Biochimica et Biophysica Acta, 307 (1973) 399–403

BBA 76318

EFFECTS OF GLUCAGON, 3′,5′-AMP AND 3′,5′-GMP ON ION FLUXES AND TRANSMEMBRANE POTENTIAL IN PERFUSED LIVERS OF NORMAL AND ADRENALECTOMIZED RATS

NAOMI FRIEDMANN and GEORGE DAMBACH

Departments of Biochemistry and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19174 (U.S.A.)

(Received November 22nd, 1972)

SUMMARY

The effects of glucagon, 3′,5′-AMP, 3′,5′-GMP and dexamethasone on ion fluxes and transmembrane-potential changes were compared in perfused livers from normal and adrenalectomized rats. Glucagon and cyclic nucleotide administration resulted in a similar redistribution of Na^+ and K^+ and membrane hyperpolarization in both groups. Dexamethasone at a dose which restores the gluconeogenic response after adrenalectomy, had no effect on either the ion movements or membrane potential and did not alter the responses to cyclic nucleotides or glucagon in either normal or adrenalectomized rat livers. These results suggest that the permissive effect of glucacorticoids on gluconeogenesis might be related to an event following ion movement.

INTRODUCTION

It has been demonstrated that administration of glucagon, 3′,5′-AMP or 3′,5′-GMP to the perfused liver is followed by an increased rate of gluconeogenesis[1–3]. The stimulatory effect of these agents on gluconeogenesis seems to be associated with a large-scale ion movement and hyperpolarization of the cell membrane[4–8]. Interference with the ion redistribution either by local anesthetics, or by varying the ionic composition of the perfusate blocks the increase in glucose output[5,6]. The ability of glucagon or 3′,5′-AMP to exert their effect on gluconeogenesis depends on the presence of glucocorticoids. While the exact mechanism by which glucocorticoids influence these processes is not known, it seems to be established that their role is a permissive effect rather than a regulatory one[9–15]. The observed relationship between the hormonally-induced gluconeogenesis and ion movements indicates the possibility that the glucocorticoid influence on gluconeogenesis is correlated with an effect on ion fluxes. Results from our experiments testing this hypothesis are given in this report.

METHODS

Male Sprague–Dawley rats weighing 80–100 g were used. Adrenalectomized rats were given 0.9% NaCl to drink. 5–8 days later after overnight fast they served

as liver donors. Normal animals were also fasted overnight prior to experiments. The liver perfusion technique has been described in detail (ref. 5, also, see legends for Figs 1 and 2). The standard medium consisted of Krebs–Ringer bicarbonate buffer containing 4% bovine albumin (Fraction V, N.B.C.). Membrane potentials were measured by impalement of superficial cells with glass capillary microelectrodes filled with either 2 M potassium citrate or 3 M KCl. 15–60 MΩ resistance electrodes were used. Although it was possible to maintain stable cell penetrations for long periods (5–20 min) electrodes were routinely withdrawn from cells after attaining a stable potential level; thus, a sampling of up to 15 cells per min was achieved. Temperature, monitored at the surface of the liver was maintained at 33 °C. Na^+ and K^+ were determined with diluted perfusate by using a Beckman flame-photometer.

RESULTS AND DISCUSSION

The effects of glucagon, 3′,5′-AMP, 3′,5′-GMP and dexamethasone on K^+ and Na^+ movement were examined. Following glucagon or cyclic nucleotide administration, a biphasic movement of Na^+ and K^+ was observed (Fig. 1). The responses in livers from the adrenalectomized animals were similar to those reported previously for glucagon and 3′,5′-AMP in normal animals[6]. We are reporting now that 3′,5′-GMP also affects Na^+ and K^+ movements in the livers from normal (not shown) and adrenalectomized animals. This effect is essentially similar to the effect of 3′,5′-AMP. Dexamethasone (2 or 7 μg/ml, a dose which has been shown to restore the gluconeogenic response in livers from adrenalectomized rats[12]) had no effect on ion movement (Fig. 1).

The control level of liver cell membrane potentials from adrenalectomized and normal animals were not significantly different (Table I). Similar values have been reported for perfused rat liver[7,8,16–18]. Because it was desirable to perform the electrophysiological measurements under conditions identical to the metabolic studies, Krebs–Ringer bicarbonate buffer with an additional 20 mM sodium pyruvate was the control perfusate. In both groups the addition of sodium pyruvate produced a hyperpolarization which developed to a steady state over a 10–15-min period (Table I). Dexamethasone, 4 μg/ml, had no effect on liver cell membrane potentials within 20 min (Table I). Glucagon, 3′,5′-AMP and 3′,5′-GMP produced transient hyperpolarization in all experiments (Table I and Fig. 2). The hyperpolarization responses in the normal and the adrenalectomized groups were not significantly different. The range of peak hyperpolarization responses was 17–21 mM and the range of maximum membrane potentials during this response was 60–77 mV. As determined in separate experiments (not shown) the hyperpolarization responses were not dependent on the presence of sodium pyruvate; the membrane potentials were less in the absence of the sodium pyruvate but the increments of hyperpolarization were similar. Hyperpolarizations were always transient, attaining peak values at 4–7 min then returning toward control levels. Frequently, a second period of membrane hyperpolarization was observed 6–12 min after the initial response.

