EFFECT OF ETHANOL AND LACTATE ON THE BASAL AND GLUCAGON-ACTIVATED CYCLIC AMP FORMATION IN ISOLATED HEPATOCYTES

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Received 11 February 1977

1. Introduction

The activation of adenylate cyclase as a consequence of the interaction of catabolic hormones with the plasma membrane is a well established concept [1]. The concomitant increase of cyclic AMP, the second messenger, mediates some of the metabolic responses to these hormones. Recently it has been shown that NADH, but not NAD or NADPH, can inhibit the adenylate cyclase activity in isolated fat-cell plasma membranes [2]. In view of evidence that plasma membranes contain NADH dehydrogenase activity [3] which can be influenced by hormones that at similar concentration stimulate adenylate cyclase activity [4] we were led to question if changes in the redox state in intact cells influence the response of these cells to hormone stimulation.

The aim of the present work therefore, was to study the influence of a change in the oxidation–reduction state on the hormone-induced cyclic AMP production in isolated cells, more specificly hepatocytes. Since these cells contain alcohol-dehydrogenase the oxidation–reduction level of the pyridine-nucleotides of the cytosol could not only be manipulated by incubation with lactate [5] but also with ethanol.

2. Materials and methods

2.1. *Isolation of rat hepatocytes*

Male rats, weighing 150–180 g and fed a regular laboratory diet and water ad libitum, were used throughout. Parenchymal liver cells were prepared by the enzymatic procedure of Christoffersen and Berg [6]. After preperfusion with Ca^{2+}-free Hank's solution, the liver was removed and perfused with buffer containing 0.05% collagenase and Ca^{2+} (4 mmol/l) for 8–10 min with a flow-rate of 30 ml/min. The cells were washed 3 times with buffer containing 0.02 mol/l Hepes and 1% BSA. The cell-yield was about 300–400 $\times$ 10^6 cells/liver. Viability of the hepatocytes was 85–90% as determined by Trypan Blue exclusion.

2.2. *Incubation procedure*

The hepatocytes were suspended in a modified Krebs-Ringer bicarbonate buffer supplemented with glucose and gassed with 95% O_2 + 5% CO_2 (pH 7.4). The composition of the buffer was as follows: 118.5 mmol/l NaCl, 4.2 mmol/l KCl, 2.0 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4, 24.9 mmol/$NaHCO_3$ and 10 mmol/l glucose. Aliquots, 0.5 ml, of buffer containing 8 $\times$ 10^6 cells/ml and ethanol or lactate added to appropriate concentration were preincubated for 45 min, at 37°C with constant shaking and under continous aeration with 95% O_2 + 5% CO_2. Glucagon and 3-isobutyl-1-methylxanthine as a phosphodiesterase inhibitor in 0.5 ml buffer was then added, giving the final concentrations indicated in the Results. The incubation was terminated after 2 min by adding 10% ice-cold trichloroacetic acid.

2.3. *Determination of cyclic AMP*

After centrifugation of the trichloroacetic acid homogenates at 2000 rev./min for 30 min, the supernatants were transferred to glass test tubes and HCl was added to final concentration of 0.1 mol/l. The samples were extracted 4 times with 2 vol. ether and

dried at room temperature. Cyclic AMP was determined by the protein-binding assay of Gilman [7] as modified by Geisler et al. [8]. The separation of free and bound radioactivity ([^{3}H]cAMP) was achieved by precipitation with 70% saturated $(NH_4)_2SO_4$ solution containing 2% $CaSO_4$. All the procedures, including counting, were performed in a single assay tube. Protein was determined in the precipitates by the method of Lowry [9].

The following materials were purchased: collagenase (type I), Hepes (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid), adenosine 3′5′-cyclic monophosphoric acid, bovine serum albumin (fraction V), from Sigma Chem Co., St Louis. Adenosine 3′5′-cyclic phosphate [^{3}H]ammonium salt from New England Nuclear, Boston. Glucagon was from Novo, Copenhagen.

