Evidence for synergism in steroid hormone-receptor association[1]

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Summary. A number of glucocorticoids stimulated oestradiol binding to liver cytosol receptor; oestradiol activated glucocorticoid receptor association at a time when it reversed triamcinolone mediated increase in liver glycogen synthesis.

The equation: steroid + receptor ⇄ S-R complex → response currently sums up the initial step in the mechanism of corticoid hormone action[2]. Liver is endowed with high affinity: low capacity glucocorticoid-specific receptors and is the site of synthesis of high capacity: low affinity blood serum transcortin[3]. More recently, evidence has been accumulating for the presence of high affinity oestradiol binding activity in the liver similar to that observed in the uterus, pituitary, hypothalamus and breast tumours[4]. In the present studies, we explore the possible role of liver oestradiol binders in relation to glucocorticoid-receptor binding and gluconeogenesis.

Materials and methods. Male, Wistar, adrenalectomised rats were used in all experiments and maintained on laboratory food and 1% NaCl ad libitum. In order to study binding, liver supernates (105,000 × g; 50 min) were incubated (60 min, 4 °C) in presence of the desired concentration of the steroid alone or together with another competing steroid. Free radioactivity was thereafter removed by the charcoal technique[2,3,5]. Aliquots of 0.5 ml were mixed with 10 ml Scintix (Isotec, France) and counted in a Packard Tricarb Scintillation Spectrometer equipped with background subtraction and quench correction. Liver glycogen assays were performed by the method of Kemp and Kits van Heijningen[6], as previously adapted[3].

2,4,6,7,-^{3}H-oestradiol (104 Ci/mmole) and 1,2,6,7,-^{3}H-corticosterone (114 Ci/mole) were products of Amersham, G.B. Nonradioactive hormonal steroids were obtained from Sigma (USA).

Results and discussion. Data in the figure show that corticosterone binding to the specific liver receptor showed a biphasic pattern; a slower rise until 10^{-8} M was followed by a sharper increase until 10^{-7} M, with final levelling off between 10^{-7}–10^{-6} M steroid concentration. These are in keeping with the known saturation characteristics of the glucocorticoid receptor[3]. However, oestradiol binding appeared to take an upward turn after 10^{-7} M concentration, indicating that the affinity of oestradiol for its receptor is smaller that that of the corticosterone-receptor complex.

Cross competition studies shown in table 1 reveal that oestradiol binding was greater in presence of cold corticosterone; a maximum of 2-fold increase was observed when 6 times greater nonradioactive corticosterone was competing with oestradiol, although the triplicate determinations agreed within < 10% of each other (the 3 values were 194,194,194 for ^{3}H-oestradiol control vs 382,392,401 for ^{3}H-oestradiol + cold corticosterone in table 1). Similar results were obtained when either triamcinolone acetonide (thereby eliminating possible influence of transcortin) cortisol or cortexolone was used in place of corticosterone (not shown).

Table 1. Activation of hormone-receptor association by a heterologous steroid

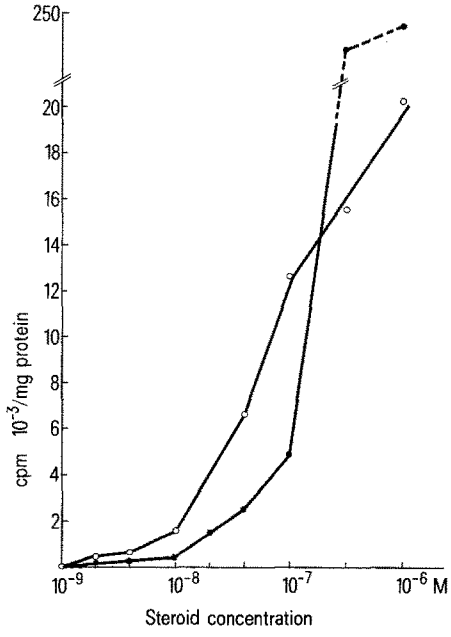
Competitor concentration (M)	cpm/mg protein Active steroid (10^{-8} M)	
	^{3}H-oestradiol	^{3}H-corticosterone
–	194	2338
10^{-8}	265	2436
3×10^{-8}	239	2246
6×10^{-8}	392	2459
10^{-7}	376	2345
3×10^{-7}	335	2493
6×10^{-7}	268	2863
10^{-6}	295	2720
3×10^{-6}	270	2426
6×10^{-6}	290	2367

Cross competition was attempted by incubation in presence of the stated concentration of the cold, heterologous steroid (corticosterone with oestradiol and vice versa). Radioactivity obtained in presence of 1000-fold excess of cold, homologous steroid was subtracted from all values to account for non-specific binding. Each point is an average of 3 separate determinations. For further details see text and references[2,3,5].

Table 2. Inhibition of rat liver glycogen metabolism by oestradiol

Treatment	Liver glycogen (mg %)	
	– Triamcinolone	+ Triamcinolone
Control	9.03 ± 0.85	18.60 ± 3.50
17-β oestradiol	2.12 ± 0.55	9.42 ± 1.19

Oestradiol (20 mg), triamcinolone acetonide (1 mg) were given i.p. 4 h prior to glycogen assay. All values are an average of 5 separate determinations. Further details are given in the text and published earlier[3]. The SE for the mean has been indicated in all cases.



