AN ENERGY-DEPENDENT CORTICOSTERONE UPTAKE SYSTEM IN THE RAT LIVER CELL

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1. Introduction

The entrance of steroid hormones into their target cells is generally considered to be a simple diffusion process through the lipid-rich cell membrane [1–3]. However, there are reports of a carrier-mediated, energy-dependent glucocorticoid uptake system in the cell [4–9]. Our studies were carried out to investigate in more detail the possibility that glucocorticoid hormones are actively taken up by the liver cell and to characterize the kinetics of the transport system. We found that isolated rat hepatocytes take up corticosterone by (i) non-saturable and (ii) saturable energy-dependent processes. One of the active systems has a K_m value well within the physiological plasma free corticosterone concentration range of the rat (high-affinity system). Oestradiol-17β inhibits non-competitively the high-affinity corticosterone uptake system.

We also made an attempt to modulate the uptake process by changing the corticosteroid status of the animal and the V_{max} of the high-affinity corticosterone uptake system appeared to be positively correlated to the level of circulating corticosterone.

2. Materials and methods

Radioactive steroids ([1,2-^{3}H]corticosterone and [1,2-^{3}H]dexamethasone) were purchased from the Radiochemical Center, Amersham (spec. act. 40 Ci/mmol and 25 Ci/mmol, respectively). Purity was checked by thin-layer chromatography. Non-radioactive steroids were from Steraloids, Wilton, NH. All glassware was siliconized (Siliclad®, Clay Adams, New York, NY).

The parenchymal liver cells were isolated from male Wistar rats (200–300 g) that had free access to food (AM2 Hope Farms) and water. The isolation procedure in [10] was used with the following modification: The liver is perfused with Ca^{2+}-free Hanks solution for 10 min, followed by collagenase (type 1) 0.05% (w/v) and Soybean trypsin inhibitor 0.01% (w/v) (type 1-S) as in [11,12]. The collagenase and the trypsin inhibitor were from Sigma, St Louis, MO. After isolation the liver cells were suspended (~10^6 cells/ml) in the incubation medium [10], the suspension being gently stirred at 27°C under 95% O_2 and 5% CO_2. After incubation the viability of the cells was >85% as judged by Trypan Blue exclusion.

The steroid uptake was started by adding 0.6 ml/cell suspension to 0.6 ml steroid solution (10–4000 nM in incubation medium) in plastic tubes at 27°C and ended after 45 s by pipetting 1 ml incubation mixture on a glass fiber disc (Whatman GF/C) mounted on a Millipore filter manifold connected to a water suction pump. The cells and the filter were immediately washed twice with 4 ml icecold incubation medium, whereafter they were transferred to a counting vial. After addition of 10 ml Instagel (Packard) the vial was vigorously shaken for 20 min. and radioactivity was counted in a liquid scintillation spectrometer (Packard model 3375).

Plasma corticosterone was measured by a competitive protein binding method [13]. Protein was determined according to [14].

Adrenalectomy, 14 days prior to the experiment, was performed by bilateral lumbotomy and the rats were subsequently maintained on NaCl 0.9% in water. Hypercorticosteronism was induced by daily subcutaneous injections of corticosterone acetate (2.5 mg/100 g rat), dissolved in 0.5 ml propylene glycoll (UCB, Belgium) for 2 weeks.

Correction of the total uptake for non-saturable

component(s) was performed by subtracting a straight line drawn through the origin and the value of the uptake, 682 pmol . min^{-1} . mg $protein^{-1}$, in incubation medium containing 19.2 mM corticosterone. The method in [15] was used for cross correction of the kinetic constants of the uptake systems.

The two-tailed Student's t-test was used for statistical evaluation of the data obtained after in vivo pretreatment.

3. Results

The uptake of corticosterone is very rapid and within 1 min almost linear with time (fig.1). Up to 1 μM the relationship between the concentration of corticosterone and the uptake is non-linear (fig.2). A Lineweaver-Burke plot of the data (corrected for the non-saturable uptake) indicated the presence of 2 uptake systems (fig.3).

In table 1 the K_m and V_{max} of the high-affinity system are given for: (1) normal cells; (2) normal cells that were pre-incubated with 2 mM KCN; (3) normal cells incubated in the presence of 50–2000 nM oestradiol-17β. In a Dixon plot (not shown here) oestradiol-17β appeared to inhibit the uptake of corticosterone non-competitively (K_i ~20 nM).

