Specificity of the dexamethasone-induced steroid receptor in *Tetrahymena*

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Summary. The dexamethasone-induced steroid receptor of *Tetrahymena pyriformis* specifically binds triamcinolone, which is itself a fluorinated glucocorticoid. It also binds dihydro-epi-androsterone (DHEA) but no or very little testosterone, digoxin or ouabain. It follows that the specificity of the induced steroid receptor of *Tetrahymena* may only partially be comparable with that of the mammalian steroid receptor.
Key words. Dexamethasone; DHEA; steroid receptor; *Tetrahymena.*

Unicellular organisms are able to respond to hormones characteristically occurring in higher vertebrates [1], and show after primary interaction with the hormone a changed response to it on reexposure [2]. This changed behaviour persists over many generations [3], owing in all probability to development of a specific receptor for the vertebrate hormone [4, 5]. The specificity of the receptors presented by unicellulars for various vertebrate hormones has been substantiated experimentally. For example, the binding of insulin to receptors has been demonstrated in *Acetabularia mediterranea* [6], *Neurospora crassa* [7], *Saccharomyces cerevisiae* [8] and *Tetrahymena* [9]. Other authors [10, 11] observed the presence of hormones characteristic of higher vertebrates in various unicellular organisms.

Earlier studies in this institute have shown that, although no steroid receptor occurs in *Tetrahymena,* treatment with certain steroids induces the formation of a steroid receptor [12], whose specificity has been substantiated by displacement of labeled hormone by 'cold' hormone. This prompted us to investigate whether or not the dexamethasone-induced steroid receptor of *Tetrahymena* was able to recognize and/or to bind non-glucocorticoid steroid hormones, too.

Mass cultures of *Tetrahymena pyriformis,* maintained in 0.1% yeast extract containing 1% peptone medium (Difco, Michigan, USA) for 24 h at 28 °C, were used. Part of the culture was not treated, to serve as control, part was treated with 2×10^{-6} M dexamethasone for 72 h, under mild shaking. Thereafter the treated and untreated cultures were washed in the same way in three changes of sterile Losina solution, and were returned to plain medium for 48 h at 28 °C. The cells were then centrifuged at 1000 × g for 15 min, washed in 1.5 mM EDTA and 1.0 mM dithiotreitol containing Tris-HCl buffer (pH 7.4), resuspended in a minimal amount of the latter buffer solution, and homogenized in a glass-teflon Potter homogenizer at 0 °C. The homogenate was centrifuged for 1 h at 100,000 × g, and the cytosolic fraction so obtained was examined for protein content with the Coomassie-blue technique [13]. The following ^{3}H-labeled hormones were used in the binding studies: dihydro-epi-androsterone (DHEA, sp. act. 2.2 TBq/mmol); triamcinolone (sp. act. 0.814 TBq/mmol); testosterone (sp. act. 3.1 TBq/mmol); ouabain (sp. act. 1.37 TBq/mmol); digoxin (sp. act. 0.999 TBq/mmol).

All labeled hormone preparations were products of Amersham, UK: except testosterone, which was produced by Technabexport, USSR. For binding studies 675–700 µg cytosolic protein was incubated in presence of 10 nM labeled hormone and 1 µM nonlabeled (cold) hormone for 3 h at 0 °C. (The reason for using only a single large dose of cold hormone for displacement of labeled hormone on the receptor was that the curve differed considerably from that for hormone displacement or hormone saturation on steroid receptors of the cells of higher organisms [12]; it was impossible to use Scatchard analysis as different concentrations of cold hormones caused the same inhibition of binding.) Bound and unbound hormone were separated by the dextran-coated charcoal technique. Aliquots of supernatants were pipetted into scintillation cuvettes containing Turner cocktail (toluol-triton X mixture in ⅔–⅓ proportion with PPO and POPOP), and the scintillation counts were determined in a Beckmann LS 9000 counter. Each assay was performed twice, in three replicates on both occasions. The radioactivity measured in the presence of 1 µM nonlabeled hormone was regarded as non-specific binding. The significance of inter-group differences was assessed by Student's t-test.

In accordance with earlier studies along these lines [12], there was no indication of a specific binding in the control cultures, (except for DHEA, where low but significant binding was present), whereas the cultures pretreated with dexamethasone bound both triamcinolone and DHEA specifically. The total amount of bound hormone was also relatively low in these cases, compared to the amount applied for treatment, but the highest binding values were again obtained with triamcinolone, a fluorinated glycocorticoid closely related to dexamethasone. This close relationship can explain not only the higher binding value obtained with triamcinolone, but also the fact that the binding of triamcinolone was as specific as that of dexamethasone itself on second exposure.