3. Results

The basal content of cAMP in liver cells and its incubation medium was about 0.7 pmol/mg liver protein or 1 pmol/10^6 hepatocytes. Addition of glucagon in concentrations between 1.4×10^{-10} and 1.4×10^{-7} mol/l gave after 2 min a dose-dependent increase in the cyclic AMP content to a maximum of 10–20-times the basal level (fig.1). Preincubation with

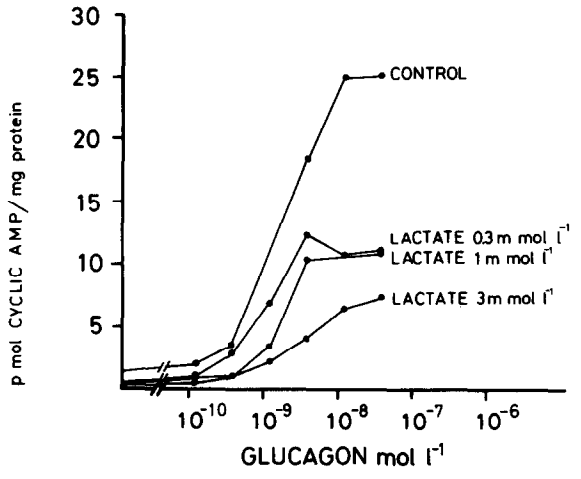

Fig.1. Effect of lactate on the basal and glucagon-stimulated cyclic AMP formation in hepatocytes. Rat liver cells (4×10^6/tube) were preincubated without and in the presence of 0.3, 1 and 3 mmol/l lactate for 45 min at 37°C. Glucagon in different concentrations and 3-isobutyl-1-methylxanthine (10^{-4} mol/l) were then added for 2 min incubation. 4×10^6 Cells contain about 6 mg protein.

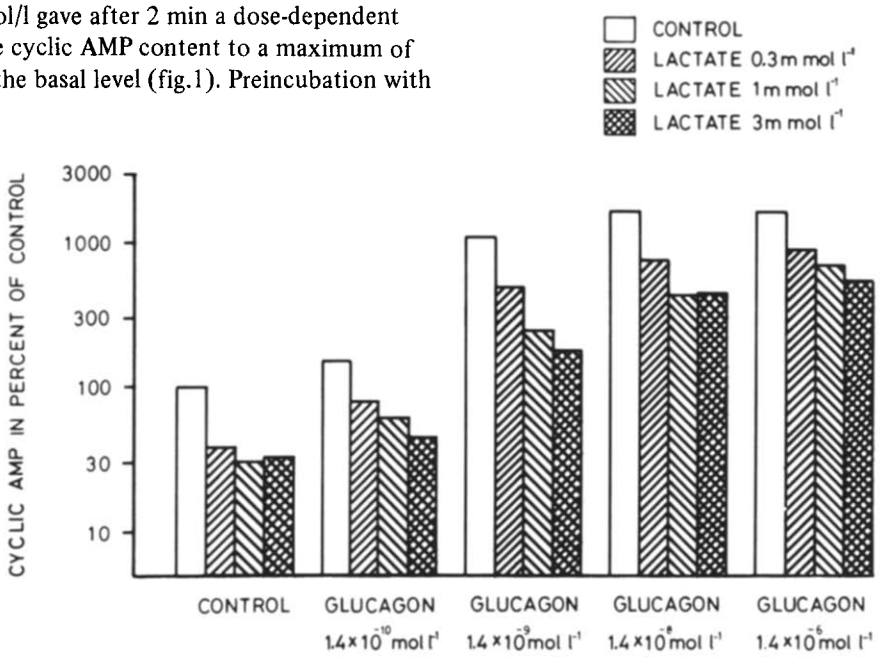

Fig.2. Inhibition of cyclic AMP formation by lactate in hepatocytes incubated without and with increasing concentrations of glucagon. The values are the means of two experiments. The cyclic AMP values are plotted on a log scale in % control without glucagon and lactate. The conditions were as in the legend to fig.1.

lactate in concentrations 0.3, 1 and 3 mmol/l for 45 min produced a dose-dependent decrease of both the basal and glucagon-stimulated increase in cAMP content (fig.1). The percentage decrease was similar in both basal and glucagon-stimulated cAMP levels as caused by lactate preincubation. In fig.2 are summarized the results obtained in 2 experiments. Preincubation with 3 mmol/l lactate for 45 min reduced the cAMP content 70–80% at all glucagon concentrations. Different lactate concentrations gave a dose-dependent inhibition.

Preincubation with ethanol also decreased both the basal and the glucagon-stimulated increase in cyclic AMP levels (fig.3). This inhibition increased with increase of alcohol concentrations from 3–6 mol/l. With ethanol, however, the percentage inhibition of cAMP content varied between experiments. Furthermore, the inhibition varied with the glucagon concentration as well.

