate experiments with dye indicators, $^{45}Ca^{2+}$ and ^{131}I-labelled sodium iodohippurate, it takes approximately 2 min for a drug added to the reservoir to reach the liver. Initially, both Na^+ and K^+ are taken up by the liver (as illustrated by a lowering of the Na^+ and K^+ concentration of the perfusate). These changes were associated with an increased $^{45}Ca^{2+}$ efflux and a decrease in perfusate pH. The duration of this initial uptake period is about 1 min in fasted animals (Fig. 1C) and about 2–3 min in fed animals (Fig. 1B).

Following the uptake phase, there was a significant efflux of K^+ and Na^+ associated with an uptake of H^+ as indicated by higher perfusate pH. The changes

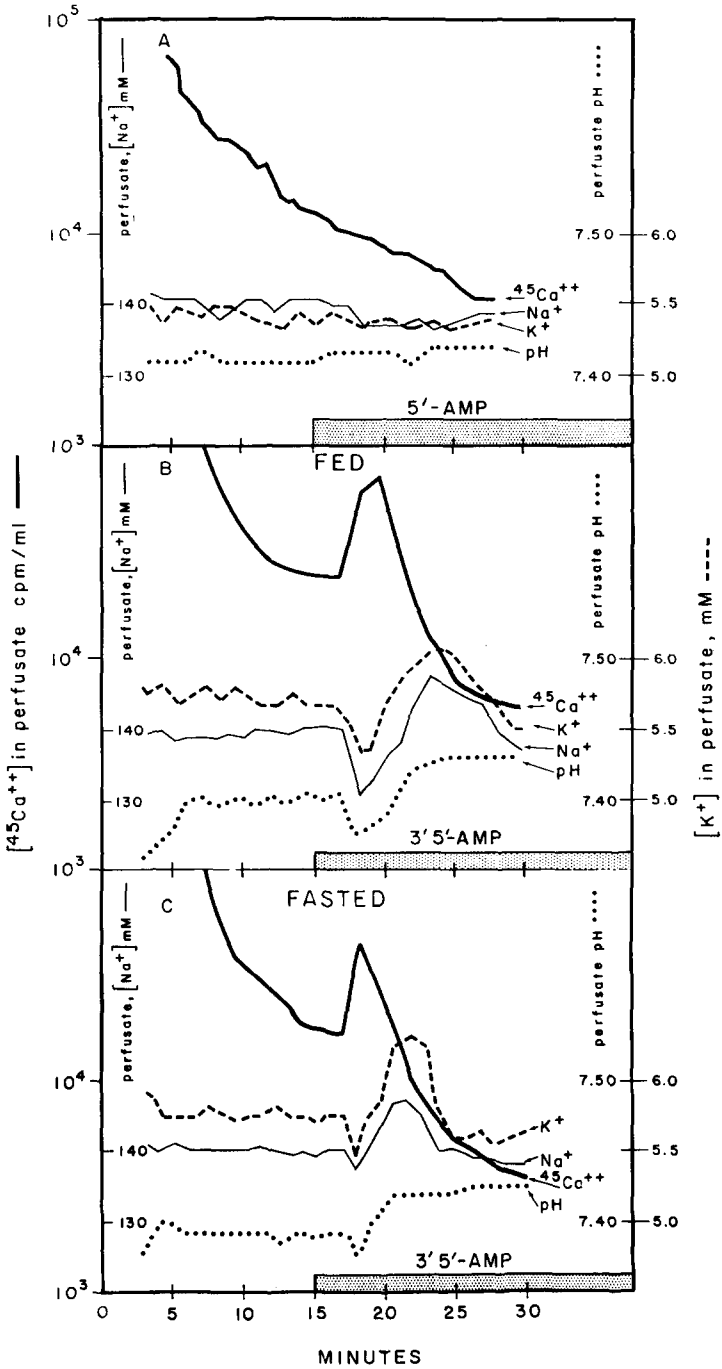

Fig. 1. The effects of 3′,5′-AMP and AMP on ion fluxes. Livers from fed or fasted rats were perfused for 60 min in a recirculating system with a standard medium containing 0.5 μCi/ml $^{45}Ca^{2+}$. The test period shown in the figure was then begun by switching to fresh isotope-free medium in a non-recirculating system and collecting the effluent over 1-min intervals. Where indicated 3′,5′-AMP or AMP was added to the reservoir to give a final concentration of $5 \cdot 10^{-4}$ M. The figures are the average of 3–7 experiments.