In earlier studies [12] DHEA, too, was able to induce formation of steroid receptors. In the present study evidence was obtained of the specific binding of DHEA to the dexamethasone-induced steroid receptor. Taking into consideration that DHEA, the precursor of testosterone, is not a glucocorticoid, and testosterone failed to bind specifically to the dexamethasone-induced steroid receptor, we speculated

Binding of steroids to the dexamethasone-induced steroid receptor of *Tetrahymena* (total bound and unspecific binding in cpm; the specific binding in percent of total bound)

Steroid (^{3}H)	Control			Induced		
	Total bound cpm ± SD	Unspecific binding cpm ± SD	Specific binding %	Total bound cpm ± SD	Unspecific binding cpm ± SD	Specific binding %
Triamcinolone	1057 ± 69.2	1323 ± 102.5^0	–	1449 ± 47.3	1075 ± 122.38	26.1**
DHEA	533 ± 36.2	481 ± 47.1	10.0*	845 ± 69.2	651 ± 16.1	23.0**
Testosterone	339 ± 11.7	345 ± 23.9^0	–	430 ± 12.0	410 ± 20.2^0	–
Digoxin	431 ± 14.7	433 ± 17.5^0	–	462 ± 18.3	453 ± 7.4^0	–
Ouabain	425 ± 17.3	452 ± 15.9^0	–	394 ± 16.6	416 ± 12.3^0	–

Significance: * $p < 0.05$; ** $p < 0.01$. ^{0} The difference between the unspecifically bound and the total bound hormone is not significant.

that either pretreatment with dexamethasone had activated the DHEA receptor as well, or the configuration of the induced steroid receptor presented by *Tetrahymena* was equally suitable for binding fluorinated glucocorticoids and the less hormone-like DHEA, but not suitable for specific binding of testosterone.

The induced receptor also failed to bind ouabain and digoxin specifically. It rather showed a greater non-specific binding (e.g., increased binding in presence of cold hormone) similar to that observed on treatment of the control cells with triamcinolone. Digoxin and ouabain are non-hormones, but both have a sterane structure, which accounts for cross-reactions with dissimilar steroid hormones and non-hormones of sterane structure on the steroid receptors of newborn rats[14,15]. No such cross-reactions were detected in *Tetrahymena*.

Thus the present experimental observations permit the conclusion that the induced steroid receptor of *Tetrahymena* is not a general steroid receptor since, although it does specifically bind certain hormones, this specificity cannot be reconciled with either the functional or the structural categories of vertebrate hormones. The speculation that *Tetrahymena* would present induced receptors only to those steroid hormones which do naturally occur in its body[10,11] is not plausible either; it does in fact contain DHEA and, in a lesser amount, testosterone as well, but while it does bind DHEA, it fails to bind testosterone.

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Dystrophin and the integrity of the sarcolemma in Duchenne muscular dystrophy

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Summary. It is suggested that in Duchenne muscular dystrophy the absence of dystrophin, which is probably a cytoskeletal protein underlying the sarcolemma, causes changes in stretch-activated cation channels rather than direct mechanical tearing of the surface membrane.
Key words. Dystrophin; calcium; skeletal muscle; muscular dystrophy.

The determination of the complete sequence of dystrophin, the missing protein product of human Duchenne muscular dystrophy (DMD)[2] shows that it is probably rod-shaped, about 150 nm in length and that it can be separated into four domains: (i) the N-terminal domain which is conserved with the actin-binding domain of α-actinin, (ii) a section that is predicted to be rod-shaped and formed of a succession of 25 triple helical segments similar to the repeat domains of spectrin and intermediate filaments, (iii) a cysteine-rich segment that is similar to the COOH domain of *Dictyostelium* α-actinin, (iv) a 420 amino acid C-terminal. Since dystrophin shares many features with spectrin and α-actinin, it has been suggested that it is a cytoskeletal protein[2] and it is also present in kidney, lung and brain, although its abundance is only about 1% of that in skeletal muscle[3]. Recent studies demonstrate that it is predominantly localised in the membrane of striated muscle cells[4–6], although earlier reports have indicated that it is mainly associated with the T-tubule system[7,8]. Dystrophin is also missing in the X-linked mouse muscular dystrophy, *mdx*[7]. These findings have led to suggestions that the function of dystrophin in normal muscle is to protect the membrane against the stresses associated with contraction[9] by providing mechanical strength for the sarcolemma[6]. Consequently, it is suggested that in DMD (where dystrophin is missing) the sarcolemma is subjected to local tearing[9], or locally separated from the basal lamina so causing focal lysis of the sarcolemma[6], or develops small gaps with the basal lamina preserved[4], or develops a membrane instability[5], causing focal plasmalemma breaks[10]. Such suggestions are consistent with the observed release of creatine kinase (CK) and other cytosolic proteins in DMD. These findings show interesting parallels with studies on eccentric contractions of human muscle where, after 20-min exercise, ultrastuctural damage was detectable and was more extensive in biopsy samples taken 24–48 h later. Contralateral muscles contracting concentrically were undamaged[11]. Muscles that are particularly affected in DMD are those that normally undergo eccentric contractions[11]. Cellular damage caused by eccentric contractions are also accompanied by large increases in plasma CK levels[12–14]; and it has been suggested that these damaging effects are also initially induced by mechanical damage to the sarcolemma[11,13,15].

A further similarity between these two types of muscle damage concerns selective fibre atrophy. Fast muscle fibres (Type II B) are preferentially affected in DMD[16] and only a small proportion of the fibres showed degeneration following eccentric contraction[13], although all fibres display equal staining for dystrophin[4–6].

Release of CK is also a typical feature of the experimental muscle damage in vitro that follows rises in intracellular calcium concentration where there is no mechanical damage to the sarcolemma; it is a characteristic of the oxygen- and calcium-paradoxes of the mammalian heart and of A23187-induced damage in skeletal muscle[17,18]. Cytosolic proteins