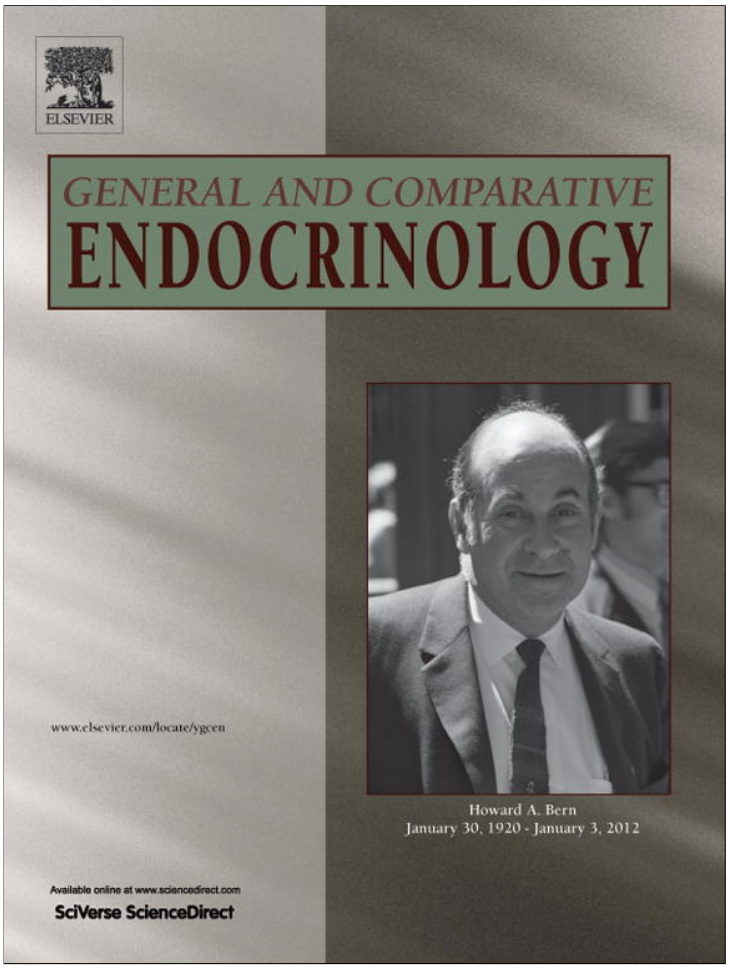

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Pharmacological characterization of intracellular glucocorticoid receptors in nine tissues from house sparrow (*Passer domesticus*)

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ABSTRACT

Glucocorticoid hormones play a key role in the stress response, but plasma concentrations vary based on physiological, environmental, or social parameters. However, hormone titers alone do not determine organismal response. To enhance our understanding of glucocorticoid actions we can examine 'downstream' factors in the organismal stress response, measuring glucocorticoid receptors across target tissues. Here, we characterized intracellular binding sites for CORT (corticosterone, the avian glucocorticoid) in house sparrow (*Passer domesticus*) brain, liver, skeletal muscle, spleen, fat, testes, ovary, kidney and skin. We used radioligand binding assays to identify total capacity, relative density and affinity for CORT of intracellular receptors in each tissue. Most evidence supported two binding sites similar to mammalian low-affinity glucocorticoid receptor (GR) and a high-affinity mineralocorticoid receptor (MR) for brain, liver, kidney and testes, and only a GR-like receptor for muscle, spleen, fat, ovary and skin. However, kidney data were somewhat more complicated, possibly hinting at a mineralocorticoid function for CORT and/or GR in birds. In all tissues, GR and MR affinities were close to published house sparrow values ($K_d \sim 6$ nM for GR, and ~0.2 nM for MR). Taken together, these data show that CORT receptor distribution appears to be as widespread in birds as it is in mammals, and suggest that independent regulation of peripheral receptors in different target tissues may play a role in CORT's diverse physiological effects.