Our present data demonstrate that glucagon, 3′,5′-AMP or 3′,5′-GMP administration is followed by a redistribution of Na^+ and K^+ and cell membrane hyperpolarization in the perfused livers of adrenalectomized and normal animals.

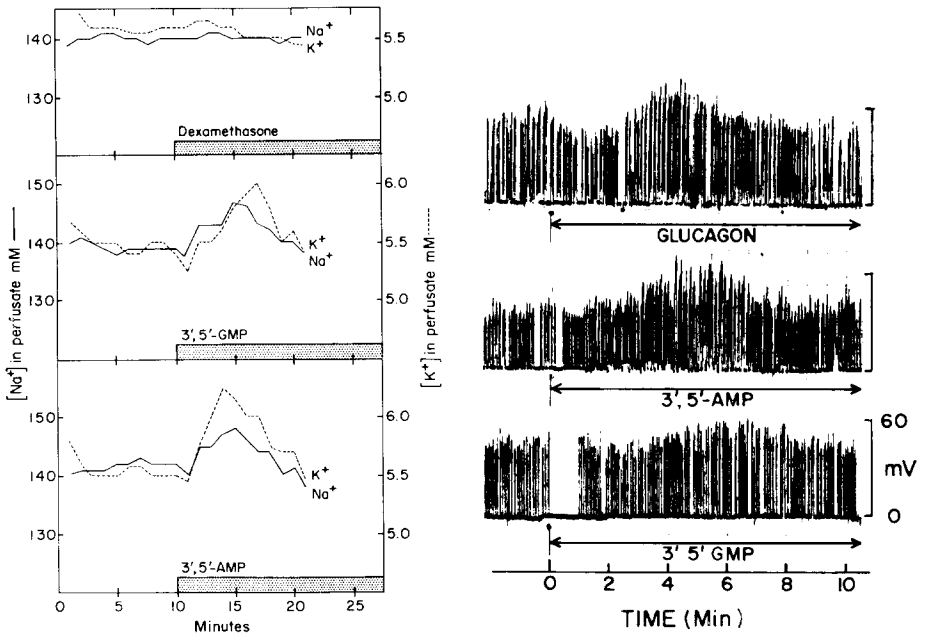

Fig. 1. The effects of 3',5'-AMP, 3',5'-GMP and dexamethasone on ion fluxes. Livers from fasted adrenalectomized rats were perfused for 45 min in a recirculating system with the standard medium. The test period shown in the figure was then begun by switching to fresh medium in a non-recirculating system and collecting the effluent over 1-min intervals. Where indicated 3',5'-AMP or 3',5'-GMP was added to the reservoir to give a final concentration of $5 \cdot 10^{-4}$ M. The concentration of dexamethasone was 2 μg per ml. Each figure represents the average of three experiments.

Fig. 2. The effects of 3',5'-AMP, 3',5'-GMP and glucagon on liver membrane potential. Livers were perfused with Krebs-Ringer bicarbonate buffer in a recirculating system. After 25 min, sodium pyruvate was added to the reservoir to give a final concentration of 20 mM. 40 min after the beginning of the experiments the test substances were added (Time=0). The final concentration of 3',5'-AMP or 3',5'-GMP was $5 \cdot 10^{-4}$ M and of glucagon was 0.1 μg per ml. The reference potential level (extracellular perfusate) is indicated by the base line in each record. Deflections from the base line indicate individual cell penetrations. (D.C. potential recording, upward is negative).

The glucocorticoid, dexamethasone, had no effect on either the K^+ and Na^+ movement or the membrane potential, and, further, did not alter the electrolyte movement and membrane hyperpolarization responses to cyclic nucleotide or glucagon administration in either normal or adrenalectomized animals. Previous studies showed that the efflux of Ca^{2+} which occurs in response to glucagon or 3',5'-AMP is neither dependent upon the presence of glucocorticoids nor affected by them[4]. These data indicate that the response of the adrenalectomized liver to glucagon or cyclic-nucleotide administration in respect to ion movement either measured directly or reflected by changes in membrane potential, is unimpaired.

Miller *et al.*[19] have shown that hearts from adrenalectomized rats had an impaired glycogenolytic response to epinephrine which was restored by raising the perfusate Ca^{2+} concentration from 2.5 to 3.5 mM. The depressed phosphorylase

TABLE I

MEMBRANE POTENTIALS

Results are expressed in mV. Number of experiments in parentheses. Statistical probability (p) was calculated according to Student's t-test, comparing membrane potential values from normal and adrenalectomized condition. The upper P value corresponds to comparison of the first entries in each column**, and the lower P value corresponds to the comparison of the second entries in each column***.

