Membranotropic Effects of Glucocorticoids. Effect of Hydrocortisone on D-Glucose Uptake by Isolated Hepatocytes

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Hydrocortisone immobilized on polyvinylpyrrolidone did not affect D-glucose uptake by hepatocytes, while free hydrocortisone in a concentration of 10 μM or higher decreased the initial rate of this process. Hydrocortisone-induced inhibition was dose-dependent and related to a decrease in the maximum rate of D-glucose uptake. Polyvinylpyrrolidone-immobilized hydrocortisone potentiated the effects of free hormone. Membranotropic effect of hydrocortisone on D-glucose transport into hepatocytes was associated with a decreased number of glucose carrier molecules or their turnover, rather than with lowered affinity of transport systems for glucose.

Key Words: *hydrocortisone; membranotropic effect; hepatocytes; glucose transport*

It is well known that steroid hormones display membranotropic activity (for example, effect of progesterone on Ca^{2+} concentration and activities of tyrosine kinase and phospholipase C in gametes [11], nongenomic effect of aldosterone on transmembrane Na^+ transport in epitheliocytes of renal tubules [6], early effects of vitamin D_3 on the levels of Ca^{2+} and cAMP in bone cells [5]). There are data on the involvement of membrane receptors for estrogens and progestins in the regulation of membrane-bound enzymes, adenylate cyclase, protein kinase C, and 5'-nucleotidase, under normal conditions and during tumor growth [4].

However, the role of extranuclear effects of glucocorticoids in their physiological and pharmacological effects is still poorly understood.

Here we studied membranotropic activity of hydrocortisone during regulation of D-glucose transport in isolated hepatocytes. This effect of hydrocortisone is associated with the cell plasma membrane and appears at the early period characteristic of membranotropic effects.

MATERIALS AND METHODS

To confirm the presence of hydrocortisone-binding sites on the plasma membrane, we analyzed competitive properties of hydrocortisone immobilized on polyvinylpyrrolidone with a molecular weight of 23 kD (PVP-hydrocortisone), which does not enter the cell.

Hepatocytes were isolated after *in situ* collagenase treatment as described elsewhere [3]. Cell viability estimated by trypan blue exclusion [9] was not less than 80%.

The cell suspension (1.5×10^6 cells/ml, 400 μl) in Hanks' solution was added to the mixture consisting of 20 μl 10^{-8} M ^{3}H-hydrocortisone and 10 μl unlabeled hydrocortisone or PVP-hydrocortisone and then incubated for 2 min to evaluate the interaction of ^{3}H-hydrocortisone or PVP-hydrocortisone with hepatocytes. The reaction was stopped by adding 10 ml Hanks' solution. The suspension was centrifuged at 250*g* for 1 min. The precipitate was resuspended in 300 μl 96% ethanol and kept at 4-5°C for 10-12 h to extract ^{3}H-hydrocortisone. The samples were then centrifuged, and the supernatant (200 μl) was placed into counter vials with 5 ml toluene scintillator.

^{14}C-glucose (4 μCi/sample) and unlabeled D-glucose was added into tubes containing 6×10^5 cells to a final concentration of 2 mM to study glucose uptake by hepatocytes. The volume of incubation mixture was 250 μl. The reaction was stopped with 10 ml cold Earle's solution. The samples were centrifuged at 250*g* for 1 min. Cell lysis was induced by adding 300 μl

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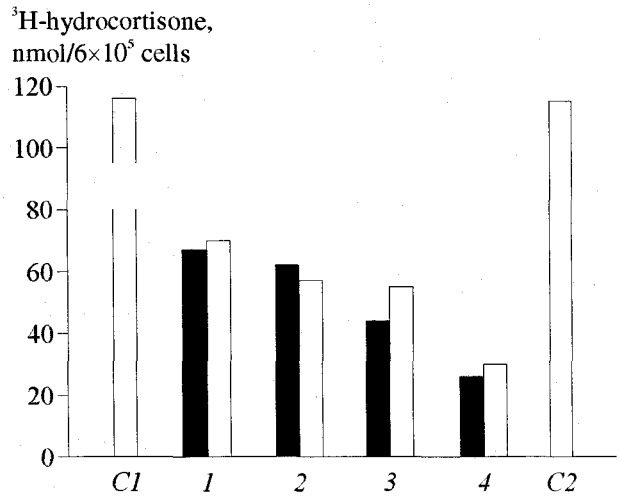