For the uptake of dexamethasone by liver cells

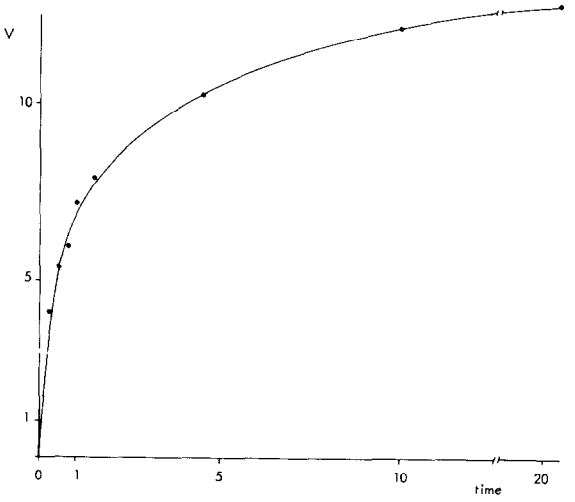

Fig.1. Time course of corticosterone uptake by isolated rat liver cells. Time in minutes, V = uptake in pmol . min^{-1} . mg $protein^{-1}$. Corticosterone in the incubation medium was 200 nM.

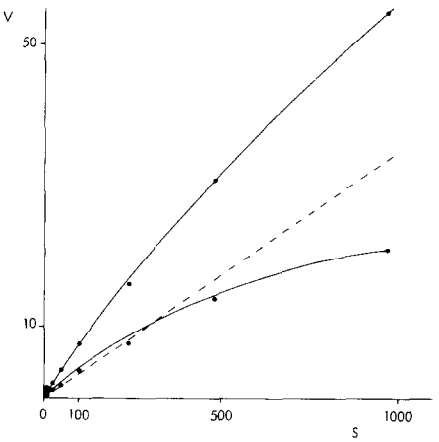

Fig.2. Relationship between the concentration of corticosterone in the incubation medium and the total uptake of corticosterone (upper curve). The broken line, drawn through the origin and the point representing V = 682 pmol . min^{-1} . mg $protein^{-1}$ at S = 19.2 mM, indicates the non-saturable part of the uptake. Subtraction of the broken line from the upper curve yields the lower curve which shows the saturable part of the uptake. S (nM); V (pmol . min^{-1} . mg $protein^{-1}$); n = 6.

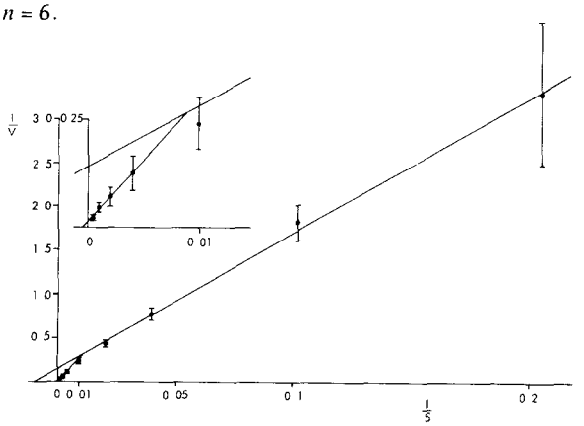

Fig.3. Lineweaver-Burk plot of the uptake data, after correction for the non-saturable part (mean ± 1 SD).

Table 1
High-affinity system of corticosterone uptake by rat liver cells – Effect of metabolic inhibition and competition

Experimental group	V_{max}[a]	K_m[b]	n[c]
Normal control	1.57	42	6
2 mM KCN	zero		3
50 nM oestradiol-17β	0.29	27	3
200 nM oestradiol-17β	zero		3
2000 nM oestradiol-17β	zero		3

[a] pmol corticosterone . min^{-1} . mg $protein^{-1}$
[b] nM corticosterone
[c] number of rats

Table 2
High-affinity system of corticosterone uptake by rat liver cells – Effect of in vivo manipulation of the corticoid state of the animal

Experimental group	V_{max}[a]	K_m[b]	Plasma corticosterone (nM)	n[c]
Normal control	1.49 ± 0.44	41 ± 10	410 ± 228	12
Adrenalectomized	0.95 ± 0.38	18 ± 5	64 ± 72	7
Propylene glycol	0.50 ± 0.08	23 ± 4	376 ± 188	6
Corticosterone in propylene glycol	1.06 ± 0.70	19 ± 9	1436 ± 653	8

[a] pmol corticosterone . min^{-1} . mg $protein^{-1}$
[b] nM corticosterone
[c] number of rats

Mean ± SD

from normal rats (studied in exactly the same way as corticosterone) no high-affinity system was found (data not shown).