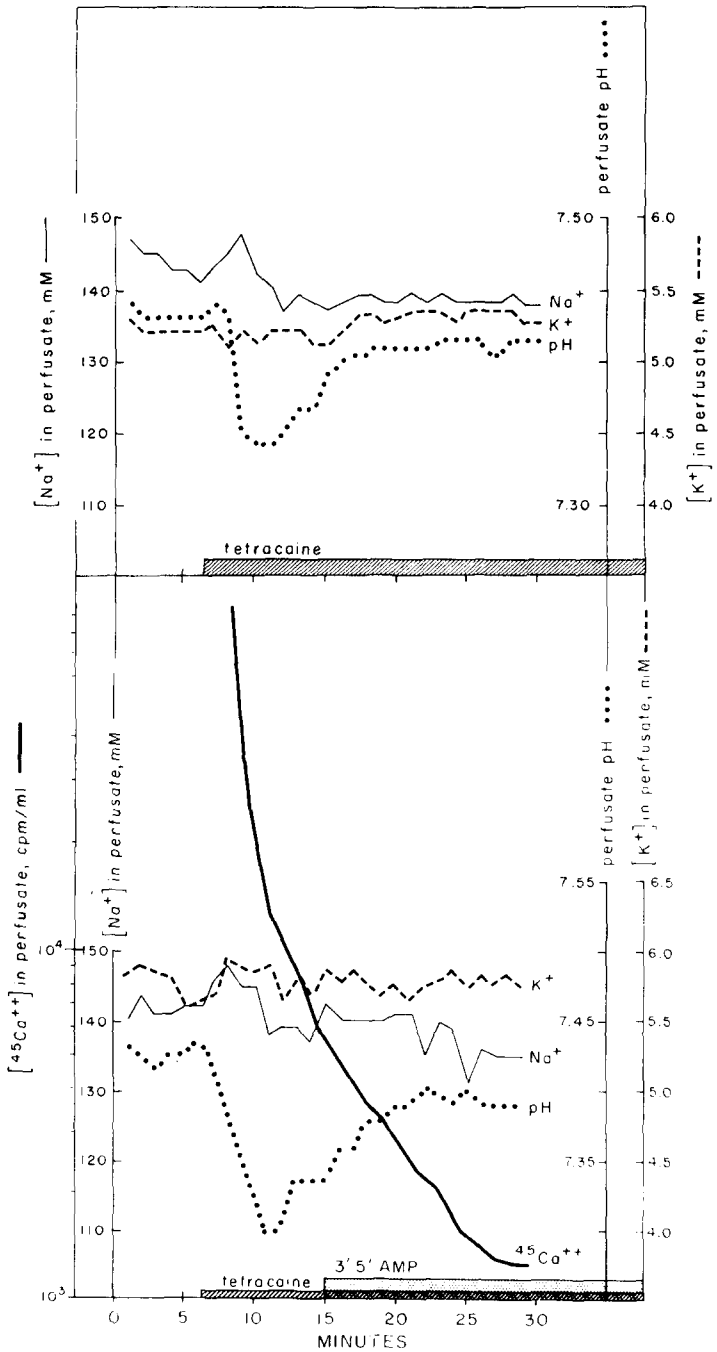

Fig. 2. The blocking effect of tetracaine on 3′,5′-AMP-induced ion fluxes. Livers from fed animals were perfused. Experimental conditions as in Fig. 1. Where indicated, tetracaine was added to give a final concentration of $1 \cdot 10^{-3}$ M. The concentration of 3′,5′-AMP was $5 \cdot 10^{-4}$ M. The figure represents the average of 3 and 4 experiments.

in pH were especially significant considering the fact that they occur in a buffer system. The H^+ uptake seems to persist longer than the K^+ and Na^+ release. In experiments similar to those depicted in Fig. 1 employing livers from fasted animals 8.8 ± 0.9 μg C^2a^+ per g, 302 ± 42 μg K^+ per g, 360 ± 57 μg Na^+ per g were released.

