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IS CORTISOL METABOLIZED AS IT DISSOCIATES FROM GLUCOCORTICOID RECEPTORS IN THYMUS CELLS?

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SUMMARY

To investigate the possibility that metabolic transformations of a steroid hormone are associated with its release from nuclear receptors, rat thymus cells were incubated with [^{3}H]-cortisol at 37°C to form nuclear glucocorticoid–receptor complexes. Then, by means of successive timed dilutions with buffer at 37°C, the receptor–bound radioactivity was isolated after it had been allowed to dissociate from the receptors and leave the intact cells under physiological conditions. Analysis of this radioactivity showed that it consisted of essentially pure [^{3}H]-cortisol. It is therefore concluded that no metabolic transformation is necessary for or accompanies the release of cortisol from the nuclear glucocorticoid receptors of thymus cells.

INTRODUCTION

All mammalian steroid hormones have in recent years been found to form complexes with specific protein receptors in target cells. The hormone–receptor complexes ultimately become attached to acceptor sites in the target cell nuclei, where they apparently initiate synthesis of certain kinds of mRNA that in turn lead to synthesis of proteins the activities of which give rise to observable hormonal effects [1, 2].

Careful analyses have shown that hormones such as estradiol and cortisol become attached and remain attached to receptors in their respective target tissues without undergoing metabolic transformations. For example, estrogen receptors in mouse uteri have high affinity for estradiol, which may be extracted untransformed after becoming associated with the receptors [1, 3]. Similarly, glucocorticoid receptors in rat thymus cells have high affinity for cortisol, which can also be extracted untransformed from the steroid–receptor complex [4–6].

In connection with investigations on the relation between estradiol and estrone in human tissues, Siiteri, Ashby and Macdonald [7] have found that nuclei from endometrial cells have measurable 17β-estradiol dehydrogenase activity, and that a significant fraction of the [^{3}H] released by isolated endometrial nuclei obtained from tissue exposed to [^{3}H]-estradiol is in the form of [^{3}H]-estrone. They have consequently raised the general question of whether, under physiological conditions, a metabolic transformation such as the conversion of estradiol to estrone may accompany or be necessary for release of a steroid hormone from its nuclear binding site. Neither the studies with estrogens and glucocorticoids described above, nor, as far as we know, any other studies with steroid hormones, provide a direct answer to this basic question, which is of importance owing to the relation it may have to the transformations that the hormone induces in the nucleus, its close connection with the problem of how hormone actions are terminated, and the possible existence of a hormone–receptor cycle [8–10].

In all cases that we are aware of, the nuclear receptor–bound steroid has been obtained for analysis following manipulations—such as disruption of cells, or extraction of the target tissue with organic solvents —that could preclude the steroid's leaving the nuclear site by normal physiological processes. Metabolic transformations due to such processes might therefore not have been detected.

Our purpose in the present work has been to analyze a steroid after it has dissociated from the hormone–receptor complex under conditions that approach physiological. To this end we have employed the isolated rat thymus cell system in which, as we have shown previously, glucocorticoids added at physiological concentrations exert metabolic effects that are closely related to their effects *in vivo* [11]. This system is particularly well suited for a direct experimental approach to the question posed above, since cortisol that is bound to the glucocorticoid receptors in the thymus

cells can be identified by the fact that it dissociates from the cells with a characteristic time-constant at 37°C of about 3 min, permitting clear separation of receptor–bound cortisol from nonspecifically-bound cortisol. The latter dissociates with a time constant of less than 15 s [4, 5]. As mentioned, our earlier results have shown that in thymus cells incubated with cortisol, the steroid that is bound to the receptors remains in the form of cortisol. The results we now present show that the steroid released from the receptors under normal incubation conditions is also still cortisol. These results have previously been described briefly [8].

METHODS

A suspension (0·5 ml) of thymus cells from adrenalectomized rats was prepared and incubated as described before [5] in Krebs–Ringer bicarbonate buffer (gassed with 5% CO_2: 95% O_2, and containing 5 mM glucose) at 37°C, with a total of 850,000 c.p.m. [1,2-^{3}H]-cortisol (New England Nuclear, 44 Ci per mol). The concentration of free cortisol in the medium after 10 min incubation (by which time equilibrium is attained between free and bound steroid [5]) was 8 nM.

After 20 min incubation the cell suspension was diluted 50-fold with 25 ml buffer at 37°C, in order to bring about rapid dissociation of the nonspecifically-bound [^{3}H]-cortisol. The dilute suspension was immediately placed in a clinical centrifuge and centrifuged at about 600 ***g*** for 2 min. By this means most of the slowly-dissociating, receptor–bound cortisol was sedimented with the cells, while the [^{3}H]-cortisol originally in the medium or bound nonspecifically remained in the supernatant. Aliquots of each fraction were removed for scintillation counting [5].