In table 2 the influence of in vivo manipulation of the corticosteroid status of the animal on the high-affinity system is shown. Normal control animals yielded exactly the same values as sham-operated animals. Therefore these results were taken together. The uptake of corticosteroid by control cells showed a higher V_{max} as well as K_m than that by cells from adrenalectomized rats ($p < 0.02$ and < 0.001, respectively). Comparison of propylene–glycol and corticosterone in propylene–glycol injections showed that excess corticosterone raised V_{max} significantly ($p < 0.05$), while K_m was not affected.

4. Discussion

Our results indicate the presence of a saturable energy-dependent corticosterone uptake system in rat liver cells. This system accounts for ≥60% of the total steroid uptake at physiological free plasma corticosterone levels (15–72 nM) in the rat. Our results agree but partly with the energy-dependent uptake process for cortisol described in [6] although no influence of KCN on the uptake of corticosterone was found [7]. This might be because the cells were exposed for only 10 s to corticosterone. An error of only ~1 s may then obscure differences. The differences between the absolute values of V_{max} and K_m reported for corticosterone uptake [7] and our values may be because the data in [7] were not cross-corrected. Without cross-correction our data agree well with those in [7]: we then find for corticosterone a V_{max} of 6.61 ± 2.21 pmol corticosterone . min^{-1} . mg $protein^{-1}$ and a K_m of 103 ± 13 nM.

It has been suggested that transcortin plays a role in the corticosterone uptake process [16,17]. The results of the inhibition studies with oestradiol-17β and KCN make this hypothesis less likely as oestradiol-17β does not bind to transcortin [18] and binding of corticosterone to transcortin is not energy-dependent.

The results of the in vivo manipulation show that the level of circulating corticosterone is positively correlated with the V_{max} of the high-affinity uptake system, as the V_{max} of the adrenalectomized animals is lower than that of the normal controls, while the V_{max} of propylene glycol controls is lower than that of corticosterone-injected animals. These data suggest that the level of circulating corticosterone helps to regulate the activity of the uptake system in the liver. This regulation may indicate an adaptation of corticosteroid metabolism, as the liver cell is not only a target for corticosteroid hormone, but also the major site of metabolism. An increase of the metabolic clearance rate of cortisol in patients with Cushing's syndrome was described in [19]. At least part of the adaptation of corticosteroid metabolism could be accounted for by the variations in the uptake system of the liver cell.

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References

[1] Munck, A. (1971) Persp. Biol. Med. 14, 265–289.
[2] Erttmann, R. R. and Damm, K. H. (1975) Arch. Int. Pharmacodyn. 214, 232–239.
[3] Baulieu, E. E., Alberga, A., Rochefort, H., Raynaud, J. P., Jung, I. and Truong, H. (1971) in: Advances in the Biosciences (Raspé, G. ed) vol. 2, p. 244, Pergamon, Oxford.
[4] Gross, S. R., Aronow, L. and Pratt, W. B. (1970) J. Cell. Biol. 44, 103–115.
[5] Milgrom, E., Atger, M. and Baulieu, E. E. (1973) Biochim. Biophys. Acta 320, 267–283.
[6] Rao, M. L., Rao, G. S., Holler, M., Breuer, H., Schattenberg, P. J. and Stein, W. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 573–584.
[7] Rao, M. L., Rao, G. S., Eckel, J. and Breuer, H. (1977) Biochim. Biophys. Acta 500, 322–332.
[8] Harrison III, R. W., Fairfield, S. and Orth, D. W. (1975) Biochemistry 14, 1304–1307.
[9] Harrison III, R. W., Fairfield, S. and Orth, D. W. (1977) Biochim. Biophys. Acta 466, 357–365.
[10] Berry, M. N. and Friend, D. S. (1969) J. Cell Biol. 43, 506–520.
[11] Crane, L. J. and Miller, D. L. (1977) J. Cell Biol. 72, 11–25.
[12] Grohlich, D., Morley, C. G., Miller, R. J. and Bezkorovainy, A. (1977) Biochem. Biophys. Res. Commun. 76, 682–690.
[13] De Jong, F. H. and Van der Molen, H. J. (1972) J. Endocrinol. 53, 461–474.
[14] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[15] Spears, G., Sneyd, J. G. T. and Loten, E. G. (1971) Biochem. J. 125, 1149–1151.
[16] Keller, N., Richardson, U. I. and Jates, F. E. (1969) Endocrinology 84, 49–62.
[17] Koblinsky, M., Beato, M., Kalimi, M. and Feigelson, P. (1972) J. Biol. Chem. 247, 7897–7904.
[18] King, R. J. B. and Mainwaring, W. I. P. (1974) in: Steroid Cell Interactions, p. 102, Butterworths, London.
[19] Dazord, A., Saez, J. and Bertrand, J. (1972) J. Clin. Endocrinol. Metab. 35, 24–34.