Effect of glucose, NADH and NADPH on cortisol metabolism by mononuclear cells

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RECEIVED 8 July 1985

ABSTRACT

Mononuclear cell preparations are capable of metabolizing cortisol to three metabolites which lack the immunosuppressive effect of their precursor. In the present study we noted a linear correlation, up to a point, between glucose concentration and the rate of human mononuclear cell cortisol metabolism *in vitro*. The mechanism by which glucose exerts its effect was investigated further. We observed that: (1) the effect of glucose on mononuclear cell cortisol metabolism was not influenced by insulin; (2) NADPH and NADH enhanced cortisol metabolism by disrupted cells, irrespective of whether the homogenates were dialysed or not; (3) lactate and ATP inhibited mononuclear cell cortisol metabolism and (4) almost all the glucose used was converted to lactate. It is concluded that mononuclear cell cortisol metabolism can depend on both nucleotides.

J. Endocr. (1986) **109,** 181–185

INTRODUCTION

The destructive effect of cortisol on human and animal lymphocytes, as well as its ability to reduce the number of lymphocytes in human peripheral blood, has been demonstrated repeatedly (Berliner & Dougherty, 1961; Dougherty, Berliner & Berliner, 1961; Fauci & Dale, 1974; Yu, Clements, Paulus *et al.* 1974; Fan, Yu, Targoff & Bluestone, 1978; Haynes & Fauci, 1978; Thomson, McMahon & Nugent, 1980; Riley, 1981; Thompson, Harmon & Zawydiwski, 1983). In addition, the ability of lymphocytes to effect alterations in the molecular structure of cortisol has been reported by a number of workers including Berliner & Dougherty (1961). Dougherty *et al.* (1961), Jenkins & Kemp (1969) and Klein, Kaufmann, Mannheimer & Joshua (1978). Jenkins & Kemp (1969) and Klein *et al.* (1978) demonstrated that human lymphocytes are capable of reducing cortisol to tetrahydrocortisol, 20-α-dihydrocortisol and 20-β-dihydrocortisol.

Berliner & Dougherty (1961), Dougherty *et al.* (1961) and Langhoff & Ladefoget (1983) found that the cortisol metabolites have no suppressive effect upon lymphocytes. Berliner & Dougherty (1961) have postulated that the ability of lymphocytes to effect changes in cortisol constitutes an important homeostatic mechanism in the regulation of the lymphocyte population.

It is known that NADPH is the hydrogen donor in steroid reduction reactions; however, Graef, Golf & Tuschen (1981) demonstrated that NADH too can be used as a coenzyme for the reduction of 4-ene-3-oxosteroids by liver microsomes.

In the present study we investigated the effect of glucose, NADH and NADPH on mononuclear cell cortisol metabolism. Intact cells were used for measuring the effect of glucose whereas disrupted cells were used for measuring the effect of the nucleotides.

MATERIALS AND METHODS

The effect of glucose, NADH, NADPH, lactic acid, insulin and ATP on the metabolism of cortisol by mononuclear cell preparations was examined. Cells were prepared from leucocyte-enriched blood (buffy coats) obtained from a blood bank (Canadian Red Cross Society, Toronto) within 2 h of blood drawing. They were isolated by centrifugation at 400 *g* using the Ficol-Isopac method of Boyum (1968) (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.). Phosphate-buffered saline (PBS; pH 7·4), containing penicillin (100 i.u.; Glaxo Laboratories, Toronto, Canada) and