Fig. 1. Effects of hydrocortisone (dark bars) and PVP-hydrocortisone (light bars) in concentrations of 2×10^{-8} (*1*), 5×10^{-8} (*2*), 10^{-7} (*3*), and 10^{-6} (*4*) on ^{3}H-hydrocortisone uptake by hepatocytes. *C1*: without unlabeled steroids; *C2*: in the presence of polyvinylpyrrolidone (4×10^{-7} M).

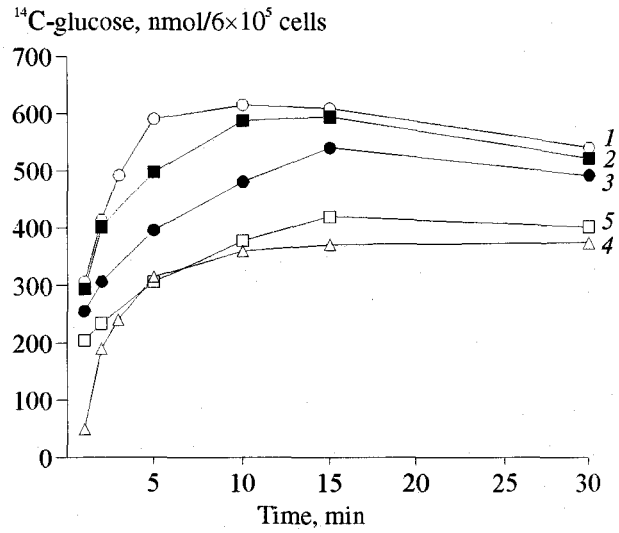

Fig. 2. Effects of hydrocortisone and PVP-hydrocortisone on glucose transport into isolated hepatocytes: control (*1*) and with 1 μM hydrocortisone (*2*), 10 μM hydrocortisone (*3*), 10 μM hydrocortisone and 10 μM PVP-hydrocortisone (*4*), and 20 μM hydrocortisone (*5*).

88% formic acid to the precipitate; the lysate (200 μl) was then placed into counter vials with 5 ml toluene scintillator.

Radioactivity of samples was measured on a Beta-2 liquid scintillation radiometer. Reading time was adjusted so that the number of recorded impulses was above 3000 and the error was 4%.

RESULTS

Both PVP-hydrocortisone and free unlabeled hydrocortisone inhibited ^{3}H-hydrocortisone binding to hepatocytes. The competitive effect was dose-dependent and increased with increasing the concentrations of unlabeled and immobilized ligands (Fig. 1). The carrier polymer alone did not affect ^{3}H-hydrocortisone uptake by hepatocytes.

PVP-hydrocortisone and free unlabeled hydrocortisone produced practically similar inhibitory effects (Fig. 1). These data suggest that free and immobilized ligands interact with hepatocytes in a similar manner (at least at the initial stages).

Hepatocytes rapidly took up ^{14}C-glucose added to the suspension, and 5 min later glucose uptake by cells reached a plateau (Fig. 2, *1*). The content of ^{14}C-glucose in cells then decreased probably due to its utilization [7].

Thirty-minute preincubation of cells with 1, 10, and 20 μM hydrocortisone led to a dose-dependent reduction in the initial rate of D-glucose transport into hepatocytes (Fig. 2). Hydrocortisone in a concentration of 10 μM significantly inhibited D-glucose uptake by hepatocytes and decreased the initial rate D-glucose transport by 35%. The initial rate of D-glucose uptake decreased by 50% with increasing the concentration of hydrocortisone to 20 μM. Exposure of cells to a medium containing 1 μM hydrocortisone produced no effect on the kinetics of D-glucose uptake by hepatocytes.