4. Discussion

The basal level of cyclic AMP in the isolated hepatocytes was similar to that found by others [6,10].

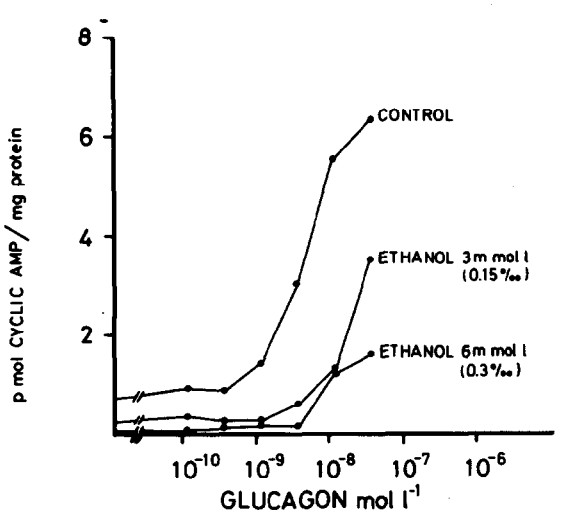

Fig.3. Effect of ethanol on the basal and glucagon-stimulated cyclic AMP formation in hepatocytes. The hepatocytes (4×10^6/tube) were preincubated without or with 3 mmol/l and 6 mmol/l ethanol for 45 min at 37°C. Glucagon in different concentrations and 3-isobutyl-1-methyl-xanthine (10^{-4} mol/l) were then added for 2 min incubation.

Addition of glucagon in the presence of phosphodiesterase inhibitor gave a rapid and dose-dependent increase in cyclic AMP accumulation; the sensitivity to glucagon was in accordance with that reported by others [6,11,12].

Preincubation with lactate or ethanol decreased both the basal and glucagon-stimulated cyclic AMP accumulation in isolated rat hepatocytes. This result is in accordance with earlier findings, that NADH caused an inhibition of hormone-stimulated adenylate cyclase activity in isolated plasma membranes [2]. The impermeability of intact cells for NADH was overcome by adding lactate or ethanol, which cross the cell-membrane and increase the NADH/NAD ratio in the cytosol [5,13]. The oxidation of ethanol to acetaldehyde and then to acetate [14] in liver cells, catalyzed by alcohol dehydrogenase and acetaldehyde dehydrogenase respectively, requires NAD as a hydrogen-acceptor and generates NADH.

It is well established that ethanol in vivo reduces hepatic gluconeogenesis and causes hypoglycemia [13]. The effect of ethanol in vivo on gluconeogenesis is contrary to the glucagon effect, which at least partly is mediated by cyclic AMP. The present finding that ethanol decreases cyclic AMP generation offers an explanation for the hypoglycemic effect of ethanol.

There is some controversy about the effects of alcohol on cyclic AMP formation. Using high concentrations of alcohol Gorman and Bitensky [15] found stimulation of adenyl cyclase in both whole liver homogenates and washed particle enzyme whereas Mashiter et al. [16] only found stimulation in plasma membrane preparation but not in homogenates. In contrast to this, acute administration of ethanol to rats decreased the cyclic AMP level in liver cells [17], and inhibited the formation of cyclic AMP from ATP prelabelled with [^{3}H]adenine [18].

The inhibitory effect of lactate on cyclic AMP accumulation had been shown previously in adipocytes but the authors had no explanation for this [19]. In contradiction to the present results Jarret et al. [20] reported that a high ratio of lactate/pyruvate in the incubation medium increased the rate of glucagon-induced cyclic AMP formation in hepatocytes. This discrepancy can be explained by the facts that no equilibration period with lactate/pyruvate preceded their incubation and no cyclic AMP phosphodiesterase inhibitors were used.

In conclusion, the present study indicates that changes in the intracellular redox situation influence cyclic AMP generation and thereby the metabolic response to adenylate cyclase stimulating hormone. This is in accordance with the previous finding that NADH inhibits adenylate cyclase in liver plasma membranes. The mechanism by which an increase in NADH/NAD ratio can act on the membrane-bound adenylate cyclase is not clear, but some kind of coupling may exist between the hormone sensitive plasma membrane-bound NADH dehydrogenase and adenylate cyclase.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (No. 19X-04224 and 03X-04539) and Nordic Insulin Foundation.

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