  Printed in Great Britain
0022–0795/86/0109–0181 $02.00/0

streptomycin (100 i.u.; Allen & Hanburys, Toronto, Canada), was used for washing (twice) and suspending the cells. The mean percentage of lymphocytes obtained was 83 (maximum 89, minimum 74), whereas the percentage of monocytes and neutrophils was 16 and <1 respectively. Cortisol metabolism by monocytes was found to be lower than that of lymphocytes. The contribution of monocytes was therefore minimal (authors' unpublished data). Nucleotides are membrane impermeable (Suzuki & Kakinuma, 1983); the cells were, therefore, divided into three groups: (1) intact cells, (2) disrupted cells (frozen and thawed three times) and (3) disrupted cells which had then been dialysed against Hank's balanced salt solution (1 : 100 for 24 h). The dialysis was undertaken to remove intracellular NADP in order to see whether NADH can directly influence mononuclear cell cortisol metabolism. Each preparation of cells was divided into flasks, so that each flask contained an average of 5×10^7 cells in either 2 ml (intact cells) or 0·7 ml (disrupted cells) of suspension. The cells were used for measurements of cortisol metabolism (each flask contained 2·0 μCi [1,2-^{3}H]cortisol; sp. act. 14·54 Ci/mol; New England Nuclear, Boston, MA, U.S.A.) and for analysis of glucose utilization. For the measurement of cortisol metabolism the sealed flasks were incubated in a shaking bath at 37 °C for 17 h. The time of incubation was chosen after a preliminary experiment in which cortisol metabolism was plotted against time. The reaction was hyperbolic with time, reaching a plateau after 17 h. The average metabolism of cortisol was 12%. No significant death of cells was discovered after this period of time, using trypan blue exclusion as an indicator. At the end of the incubation period the contents of each flask were extracted with 10 ml chloroform and evaporated to dryness under nitrogen. The residue was dissolved in ethanol and applied on silica gel HF-254 thin-layer plates (E. Merck, Darmstadt, F.R.G.). The plates were developed in chloroform : methanol (90 : 10, v/v). Since all the cortisol metabolites are located on one spot of the plate (Klein *et al.* 1978), u.v. light (254 nm) was used for detecting the spots of cortisol and its metabolites. The product and substrate spots were scraped off and extracted with ethanol (final volume 5 ml). Samples of 0·2 ml were transferred into scintillation vials and the radioactivity measured in a liquid scintillation spectrometer.

For the analysis of glucose utilization the sealed flasks were incubated for 17 h with either unlabelled glucose (5·5 mmol/l) or 1·0 μCi [D-U-^{14}C]glucose (Amersham Corporation, Oakville, Ontario, Canada) as appropriate. The unlabelled glucose was used for measuring glucose disappearance from the medium (glucose oxidase method using an oxygen electrode; Beckman-ASTRA-8 Analyser) and lactic acid formation (Rosenberg & Rush, 1966), whereas the labelled glucose was used for measuring CO_2 generation (Tsan, Chen, Newman *et al.* 1975). Controls containing all the substances, except mononuclear cells, were run in parallel to rule out the influence of any extraneous factors.

RESULTS

Glucose utilization

In the case of intact cells given 11 μmol glucose, 6·32 ± 0·5 (mean ± S.E.M.) μmol glucose were utilized and 12·46 ± 0·8 μmol lactate were produced by 5×10^7 mononuclear cells in 17 h. Similar rates (6·1 ± 0·5 μmol glucose utilized and 12·3 ± 0·3 μmol lactate formed) were obtained with disrupted cells. In addition, disrupted cells converted 0·4% of the consumed glucose to CO_2, whereas 0·8% of the glucose was converted to CO_2 by the intact cells. All extracts of the cell suspensions preincubated with labelled cortisol showed two radioactive peaks: a major, less polar peak, corresponding to cortisol itself and a minor, more polar peak, corresponding to the total amount of metabolized cortisol.

FIGURE 1. Effect of glucose on cortisol metabolism in 'starved' (■) or 'non-starved' (●) human mononuclear cells. 'Starvation' was carried out by incubating the cells in phosphate-buffered saline alone at 37 °C for 3 h before the start of the experiment, while the 'non-starved' cells are means ± S.E.M. of duplicate determinations in three separate experiments. Values for the 'starved' cells represent the means of duplicate determinations (S.E.M. not calculated). Final volume of medium was 2 ml; amount of cortisol added was 140 nmol.

Cortisol metabolism

Intact cells

Figure 1 shows the effect of glucose concentrations on cortisol metabolism by mononuclear cells which were either kept at 4 °C or 'starved' before the start of incubation. 'Starvation' of cells was carried out by incubating the cells in PBS alone at 37 °C for 3 h before the start of the experiment. The results show that at a glucose concentration of 1 mg/ml (5·55 mmol/l) the highest rate of cortisol metabolism was obtained by both cell groups. The increase in cortisol metabolism was followed by a plateau (more apparent in the 'starved' cells) and then a drop in activity. 'Starvation' caused a decrease in the activity of the control cells (with no glucose added) which was restored by the addition of glucose. Insulin (0·15 i.u. beef and pork insulin/ml; Connaught Laboratories, Willowdale, Ontario, Canada) had no effect on the rate of cortisol metabolism of 'non-starved' cells in the presence of various concentrations of glucose (0, 0·25, 1·0, 2·5 and 5 mg/ml).