Preincubation with 1, 10, and 20 μM PVP-hydrocortisone for 1 h did not change the kinetics of ^{14}C-D-glucose transport in hepatocytes. It can be assumed that hydrocortisone produces the effect only after the entry into the cell or certain orientation on the membrane.

Incubation with free hydrocortisone and PVP-hydrocortisone showed that PVP-hydrocortisone in a concentration of 10 mM potentiated the inhibition of D-glucose uptake by hepatocytes caused by free hydrocortisone (Fig. 2, *4*). In this case, the initial rate of D-glucose transport decreased by 50% (as under the effect of 20 mM free hydrocortisone alone).

Lineweaver-Burk linearization showed that the initial rate of D-glucose transport in isolated hepatocytes increased with increasing its concentration in the incubation medium from 1.5 to 10 mM. The Michaelis constant (C_M) for D-glucose uptake by intact hepatocytes was 26.5±5.5 mM, and the maximum rate of uptake (V_{max}) was 5.8 μmol/6×10^5 cells/min (Fig. 3). This value of C_M is within the biological range and closely agrees with previously reported values (30 mM for D-glucose uptake by isolated hepatocytes [2] and 17 mM for perfused liver [10]).

Hydrocortisone in a concentration of 10 μM practically did not change C_M (20.6±3.2 mM), but decreased V_{max} to 2.9 μmol/6×10^5 cells/min (Fig. 3). These data indicate that hydrocortisone reduced the carrier turnover rate or decreased its content in the membrane, rather than lowered the affinity of transport systems

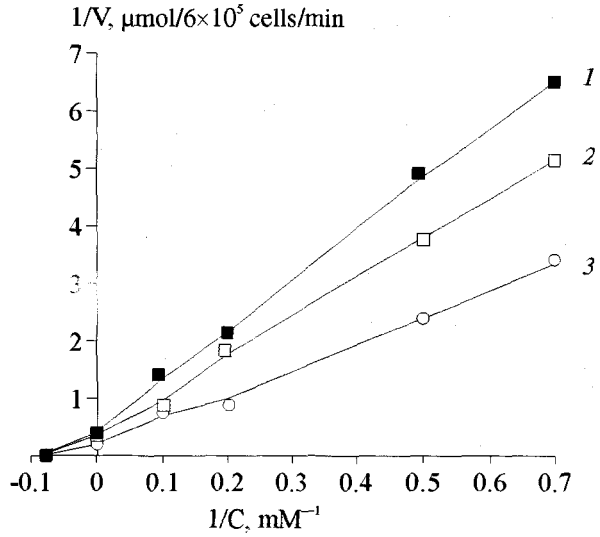

Fig. 3. Initial rate (V) of ^{14}C-D-glucose uptake as a function of its concentration (C) in the hepatocyte suspension: intact hepatocytes (*1*); in the presence of 10 μM hydrocortisone (*2*) and 10 μM hydrocortisone and 10 μM PVP-hydrocortisone (*3*).

for glucose. Previous studies of D-glucose transport into adipocytes showed similar results [8].

PVP-hydrocortisone did not markedly change C_M (20.8±2.1 mM), but decreased V_{max} of glucose uptake to 2.4 μmol/6×10^5 cells/min (Fig. 3). These findings indicate that the potentiating effect of PVP-hydrocortisone and the inhibitory influence of hydrocortisone involve the same mechanisms. It can be also assumed that the potentiating effects are realized via classic mechanisms associated with activation of membrane-bound enzymes and the formation of secondary messengers in the effector cell. This suggestion seems to be credible because there are data that PVP-hydrocortisone in concentrations of 1 and 3 μM elevates the content of cAMP in thymocytes [1].

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