1. Introduction

In birds, the hypothalamus–pituitary–adrenal (HPA) axis is responsible for the release of the glucocorticoid hormone corticosterone (CORT). At baseline levels, CORT is important in regulating basic metabolism; at higher concentrations, it plays a key role in the vertebrate stress response [29,46]. There are predictable changes in CORT titers depending on time of day [9,13] and season [40]. There are also less predictable changes in plasma CORT concentrations depending on species, population [41], and the extent of chronic stress [4,15,39]. In these cases, the circumstances in which CORT secretion can be expected to increase or decrease are not always clear.

To enhance our understanding of CORT action we can focus "downstream" of the HPA axis. Classic signaling theory emphasizes that for a signal to have an effect, it must be perceived by a receiver [14]. CORT receptors in different target tissues are those receivers, yet receptors are rarely characterized in non-traditional model species. Furthermore, because steroid hormones like CORT show remarkable conservation across different vertebrate lineages, we might expect selection to operate by modulating the responsiveness of different target tissues, for example, through changes in receptor capacity [26,27].

In mammalian systems, many of CORT's actions on the body occur via two intracellular receptors, Type 1 or mineralocorticoid receptors (MR), and Type 2 or glucocorticoid receptors (GR) [22]. Upon activation, these receptors translocate to the nucleus, form homodimers and cause changes in gene transcription [43]. MR has an approximately 10-fold higher affinity for CORT than GR, leading to the idea of a two-tier system for CORT binding under baseline and stress-induced concentrations of hormone [16,17].

CORT receptors are practically ubiquitous throughout the mammalian body [1]. MR- and GR-like receptors have also been characterized in several avian tissues, including brain [5,7,52], immune tissues [21,47], kidney [3,12] and intestine [19]. However, there are many peripheral tissues where CORT receptors have yet to be identified in birds. Furthermore, in mammals, MR distribution is rather limited compared to GR distribution, with MR occurring in high levels primarily in kidney, brain, gut and heart [23]. It is not known if MR distribution in birds is similarly limited or if MR

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may play a different role in avian systems. Neural MR has very different distributions between mammalian and avian systems examined [52], hence there is precedent for non-homology in localization or function.

Receptors can be characterized in different target tissues using pharmacological radioligand binding assays [24]. This technique has advantages over methods such as reverse-transcriptase quantitative PCR or Western blot analysis in that it is fully quantitative, giving absolute levels of functional proteins. It also offers a functional characterization, clarifying how much hormone is needed to activate the receptor, and what diversity of hormones can bind. Two distinct intracellular CORT receptors have recently been characterized in house sparrow brain [8]. One of these receptors has similar affinity to mammalian MR ($K_d \sim 0.2$ nM), the other to mammalian GR ($K_d \sim 6$ nM). However, the MR-like receptor in sparrows appears to be somewhat different from mammalian MR; for example, it does not bind to two compounds that bind mammalian MR with high affinity.

In this study, we characterized intracellular CORT receptors in a number of peripheral tissues in house sparrows. We selected tissues involved in energy balance and metabolism (liver, muscle and fat), water balance (kidney), the immune system (spleen and skin), and reproduction (ovary and testes). We have also replicated the work of Breuner and Orchinik [8] on intracellular brain receptors. We focused on specific binding to corticosterone because it is the primary avian glucocorticoid hormone [25], although there is evidence that birds may also use cortisol to a lesser extent [47].

2. Methods

2.1. Capture and chemical adrenalectomy of birds

Wild house sparrows ($n = 85$) were caught in Medford and Somerville, MA from October 2008 to September 2010 using Potter traps and mist nets. In the lab, birds were housed 2/cage under photoperiod conditions corresponding to their capture date, and fed *ad libitum* mixed seeds and chopped apples. In house sparrows, mitotane (ortho, para, dichlorodiphenyl dichloroethane) is effective in blocking endogenous CORT production that could otherwise interfere with radioligand binding assays [6,34]. Most evidence suggests that mitotane is quite specific in its effects [45]; for example, Breuner et al. [6] found that three days of mitotane treatment did not affect testicular weight or testosterone titers in house sparrows. Birds received two 100 μl intramuscular injections of mitotane (180 mg/kg body weight) dissolved in peanut oil using sonication. The first injection was given ~36 h before sacrifice, the second ~24 h before sacrifice. The birds' diets were supplemented with apples to compensate for any inability to mobilize glucose caused by chemical adrenalectomy.