After removal of the supernatant, which was discarded, the cells were immediately resuspended in 25 ml buffer at 37°C, in order to allow dissociation of the receptor–bound steroid to proceed. After 15 min the cells were sedimented again, and the supernatant, containing the dissociated steroid, was removed for analysis.

Steroid in the supernatant was extracted and analyzed as indicated in Table 1 by procedures similar to those described previously [5], except that no carrier cortisol was added. Pure [^{14}C]-cortisol was added to the supernatant before extraction and content of [^{3}H]-cortisol in the supernatant was gauged by the ^{3}H/^{14}C ratio through successive stages of purification.

Table 1. Analysis by ^{3}H/^{14}C ratios of [^{3}H]-steroid dissociated from glucocorticoid receptors in thymus cells treated with [^{3}H]-cortisol

Fraction	Total ^{3}H (c.p.m.)	^{3}H/^{14}C
Supernatant	155,000	0·50
Methylene chloride extract	143,000	0·48
Cortisol spot from		
1st chromatogram	113,000	0·52
2nd chromatogram	59,000	0·52
Cortisol acetate spot from		
3rd chromatogram	38,600	0·51

Supernatant containing the [^{3}H]-steroid was obtained as described in the text. To this supernatant, [^{14}C]-cortisol (New England Nuclear) that had been freshly purified by chromatography, was added in a small volume of ethanol. The supernatant was extracted with methylene chloride and the extract was chromatographed twice in the Bush B/50 system (Benzene–Methanol–Water, 100:50:50 by vol.). The cortisol spot from the second chromatogram was acetylated and chromatographed in the Bush LB21/80 system (Petroleum ether–Benzene–Methanol–Water, 66·6:33·3:80:20 by vol.). Results are expressed as the ratio of ^{3}H/^{14}C after each step, with a standard error of ±0·02. The middle column gives the total ^{3}H recovered at each stage. Further details are given in Ref. 5.

RESULTS

Of the initial 850,000 c.p.m. [^{3}H] in the incubation, some 665,000 c.p.m., representing both the radioactivity that was in the incubation medium and radioactivity that dissociated rapidly from the cells (i.e. nonspecifically bound radioactivity [5]) was discarded in the supernatant obtained after the first dilution with 25 ml buffer.

The rest of the radioactivity remained with the sedimented cells. Of that, 155,000 c.p.m. was recovered in the supernatant from the second dilution with 25 ml buffer. After addition of authentic [^{14}C]-cortisol, this supernatant was extracted and the extracts analyzed as shown in Table 1.

From the constancy of the ratios of [^{3}H] to [^{14}C] obtained through several stages of purification (Table 1) it may be concluded that the radioactivity that was associated with the cells and was recovered in the supernatant was practically all in the form of [^{3}H]-cortisol. Similar results, and the same conclusion, were derived from a separate experiment, not reported here, in which the only difference in procedure was that the 25 ml medium into which the cell suspension was diluted 50-fold, and the 25 ml with which the cells were subsequently resuspended, contained 10^{-6} M unlabelled cortisol.

DISCUSSION

The purpose of the present experiments was to analyze the steroid associated with the nuclear receptors

of thymus cells incubated with [^{3}H]-cortisol at 37°C, after it had been allowed to dissociate and leave the cells under physiological conditions. Taking into account the known time-constants of dissociation of the various fractions of bound steroid, nonspecifically-bound steroid and steroid free in the medium was removed first by means of an initial dilution of the cell suspension. After brief sedimentation of the cells, a second dilution was performed to allow the more slowly-dissociating receptor–bound fraction to dissociate. Throughout these manipulations the cells were maintained intact in buffer at 37°C.

From our earlier results it can be estimated that under the incubation conditions described, prior to dilution of the cell suspension about 20% or 170,000 c.p.m. of the total [^{3}H]-cortisol initially present (850,000 c.p.m.) would be bound to glucocorticoid receptors, mainly in the nucleus [4–6]. The amount actually recovered in the second supernatant (155,000 c.p.m., of which about 10% can be ascribed to residual nonspecific binding [5]) consequently represents about 80% of the radioactivity associated initially with the nuclear receptors.

The fact that, as shown by the results in Table 1, the radioactivity in the second supernatant was almost entirely in the form of [^{3}H]-cortisol, demonstrates that cortisol is released from the receptors and diffuses out of the cell without undergoing detectable metabolic transformation. We cannot exclude the possibility that cortisol might, for instance, be transformed to cortisone and then back to cortisol before leaving the cell, but other results—notably that the cells appear to be freely permeable to cortisol and cortisone, and that no appreciable interconversion of these substances takes place even after prolonged incubation [4, 5, 12]—render such a possibility quite unlikely.

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