Effects of tetracaine

Addition of tetracaine to the perfusate—in a concentration which was shown to inhibit cyclic AMP-induced $^{45}Ca^{2+}$ movement, gluconeogenesis and membrane hyperpolarization—was followed by an immediate drop in perfusate pH and a slight increase in Na^+ uptake (Fig. 2A). The local anesthetic blocked the glucagon on cyclic AMP-induced ion movements completely (Fig. 2B).

Affect of cyclic-AMP and tetracaine on intracellular pH

In order to understand the nature of the observed changes in perfusate pH, it was necessary to measure intracellular pH changes. The method of Waddel and Butler[25] was chosen because of its proven reliability[26]. After determining that [^{14}C]-DMO was fully equilibrated after a perfusion period of 40 min and the distribution remained stable, the period between 40–50 min was chosen as the experimental period (see Methods). Administration of cyclic AMP consistently lowered the distribution of [^{14}C]DMO demonstrating, that intracellular pH decreased as the perfusate pH increased (Table I). In contrast, tetracaine increased the [^{14}C]DMO space indicating an increase of intracellular pH (Table I).

TABLE I

THE EFFECTS OF 3′,5′-AMP AND TETRACAINE ON [^{14}C]DMO SPACE AND CALCULATED INTRACELLULAR pH

Livers from fasted rats were perfused with Krebs–Ringer bicarbonate buffer containing [^{14}C]DMO 10 Ci/100 ml in a recirculating system. After 40 min a lobe of the liver was taken for control value and the test substance was added. (3′,5′-AMP to give a final concentration of $5 \cdot 10^{-4}$, and tetracaine to give a final concentration of 2 mM) at 50 min additional liver samples were taken and the experiment was terminated. Results are given as mean ± S.E. Numbers in parentheses indicates number of rats.

	Control	*3′,5′-AMP*	*Tetracaine*
[^{14}C]DMO space (ml/g)	0.822 ± 0.018 (19)	0.697 ± 0.027 (4)	0.940 ± 0.041 (5)
Intracellular pH	7.28	7.17	7.37

Effects of omission of Na^+ and K^+ from the perfusate on cyclic AMP-induced ion movement

Because of the numerous reports indicating a relationship between Ca^{2+} and Na^+ movement in various systems[28,29], the possibility of such a connection in the perfused liver was checked. Livers were perfused with a medium in which Na^+ was replaced (if not otherwise noted) with an equimolar concentration of choline, and K^+ with an equimolar concentration of Na^+. In order to test whether $^{45}Ca^{2+}$ uptake into the hormone sensitive Ca^{2+} pool is either Na^+ or K^+ dependent, livers were perfused in a recirculating system using either Na^+-free or K^+-free buffer (Fig. 3).

After 60 min, the perfusate was replaced with regular Krebs–Ringer bicarbonate buffer, and the experiment was continued in the usual way using a flow-through system. In these experiments (Fig. 3), the addition of cyclic AMP was followed by a release of $^{45}Ca^{2+}$ from the livers. Because $^{45}Ca^{2+}$ was not present in the flow-through period, yet administration of cyclic AMP was followed by release, the $^{45}Ca^{2+}$ released had to be taken up to the nucleotide pool during the recirculating period. We can conclude, therefore, that the presence of either extracellular Na^+ or K^+ is not an absolute requirement for Ca^{2+} uptake into this pool.