	Krebs–Ringer bicarbonate buffer	*Krebs–Ringer bicarbonate buffer + 20 mM sodium pyruvate*	*Glucagon*	*3′,5′-AMP, $5 \cdot 10^{-4}$ M*	*3′,5′-GMP, $5 \cdot 10^{-4}$ M*	*Dexamethason 4 µg/ml*
Normal	35.2 ± 1.6*	45.8 ± 2.1	55.8 ± 1.0** 62.7 ± 2.0***	61.2 ± 2.9 64.8 ± 4.2	57.0 ± 1.2 62.7 ± 2.7	45.0
	(14)	(17)	(5)	(6)	(3)	(1)
Adrenal-ectomized	36.8 ± 2.3	44.0 ± 1.6	58.7 ± 2.0 66.5 ± 3.2	55.8 ± 1.6 62.3 ± 1.9	57.0 ± 1.1 61.5 ± 1.5	$45.3 \pm 2.($
	(6)	(18)	(6)	(6)	(2)	(4)
P	0.25	0.30	0.15 0.20	0.15 0.10	> 0.45 > 0.45	

* Mean $\pm$ S.E.

** Mean value of responses (from the average value of eight consecutive cell penetrations obtained during the peak of the response in each experiment).

*** Mean of maximum single cell membrane potentials during response.

activity was also restored to normal values. Thus, it has been proposed that the physiological role of glucocorticoids in metabolic processes is the maintenance of the intracellular ionic environment[15,19,20]. In contrast to the heart, gluconeogenesis in liver is not influenced by extracellular calcium[5]. Furthermore, the ionic movements, both directly measured and inferred from membrane potential changes, are identical in livers from normal and adrenalectomized animals and are not affected by dexamethasone. Thus, if the action of glucocorticoids in relation to gluconeogenesis in liver is mediated by regulation of the ionic environment, the alterations must be at the subcellular level rather than across the cell membrane.

The impaired gluconeogenic response observed in livers from adrenalectomized animals is caused by a defect in a step after the cyclase reaction[12,15]. The present data raise the possibility that the defect might be in a step beyond the effect of glucagon or 3′,5′-AMP on ion movement and that the permissive effect of glucocorticoids may be related to an event following ion movement.

ACKNOWLEDGEMENTS

N. Friedmann wants to thank H. Rasmussen for his continuous support. N. Friedmann is supported by the Alma Toorock Memorial Fund and by Grant U.S.P.H.S. AM-0650. G. Dambach is supported by Grant U.S.P.H.S. NS-03321-11.

REFERENCES

1 Exton, J. H., Mallette, L. E., Jefferson, L. S., Wong, E. H S., Friedmann, N., Miller, J. B. and Park, C. R. (1970) *Rec. Prog. Horm. Res.* 26, 411–457
2 Glinsmann, W. H., Hern, E. P., Linarette, L. G. and Farese, R. V. (1969) *Endocrinology* 85, 711–719
3 Exton, J. H., Hardmann, J. G., Williams, T. F., Sutherland, E. W. and Park, C. R. (1971) *J. Biol. Chem.* 246, 2658–2664
4 Friedmann, N. and Park, C. R. (1968) *Proc. Natl. Acad. Sci. U.S.* 61, 504–508
5 Friedmann, N. and Rasmussen, H. (1970) *Biochim. Biophys. Acta* 222, 41–52
6 Friedmann, N. (1972) *Biochim. Biophys. Acta* 274, 214–225
7 Friedmann, N., Somlyo, A. V. and Somlyo, A. P. (1971) *Science* 171, 400–402
8 Somlyo, A. P., Somlyo, A. V. and Friedmann, N. (1971) *Ann. N.Y. Acad. Sci.* 185, 108–114
9 Ingle, D. I. (1952) *J. Endocrinol.* 8, 23–38
10 Friedmann, N. and Wertheimer, H. E. (1966) *Metabolism* 15, 222–229
11 Feldman, S., Friedmann, N. and Wertheimer, H. E. (1966) *Nature* 210, 860
12 Friedmann, N., Exton, J. H. and Park, C. R. (1967) *Biochem. Biophys. Res. Commun.* 29, 113–119
13 Eisenstein, A. B. (1967) *Abstr. 27th Annu. Meet. Am. Diabetes Assoc. Atl. City*, in *Diabetis* 16, p. 522
14 Guder, W., Hepp, K. D. and Wieland, O. (1970) *Biochim. Biophys. Acta* 222, 593–605
15 Exton, J. H., Friedmann, N., Wong, E. H. A., Brineaux, P., Corbin, J. D. and Park, C. R. (1972) *J. Biol. Chem.* 247, 3579–3588
16 Claret, M. and Mazet, J. L. (1972) *J. Physiol.* 223, 279–295
17 Claret, M. and Coraboeuf, E. (1968) *C. R. Acad. Sci. Paris t.* 267, 642–645
18 Haylett, D. G. and Jenkinson, D. H. (1969) *Nature* 224, 80–81
19 Miller, T. B., Exton, J. H. and Park, C. R. (1971) *J. Biol. Chem.* 246, 3672–3678
20 Rasmussen, H. and Tenenhause, A. (1968) *Proc. Natl. Acad. Sci. U.S.* 59, 1364–1369