Neither NADH nor NADPH affected cortisol metabolism by intact cells. The results were similar to those obtained in cells immersed in PBS alone. These results are consistent with the fact that nucleotides are membrane impermeable (Suzuki & Kakinuma, 1983).

The addition of lactic acid (13·8 mmol/l) inhibited cortisol metabolism in intact cells: cells immersed in (1) PBS alone, (2) PBS plus glucose (5·5 mmol/l) and (3) PBS plus glucose plus lactate metabolized $9{\cdot}9 \pm 1{\cdot}7$, $23 \pm 2{\cdot}8$ and $15{\cdot}5 \pm 2{\cdot}6$ nmol cortisol respectively. There was a significant difference between groups 1 and 2 ($P<0{\cdot}001$) and groups 2 and 3 ($P<0{\cdot}025$), and groups 2 and 3 ($P<0{\cdot}001$), but not between groups 1 and 3 ($P>0{\cdot}2$; Student's *t*-test). It should be noted that the addition of lactic acid did not change the pH of the medium.

Disrupted cells

The addition of NADPH (5 μmol) or NADH (5 μmol) to disrupted cells produced 30- and 8-fold increases respectively in the metabolism of cortisol, compared with that of cells immersed in PBS (Fig. 2; $P<0{\cdot}001$ in both cases).

In the presence of NADH, ATP reduced the metabolism of cortisol to one-third of the rate obtained in the absence of ATP (Fig. 2). A slight inhibition by ATP of the NADPH-enhancing activity was also observed.

Dialysed disrupted cells

The addition of NADH (5 μmol) or NADPH (5 μmol) increased cortisol metabolism in dialysed cells: cells incubated in PBS, PBS plus NADH and PBS plus NADPH metabolized $0{\cdot}26 \pm 0{\cdot}05$, $1{\cdot}0 \pm 0{\cdot}1$ and $3{\cdot}4 \pm 0{\cdot}18$ nmol cortisol respectively ($P<0{\cdot}001$ between each group). The dialysis was undertaken to remove intracellular NADP and to ascertain whether NADH could influence cortisol metabolism by mononuclear cells directly, with no potential involvement of transhydrogenase.