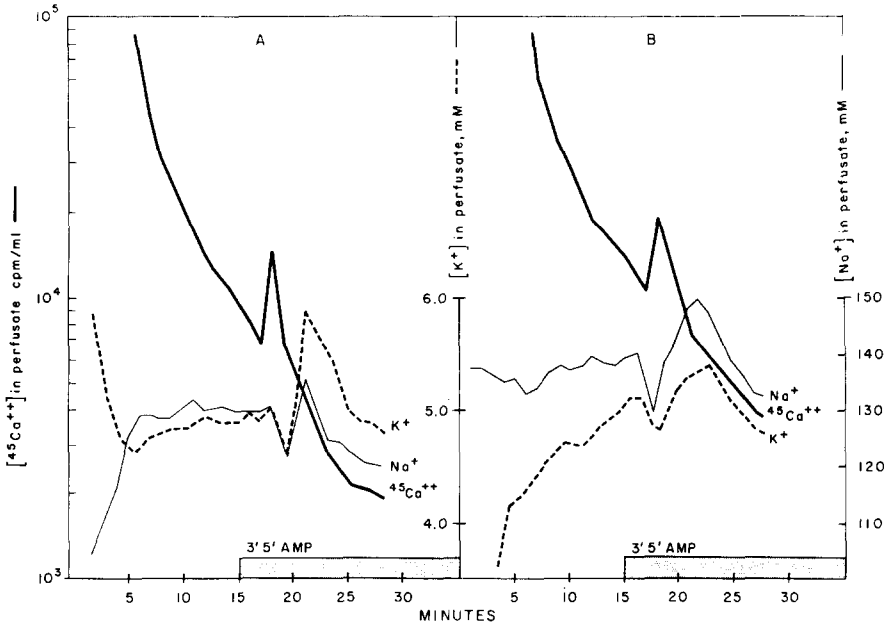

Fig. 3. The effect of omission of Na^+ or K^+ from the perfusate on the uptake of $^{45}Ca^{2+}$ into the 3′,5′-AMP sensitive pool. The experimental conditions were as in Fig. 1 using fasted liver donors, with the exception that in Fig. 3A during the loading period with $^{45}Ca^{2+}$, Na^+ was replaced with an equimolar concentration of choline. In Fig. 3B, K^+ was replaced with an equimolar concentration of Na^+. The perfusate during the test period shown in the figure was the standard medium. Each figure is the average of 3 experiments.

In order to test whether the release of $^{45}Ca^{2+}$ depends on the presence of either Na^+ or K^+, experiments similar to those previously described were carried out, with the difference that the labelling with $^{45}Ca^{2+}$ was done in regular Krebs–Ringer bicarbonate buffer, but during the release period either Na^+ or K^+ was omitted. As shown in Fig. 4, the removal of Na^+ or K^+ does not prevent the cyclic AMP-induced $^{45}Ca^{2+}$ efflux. In order to determine whether omission of Na^+ decreases the amount of Ca^{2+} released, in a different series of experiments net Ca^{2+} release was determined. In these experiments Na^+ was replaced with equimolar concentration of choline, and Ca^{2+} was omitted, as it was previously done[15]. Omission of Na^+ lowered the amount of Ca^{2+} released after cyclic AMP administration in experiments similar to the described above (6.7 ± 0.3 μg per g wet liver).