Thirty-six hours after the first injection, birds were put into cloth bags for 30 min and ~50 μl of blood taken to ensure that mitotane treatment was successful. All blood samples were stored on ice until they were centrifuged up to 6 h later. After centrifugation, plasma was removed and stored at −20 °C. We determined corticosterone concentrations in each sample using radioimmunoassay (RIA) following the methods of Wingfield et al. [55]. Briefly, a small amount of radiolabeled CORT was added to samples to determine individual recoveries, and samples allowed to equilibrate overnight. Samples were then extracted with redistilled dichloromethane, dried under nitrogen gas and re-suspended in phosphate-buffered saline with 1% gelatin. We assayed samples in duplicate using antibody B3-163 (Esoterix, Calabasas Hills, CA). This antibody is very specific, showing <1% cross-reactivity for most common cross-reactants, including aldosterone, cortisol and progesterone [30]. CORT concentrations were determined using a standard curve, and assay values corrected for individual recoveries following extraction. Average recovery was 82%; detectability was 1 ng CORT/ml plasma. Intra- and inter-assay coefficients of variation were 4% and 24%, respectively. In all birds used for this study, mitotane treatment brought stress-induced CORT titers down to less than 5 ng/mL plasma compared to ~20–30 ng/mL for house sparrows not treated with mitotane [42].

Birds were deeply anesthetized with ketamine (~80 mg/kg body weight) and xylazine (~20 mg/kg body weight), at doses shown to be effective in this species [36]. To remove circulating corticosterone binding globulin, sparrows were transcardially perfused with ice-cold heparinized saline, and tissues removed and flash-frozen on dry ice. Tissues were always taken in the same order; the mean time to take all tissues was 12 min. Tissues were stored at −80° C until assayed. All procedures were performed according to AALAC guidelines and were approved by the Tufts University Institutional Animal Care and Use Committee.

2.2. Radioligand binding assays

Assays were done following Breuner and Orchinik [7]. Briefly, on the day of the assay, tissue was thawed on ice and homogenized in ice-cold TEGMD buffer (10 mM Trisma base, 1 mM EDTA, 10% glycerol, 20 mM sodium molybdate dihydrate and 5 mM dithiothreitol, pH 7.45 at 4 °C). To strip any endogenous steroid remaining, an equal volume of TEGM buffer mixed with charcoal (1% Norit-A charcoal and 0.1% dextran) was added to homogenized tissue. Samples were spun for 1 h at 4 °C at 104,000*g* in an ultracentrifuge to separate soluble proteins (including cytosolic receptors) from nuclear, mitochondrial and microsomal proteins. We collected the supernatant (or, in the case of fat, infranatant, below a solid fatty layer) and used it immediately in radioligand binding assays.

We used two types of radioligand binding assays to characterize intracellular receptors in house sparrow tissues. For competition analyses, cytosol was incubated with 2 nM [^{3}H]CORT and unlabeled competitor (unlabeled CORT, RU486, aldosterone, or testosterone) at concentrations ranging from 0.01 nM to 1 μM. For saturation binding analyses, cytosol was incubated with [^{3}H]CORT at concentrations from 0.14 to 18 nM, and either buffer, 1 μM unlabeled CORT, or 1 μM unlabeled RU486 (a GR antagonist). Incubations with buffer represented total binding; those with unlabeled CORT, nonspecific binding; and those with RU486, MR binding only.