Kinetics of steroid binding to rat liver supernate. Aliquots of 0.5 ml were incubated in presence of the indicated concentration of either oestradiol (●) or corticosterone (○), labelled with tritium. Non-specific binding was determined by incubation in presence of a 1000-fold excess of cold, homologous steroid and is subtracted from all values calculated here as cpm/mg protein. Each point is an average of 3 separate determinations. The experiment was repeated twice. For further details see text and references[2,3,5].

In a reciprocal experiment, oestradiol increased corticosterone binding; the effect was maximal in presence of 60 times greater nonradioactive oestradiol than labelled corticosterone in the incubation mixture but never attained the level observed in the previous case. In all these experiments, the binding of the respective steroid was greatly diminished in presence of 100-fold excess of the homologous steroid, confirming that the observed effects are an expression of a specific binding to the cellular receptor.

Thus, activation of the heterologous steroid-receptor complex was a function of the affinity of the activator for its own receptor; maximum corticosterone stimulation (at 6×10^{-8} M) is to be compared with affinity (about K_d 10^{-10}) of the corticosteroid-receptor complex vs maximum oestradiol effect (6×10^{-7} M) and high affinity (about K_d 6×10^{-9}) of the oestradiol-receptor complex in the liver.

Data in table 2 show that glucocorticoid stimulation of liver gluconeogenesis was reversed by oestradiol; triamcinolone was used in this experiment because it is more potent than the natural glucocorticoid. Stimulation of receptor binding (table 1) was therefore associated with oestrogen-induced antagonism at the level of the physiological action of the glucocorticoid (table 2). This paradox cannot be resolved at the moment. Furthermore, there is no satisfactory biological parameter responsive to oestradiol in the liver.

What is the physiological significance of these findings? One possibility would eliminate the mediation of receptor function in the physiological action, but this seems highly unlikely. Another alternative would place recognition at the level of nuclear acceptors rather than the cytoplasmic receptor. Still another mechanism would lie in separate influences of the oestradiol-receptor and the glucocorticoid-receptor complex whose total sum is seen as the ultimate physiological response without indication of the preceding steps in the chain of events. The possibility may also be entertained for the presence of an effector site on any given R-S complex where a heterologous steroid could modulate stereospecific configuration. The fact that cortexolone, which is biologically inactive in vivo, was nevertheless able to activate in vitro is in favour of this latter hypothesis. Whatever the ultimate explanation, the fact that the presence of one steroid-receptor complex activates the receptor association of another, may have a physiological role, although the reasons for such a synergism have still not been elucidated; this would call for inhibition of receptor function for 1 set of steroid hormones by a specific inhibitor that is technically unfeasible at this time.

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Endocrine basis of wing casting and flight muscle histolysis in the fire ant *Solenopsis invicta*[1]

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Summary. In the imported fire ant *Solenopsis invicta*, wing casting and flight muscle histolysis are blocked by allatectomy. Treatment of alate allatectomized females with a synthetic mixture with high juvenile hormone activity induced wing casting and flight muscle histolysis. Apparently, wing casting and flight muscle histolysis in the fire ant are part of postemergence developmental program regulated, directly or indirectly, by the corpora allata.

Flight muscle histolysis in the Formicidae has been recognized as potentiating species survival by providing protein for oocyte development[2-4]. Alate virgin queens normally remain alate and retain the flight muscle until mated. Once the mating flight and insemination have occurred, the wings and flight muscle have served their primary function in dispersal and reproduction of the species. Subsequently, the wings are cast and the newly mated queen burrows into the soil after which ovarian development and flight muscle histolysis ensue[2,3]. Oocyte development in *S. invicta* is regulated by the corpus allatum (CA)[5] and in the following report, evidence is presented that flight muscle histolysis and wing casting in *S. invicta* require the presence of the CA.

Materials and methods. Data were obtained using classical techniques of allatectomy (CAX), juvenile hormone replacement, and the subsequent effect of these procedures on flight muscle histolysis was examined histologically. Wing casting, flight muscle histolysis, and oocyte development in intact animals were induced and synchronized with CO_2. Specific details of surgical procedures, CO_2 treatment, and holding conditions have been described elsewhere[5]. The juvenile hormone used was a gift from Ayerst Laboratories Montreal, Canada and is referred to, herein, as AJH. AJH is a synthetic mixture of 8 possible geometric isomers of *Cecropia* juvenile hormone. Flight muscle was fixed in Gomori 1-2-3 fixative and stained with Heidenain Iron Hematoxylin[6]. All CAX animals and their controls were treated with CO_2 beginning on the 2nd day following surgery. A few animals which cast their wings immediately after surgery are not included, however, these animals retained their flight muscle.

Results and discussion. In contrast to intact animals, repeated treatment of 5 alate CAX females with CO_2 for 10 min on each of 15 consecutive days failed to induce casting of the wings. When alate CAX females were treated with 1.5 or 10 µg AJH 10 days after surgery, all responded by casting their wings (table). 22 of the 25 animals which responded to AJH (90%) did so in the following 12 h period after topical

Effect of AJH on wing casting of alate CAX females

	Dosage 1 µg (13)	5 µg (10)	10 µg (6)	Acetone (13)
Animals casting wings	11	8	6	0
% casting	85	80	100	0

( ) indicates the number of animals treated with the indicated dosage.