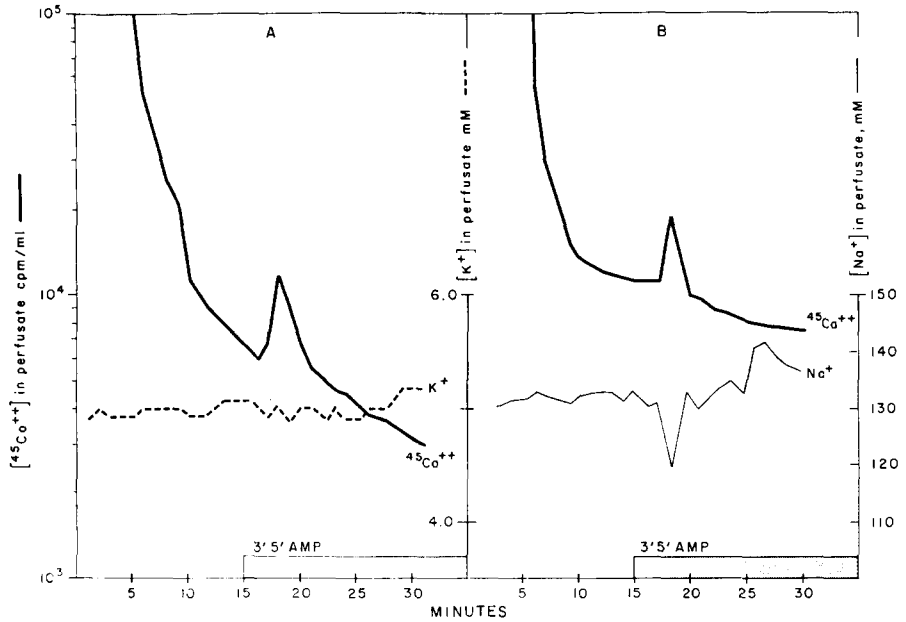

Fig. 4. The effect of omission of Na^+ or K^+ from the perfusate on the release of Ca^{2+} from the 3',5'-AMP sensitive pool. Experimental conditions as in Fig. 1, (fasted rats) with the exception that in Fig. 4A during the non-recirculating test period Na^+ was replaced with an equimolar concentration of choline while in Fig. 4B, K^+ was replaced with an equimolar concentration of Na^+. The perfusate during the recirculating period was the standard medium.

Effects of changing perfusate Na^+ and K^+ concentration on the glycogenolysis or gluconeogenetic response

Because one of the earliest demonstrated effects of glucagon and cyclic AMP in this system involves Na^+ and K^+ uptake, the relationship tetween concentration of these ions in the perfusate to metabolic events influenced by these agents was checked. Table II summarized our results in respect to glycogenolysis. While omission of K^+ from the perfusate did not interfere with the rise in glucose output, substitution of Na^+ by an equimolar concentration of choline completely blocked the hormone or

TABLE II

THE EFFECTS OF CHANGING PERFUSATE Na^+ AND K^+ CONCENTRATION ON THE GLYCOGENOLYTIC RESPONSE

Livers from fed rats were perfused in a recirculatory system. 3',5'-AMP ($5 \cdot 10^{-4}$ M) or glucagon (0.1 μg/ml) was added and the perfusion continued for an additional 45 min. The period between 0–45 min was taken for control value, and the period between 45–90 min for experimental value. Results expressed as μmoles glucose per g wet tissue per h. N.S., not significant.

Medium	*Control*	*3',5'-AMP*	*P*	*Glucagon*	*P*
Krebs–Ringer bicarbonate buffer	69 ± 3	113 ± 6	0.025	126 ± 7	0.001
Na^+-free Krebs–Ringer bicarbonate buffer	72 ± 4	71 ± 6	N.S.	71 ± 7	N.S.
K^+-free Krebs–Ringer bicarbonate buffer	77 ± 6	139 ± 22	0.01*A*	141 ± 20	0.01
Krebs–Ringer bicarbonate buffer 80 mM choline	82 ± 7	141 ± 11	0.01	—	

cyclic AMP-induced glycogenolytic response. Because of the possibility that the results obtained were due to an inhibitory effect of choline itself, rather than to a lack of Na^+ (ref. 30), experiments were performed in which Na^+ was only partially replaced by choline. According to the results (Table II) the presence of 80 mM choline had no inhibitory effect on the parameter measured. To pursue the question further, experiments were done where Na^+ was replaced either with equimolar concentration of Tris or Li^+. In the experiments in which Na^+ of the standard perfusate medium was replaced by an equimolar concentration of Tris, the basal rate of glycogenolysis was 68 ± 3 μmoles glucose per g per h and with $5 \cdot 10^{-4}$ M 3',5-AMP 65 ± 4 μmoles glucose per g per h. In those experiments in which Na^+ was replaced by Li^+, it was necessary to use Krebs–Ringer phosphate buffer instead of bicarbonate buffer. In regular Na^+-containing phosphate buffer, the basal rate of glycogenolysis was 140 ± 6 μmoles glucose per g per h, which was significantly higher than in bicarbonate buffer. $5 \cdot 10^{-4}$ M cyclic AMP increased the rate to 204 ± 26 μmoles glucose per g per h. Substitution of Na^+ with an equimolar concentration of Li^+ did not affect the basal rate, (124 ± 24 μmoles glucose per g per h), however, Li^+ completely inhibited the hormonal response (88 ± 13 μmoles glucose per g per h.) The gluconeogenic response (Table III) was also blocked by substitution of Na^+ with choline. In contrast to glycogenolysis, where the control rate was not affected by the composition of the perfusate, the control values for gluconeogenesis in choline medium was lower than in the normal medium.