Incubations were terminated by rapid filtration over GF/B filters in a Brandel harvester (model M24, Gaithersburg, MD), and rinsed 3× with 3 ml of ice-cold TEM rinse buffer (5 mM Tris base, 1 mM EDTA, 10 mM sodium molybdate dihydrate). Prior to filtration, filter paper was soaked for 1 h in TEM buffer mixed with 0.3% polyethylenimine. After filtration, filter paper was placed in Ultima Gold scintillation fluid, vortexed, and run on a liquid scintillation counter (TriCarb 1600, PerkinElmer/Packard, Waltham, MA). Samples were assayed in triplicate, and all assays used tissue pooled from two or more individuals (Table 1). Because there is no difference in intracellular receptor affinity in house sparrows by sex or season [7], we randomly pooled individuals for competition and saturation binding analyses. Binding in individual samples was standardized per mg protein using a Bradford assay with bovine serum albumin as a standard.

2.3. Tissue optimization

For each tissue, optimal time, temperature and homogenization technique for cytosolic binding assays were determined (Table 1). Homogenizers used included a glass-Teflon homogenizer (VWR, Radnor, PA), Polytron (Brinkmann Instruments Co, model PCU-11,

Table 1
Optimized conditions for homogenization and incubation of nine different house sparrow tissues for radioligand binding assays.

Tissue	Number of individuals pooled per assay	Homogenization technique	Incubation time (h)	Incubation temperature (°C)	Tissue dilution
Brain	2–3	Glass–Teflon homogenizer (10–12 strokes)	4	22	0.11 g/mL buffer
Liver	2–3	Glass–Teflon homogenizer (15–20 strokes)	6	9	0.10 g/mL buffer
Skeletal muscle	2–4	Sonication (output 6)	8	9	0.15 g/mL buffer
Kidney	2–3	Glass–Teflon homogenizer (10–12 strokes)	6	15	0.11 g/mL buffer
Spleen	7–9	Glass–Teflon homogenizer (12–15 strokes)	6	9	0.08 g/mL buffer
Fat	3–10	Polytron (output 6)	16	1	0.15 g/mL buffer
Skin	2–3	Polytron (output 6)	12	4	0.11 g/mL buffer
Testes	2–4	Glass–Teflon homogenizer (12–15 strokes)	4	22	0.15 g/mL buffer
Ovary	7–14	Glass–Teflon homogenizer (10–12 strokes)	6	9	0.15 g/mL buffer

Westbury, NY) and sonication (Branson Sonifier 450, Danbury, CT). In all tissues but fat, assays were optimized to $\geqslant$85% specific binding. In fat, assays were optimized to 80% specific binding. For all tissues, we used tissue/buffer ratios that produced cytosol with protein concentrations from 1–10 mg/mL, a range shown to produce accurate results for intracellular glucocorticoid receptor binding assays [32].

2.4. Chemicals

Sodium heparin, Trisma base, EDTA, polyethylenimine, glycerol, Norit-A charcoal, sodium molybdate dihydrate, dithiothreitol, corticosterone, aldosterone, testosterone and mitotane were obtained from Sigma Aldrich (St. Louis, MO). RU486 was purchased from Tocris Bioscience (Minneapolis, MN), Dextran from Pharmacia Biotech AB (Uppsala, Sweden) and Ultima Gold scintillation fluid and [^{3}H]CORT from Perkin Elmer (Waltham, MA). Xylazine was from Akorn, Inc. (Decatur, IL) and ketamine from Fort Dodge Animal Health (Fort Dodge, IA).

2.5. Data analysis

Binding parameter estimates from radioligand binding experiments were obtained by fitting untransformed data to appropriate equations using iterative, least-squares curve-fitting techniques in GraphPad Prism (version 5, La Jolla, CA). For each analysis, we also tested the null hypothesis that binding could be modeled as a simple bimolecular reaction (a one-site model) using a sum-of-squares F test. Tests were two-tailed, with $\alpha = 0.05$. Standard errors were calculated from the goodness of fit of data points to curves generated within an individual experiment. Each experiment was repeated at least twice with similar results; however, there was not enough tissue volume to perform competition analyses of house sparrow ovary.