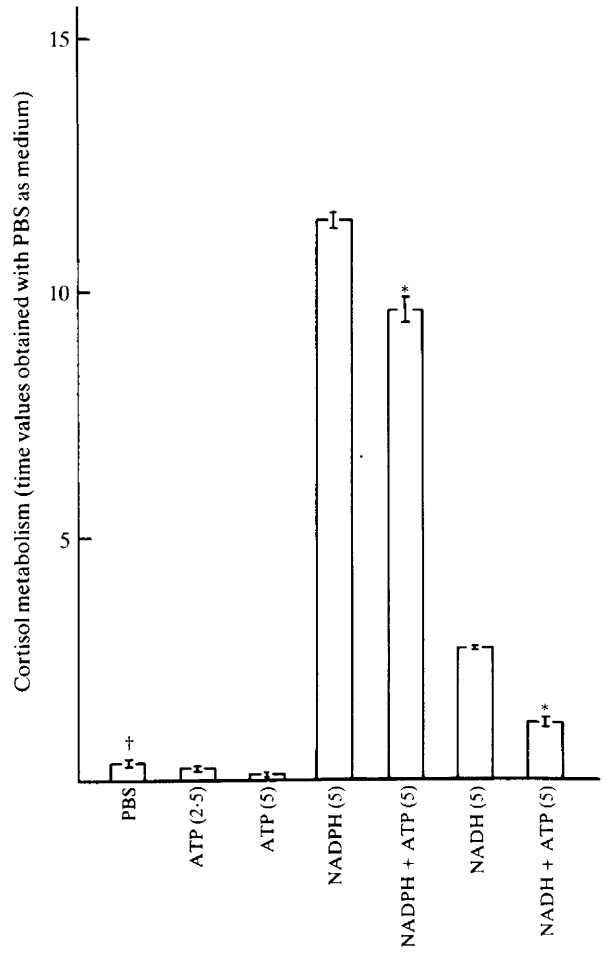

FIGURE 2. Effect of ATP on cortisol metabolism obtained with disrupted human mononuclear cells. Values are means ± S.E.M. of duplicate determinations in two separate experiments. Final volume of medium was 0·7 ml; amount of cortisol added was 140 nmol. * $P<0{\cdot}025$ compared with NADH; † $P<0{\cdot}05$ compared with 5 μmol ATP (no nucleotide added) (Student's *t*-test). PBS, phosphate-buffered saline. Amounts (μmol) of ATP, NADH and NADPH added to the medium are given in parentheses.

DISCUSSION

The large increase in mononuclear cell cortisol metabolism in disrupted cells after the addition of NADPH (Fig. 2) is readily accounted for by the fact

that the nucleotide serves as a donor of hydrogen atoms in steroid reduction. The effect of NADH on mononuclear cell cortisol metabolism is more complex and implies either the involvement of transhydrogenase in the formation of NADPH from NADH or a direct effect of NADH. Results obtained after dialysing the disrupted cells, to remove intracellular NADP, indicated that NADH can directly affect mononuclear cell cortisol metabolism with no involvement of transhydrogenase. These results support the findings of Graef *et al.* (1981) who demonstrated that NADH can be used as a coenzyme for the reduction of 4-ene-3-oxosteroids by liver microsomes. However, when glucose is converted completely to lactate there is no net production of NADH. Consequently, the conversion of 0·8% of the glucose to CO_2 implies the use of pyruvate by systems other than lactate dehydrogenase and the release of available NADH. This is supported by Berger, Berger, Siborski & Catino (1982) who showed that resting lymphocytes formed three times more NADH than NADPH. The fact that the tricarboxylic acid cycle was found to function at a low rate in resting lymphocytes and was almost unaffected by mitogens (MacHaffie & Wang, 1967; Sagone & Murphy, 1975; Tsan *et al.* 1975; Egorin, Felsted & Bachur, 1977) indicates that this cycle, in addition to its other functions, may be responsible for the NADH formation needed for cortisol metabolism by mononuclear cells. It does not, however, exclude the possibility that enough NADPH is formed by the pentose-phosphate pathway to support mononuclear cell cortisol metabolism. In any case, stimulation of lymphocytes is characterized by a rise in NADPH formation (Sagone, LoBuglio & Balcerzak, 1974) which apparently plays the main role in cortisol metabolism.

Glucose seems to play an important role in sustaining and regulating cortisol metabolism by mononuclear cells (Fig. 1). 'Starvation' probably causes a depletion of hydrogen donors (NADH and NADPH) which is restored by the addition of glucose. The maximum rate of cortisol metabolism, obtained in this experiment with 50×10^6 intact cells immersed in medium containing 1 mg glucose/ml, was 14 pmol/min, which seems to be a rather low rate of conversion. The implications of these results are, however, more significant when it is considered that the normal concentration of free cortisol *in vivo* in man is about 13 nmol/l and only a small fraction of the steroid penetrates the cells.

The inhibitory effect of ATP on mononuclear cell cortisol metabolism seems to be more significant on the NADH- rather than the NADPH-supported reactions. It could not, however, be excluded that this effect is on other metabolic levels, such as the use of the nucleotides for purposes other than cortisol metabolism.

The highest mononuclear cell cortisol metabolism was obtained at a normal glucose concentration of 1 mg/ml (Fig. 1), and was followed by a plateau and then a decline in activity. This decline could be due to an increase in the concentration of lactate, which was found to have the capacity to inhibit cortisol metabolism. This might differ from conditions *in vivo*, where lactate might leave the cells, enter the blood stream and be metabolized by the liver. Thus cortisol metabolism *in vivo* may respond to both lower and higher glucose concentrations than that observed *in vitro*.

In conclusion, it seems that the regulation of mononuclear cell cortisol metabolism is a complicated mechanism made up of stimulators and inhibitors. The dependence on glucose, at least in resting cells, may be expressed directly not only by NADPH but also through NADH.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Canadian Red Cross Transfusion Service (Toronto Centre) and to its staff for supplying buffy coats.

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