TABLE III

THE EFFECTS OF CHANGING PERFUSATE Na^+ AND K^+ CONCENTRATION IN THE GLUCONEOGENIC RESPONSE

Livers from fasted rats were perfused in a circulatory system. At 45 min potassium or sodium pyruvate was added to give a final concentration of 20 mM. The experiment was terminated at 135 min. The period between 45–90 min served as control, while the period between 90–135 min was taken as experimental period. Results expressed as μmoles glucose per g wet tissue per h. N.S., not significant.

Medium	*Control*	*3',5'-AMP*	*P*	*Glucagon*	*P*
Krebs–Ringer bicarbonate buffer	23 ± 1	37 ± 3	0.01	39 ± 3	0.01
Na^+-free Krebs–Ringer bicarbonate buffer	18 ± 1	15 ± 2	N.S.	19 ± 2	N.S.
K^+-free Krebs–Ringer bicarbonate buffer	29 ± 3	34 ± 4	N.S.	34 ± 3	N.S.

The results obtained with K^+-free perfusate are ambivalent. Omission of K^+ from the perfusate did not interfere with the glycogenolytic response (Table II). The gluconeogenetic response, however, was impaired in K^+-free perfusate (Table III). In these experiments the basal rate of glucose production was higher than in the control experiments while the stimulated rate was similar to the values obtained with regular K^+-containing perfusate.

Effect of perfusate composition on glucagon-induced cyclic AMP level

Because it seemed possible that the lack of glycogenolytic or gluconeogenic responses in Na^+-free perfusate was due to an inhibition of the cyclase reaction or a dependence of cyclic AMP uptake on external Na^+, the effect of glucagon on cyclic

TABLE IV

THE EFFECT OF OMISSION OF Na^+ OR K^+ FROM THE PERFUSATE ON THE LEVEL OF 3',5'-AMP

Livers were perfused without substrate in a recirculating system. After 45 min, where indicated, glucagon (0.1 μg/ml) was added. The livers were frozen in liquid N_2 10 min after the addition of the hormone. Results expressed as 10^{-7} mmoles per 100 mg protein. Values are given as means $\pm$ S.E. for 3 determinations.

Medium	*Control*	*Glucagon*
Krebs–Ringer bicarbonate buffer	5.06 $\pm$ 1.1	78.6 $\pm$ 15
Na^+-free Krebs–Ringer bicarbonate buffer	3.60 $\pm$ 1.5	92.5 $\pm$ 17
K^+-free Krebs–Ringer bicarbonate buffer	7.10 $\pm$ 1.3	78.11 $\pm$ 15

AMP level in Na^+ free perfusate was measured. Table IV indicates that neither extracellular Na^+ nor K^+ are required for a rise in cyclic AMP level to occur. Similar results were obtained by others[31,32].

DISCUSSION

We have shown that cyclic AMP or glucagon rapidly alter ion fluxes in the liver. Also we have found that conditions interfering with such ion fluxes, namely tetracaine and Na^+-free perfusate alter the effect of these agents on glycogenolysis and gluconeogenesis. These data suggest a possible relationship between these two facets of, cyclic nucleotide action. According to our results Na^+ and K^+ movements are parallel. It seems, therefore, that the (Na^+,K^+)-ATPase is not connected with the observed ion fluxes.