3. Results

3.1. Saturation binding analyses

Values for equilibrium dissociation constants (K_d) and receptor capacity (B_{max}) were obtained by averaging estimates from multiple independent saturation binding assays (Table 2). Two-site models describing one binding site with nanomolar affinity (GR-like) and a second with subnanomolar affinity (MR-like) best fit [^{3}H]CORT binding to cytosol from house sparrow brain, liver, kidney, and testes (Fig. 1). In the presence of 1 μM RU486, [^{3}H]CORT binding data were best fit by one-site models describing a binding site with subnanomolar affinity (MR-like) in brain, liver, testes, and two of the four kidney assays. Data from the other two kidney assays were best fit by a two-site model describing one binding site with subnanomolar affinity and another site with very low affinity (K_d = ~100–200 nM). For cytosol from skeletal muscle, spleen, fat, skin and ovary, [^{3}H]CORT binding data were best fit by one-site models with nanomolar affinity (GR-like).

3.2. Competition analyses

Similar to saturation binding analyses, data describing the inhibition of [^{3}H]CORT binding by non-radiolabelled CORT in brain (Fig. 2) and liver (data not shown) were best fit by two-site models, and data from spleen (Fig. 2), skeletal muscle, fat and skin (data not shown) were best fit by one-site models (Table 3). However, unlike saturation binding analyses, data from competition analyses of kidney and testes (Fig. 2) were best described by one-site models.

In the presence of increasing concentrations of RU486, data from all tissues were best fit by one-site models. In brain, skin and kidney, aldosterone (ALDO) was also used as a competitor, and data from these analyses were best fit by one-site models. Of note is the fact that in three independent competition binding analyses, ALDO was not able to completely displace [^{3}H]CORT binding to kidney cytosol, although it did in brain and skin. In testes, *T* was used as a competitor, and data from these analyses were best described by a one-site model. *T* was only able to displace about 60% of [^{3}H]CORT binding to testes cytosol, even at micromolar concentrations.

4. Discussion

In this study, we partially characterized specific binding sites for CORT in the cytosol of house sparrow liver, skeletal muscle, kidney, spleen, fat, skin, testes and ovary. We also replicated previous work characterizing intracellular receptors in house sparrow brain [7,8]. Overall, our data suggest high concentrations of two binding sites for CORT similar to mammalian GR and MR in brain, liver, kidney and testes, and one binding site similar to mammalian GR in skeletal muscle, spleen, fat, skin and ovary. Although competition analyses only detected one GR-like binding site in testes and kidney, this may have been due to the presence of such low concentrations of MR in these tissues that they could not be resolved in all binding studies. The presence of MR in kidney and testes as well as brain and liver is not only supported by multiple independent saturation binding analyses, but also by data from competition analyses showing that RU486 (a GR-specific antagonist) was unable to completely displace [^{3}H]CORT binding from these tissues. MR may have also occurred in other tissues (especially spleen, where RU486 displaced most but not all binding), but concentrations of this receptor were low at best. In all tissues, equilibrium dissociation constant values were similar to previously published values for house sparrow of $K_d \sim 6$ nM for GR, and ~0.2 nM for MR [8]. Although the low-affinity binding site we described is also similar to the published affinity of corticosterone binding globulin (CBG) in house sparrows (~3 nM) [7], we feel confident we are describing glucocorticoid receptor binding rather than CBG binding for two reasons. First of all, we perfused all animals, which would

Table 2
Affinity (K_d) and capacity (B_{max}) of corticosterone receptors in nine different house sparrow tissues. Values represent means ± standard error, n = number of independent saturation binding analyses with $r^2 \geqslant 0.98$ performed for each tissue.