The magnitude of the observed Na^+ fluxes was large. The mean value for perfusate Na^+ concentration before addition of cyclic AMP was 140 $\pm$ 1 mM. After the addition of the cyclic nucleotide the concentration of Na^+ at the peak of the uptake dropped to 133 $\pm$ 3 mM. While the possibility that this Na^+ uptake was associated with increased water movement cannot be excluded no apparent swelling of the liver was ever detected. The possibility that the relatively large uptake of Na^+ might be associated with intracellular sequestering rather than with increased cytoplasmatic Na^+ concentration was not checked.

A difference in the response obtained according to the nutritional state of the animal was observed. The possible importance of the nutritional state of the animal in response to hormonal stimuli has been pointed out and discussed in earlier studies[33].

The effect of glucagon (not shown) and cyclic nucleotides on perfusate pH is of particular interest. In contrast to the kidney, in which an increase in extracellular H^+ concentration clearly stimulates glucose production, such an effect was not demonstrated in the liver[34–36]. Measurements of intracellular pH after cyclic AMP administration at times corresponding to the alkalinization of the perfusate clearly shows a decrease. Thus, the alkalinization of the perfusate could be the consequence of increased H^+ uptake by the liver and subsequent decrease of intracellular pH. In contrast to cyclic AMP, the local anesthetic tetracaine clearly increases intracellular pH while decreasing the pH of the perfusate. An intracellular alkalinization following

local anesthetic administration was demonstrated in the muscle[26]. In previous studies we demonstrated that this agent blocks hormone or cyclic nucleotide-induced Ca^{2+} movement, gluconeogenesis, glycogenolysis and membrane hyperpolarization[20,23]. It blocks also Na^+ and K^+ movement (Fig. 2). The fact that the effect of tetracaine on intracellular pH, Na^+ movement and membrane potential (significant depolarization) is opposite to the effects evoked by the hyperglycemic agents has to be taken into consideration in future evaluations of the action of this agent on metabolic parameters. Because hyperglycemic agents decrease while a blocking agent, tetracaine, elevates intracellular pH, it seems that a connection between intracellular pH and carbohydrate metabolism may exist in the liver. The difficulties in demonstrating such an effect in the liver were probably due to the fact that the liver cell seems to be relatively impermeable to external H^+. Recently, however, an effect of pH was reported in this organ also[37]. In our studies, the observed pH changes coincide with the cation movements (Fig. 1). The direction of these changes is such that an exchange reaction between H^+ and the cations is not unlikely. Exchange reactions between H^+ and monovalent and divalent cations were demonstrated in various systems[38-42].

Our observations that glucagon and cyclic AMP have no hyperglycemic effects in Na^+-free buffer indicate that Na^+ influx may play a role in these processes in a yet unknown fashion. A connection between extracellular Na^+ and the ability of cyclic AMP to elicit metabolic effects has been demonstrated in many tissues[43-47]. In the liver glucagon or theophylline increased the transport of amino acids with a Na^+-dependent transport system. Glucagon had no effect on the transport of amino acids with Na^+-insensitive transport system[47]. Because glucagon increased cyclic AMP level in Na^+-free medium, the possibility that Na^+ is necessary for the cyclase reaction is ruled out. It seems, therefore, that the lack of Na^+ affects a later step or steps in the glycogenolytic and gluconeogenetic pathways. Whether the lack of hyperglycemic response following glucagon or cyclic AMP administration in Na^+-free perfusate is due directly to lack of Na^+ in a critical step, or to some other ions affected by Na^+, remains to be elucidated.

As we already emphasized, ion movements are very closely interrelated. Intracellular Ca^{2+} concentration has been shown to affect permeability to Na^+[29]. In our experiments Ca^{2+} release was slightly reduced in choline buffer and an exchange between Na^+ and H^+ is indicated. A better understanding of the relative importance of the individual ion movements depends on a more detailed knowledge of the pattern of ion redistribution.

A connection between hormonally induced ion redistribution and metabolic effects is indicated by our data. The elucidation of the link between these processes needs further studies.

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