Tissue	Number of binding sites	Binding site K_d (nM)	B_{max} (fmol/mg protein)
Brain	2 sites (n = 3)	*low affinity:* 10.0 ± 5.4 *high affinity:* 0.20 ± 0.10	*low affinity:* 111.6 ± 41.2 *high affinity:* 25.0 ± 9.7
Liver	2 sites (n = 3)	*low affinity:* 12.4 ± 0.5 *high affinity:* 0.49 ± 0.04	*low affinity:* 63.2 ± 9.2 *high affinity:* 13.2 ± 1.8
Skeletal muscle	1 site (n = 3)	4.9 ± 0.6	122.7 ± 31.4
Kidney[a]	2 sites (n = 4)	*low affinity:* 8.5 ± 2.9 *high affinity:* 0.22 ± 0.11	*low affinity:* 394.8 ± 24.3 *high affinity:* 32.4 ± 11.4
Spleen	1 site (n = 3)	1.9 ± 0.4	214.7 ± 47.0
Fat	1 site (n = 4)	10.6 ± 2.5	220.9 ± 54.7
Skin	1 site (n = 3)	4.2 ± 0.9	189.6 ± 42.4
Testes	2 sites (n = 3)	*low affinity:* 13.5 ± 4.7 *high affinity:* 0.07 ± 0.02	*low affinity:* 64.1 ± 14.6 *high affinity:* 14.2 ± 3.2
Ovary	1 site (n = 2)	2.0 ± 0.9	122.2 ± 59.7

[a] In two of the four analyses, in the presence of 1 μM RU486, a third binding site with very low affinity (~100–200 nM) was detected.

have flushed tissues of circulating plasma and CBG. Secondly, even in tissues that might contain embedded CBG (such as liver, one of the sites of CBG synthesis [53]) we were able to displace low-affinity binding using a 1 μM concentration of RU486. RU486 is a glucocorticoid receptor antagonist, but it does not bind to CBG [2].

This study adds to a growing body of literature showing that intracellular glucocorticoid receptors are as ubiquitous in birds as they are in mammals [12,19,47]. CORT is involved in behavior, energy mobilization, immune function, and many other physiological processes [29,33,46]. Independent regulation of peripheral receptors across target tissues likely drives the diverse effects of CORT.

One aim of this study was to determine whether MR distribution is as limited in birds as it is in mammals. We found evidence for relatively high MR concentrations in brain, kidney and liver, tissues also containing high MR concentrations in mammals. However, MR presence in a tissue does not necessarily mean it is a target for CORT. In mammals, some tissues contain a high concentration of an enzyme that inactivates CORT, 11β-hydroxysteroid dehydrogenase (11β-HSD), leaving MR free to bind ALDO [20,35]. There is also evidence for high 11β-HSD activity in chicken kidney [28], suggesting avian MR might also primarily function through binding to ALDO in this tissue.

A more limited distribution for MR compared to GR is consistent with a two-tier model of CORT's effects [16,17]. Some tissues may be affected by both baseline and stress-induced concentrations of CORT, whereas others will only be a target for CORT when animals encounter environmental stressors and need to mobilize energy and undergo other physiological changes to re-establish homeostasis [29,54]. For example, we found that house sparrow muscle contained GR but little to no MR, suggesting muscle is only a target for stress-induced CORT. This is consistent with studies in mammals [51] and birds [31] showing that significant muscle catabolism occurs only at high CORT concentrations. However, high concentrations of a given receptor are not always necessary for important physiological effects. For example, even though GR but not MR were described in human adipocytes using radioligand binding assays [37,38], MR can play a key role in adipose differentiation and function, and potentially the development of metabolic syndrome [11,56]. Hence, quantifying changes to both GR and MR is important even in avian tissues where MR capacity is low.

There were a few unexpected results in this study. First, although saturation binding analyses detected both GR and MR in house sparrow kidney, we found ~10 times more GR than MR. Given MR's importance in regulating sodium balance (via ALDO binding) in mammals [22], we expected to see greater MR capacity than GR capacity in house sparrow. There were other counterintuitive results in kidney - in two of our four saturation binding analyses, in the presence of high concentrations of RU486, we detected a third binding site for [^{3}H]CORT with very low, likely non-biologically relevant affinity (K_d = ~100–200 nM). Also, unlike in brain and skin, even 1 μM concentrations of ALDO were unable to completely outcompete [^{3}H]CORT binding in kidney. Some of these unusual results could be due to 11β-HSD or another enzyme's conversion of CORT to a less active metabolite in kidney (our assays were not able to eliminate these enzymes), or the existence of multiple receptor subtypes in this tissue.

However, in other vertebrate orders, regulation of sodium balance in the kidney is more complicated than it is in mammals. For example, in reptiles, amphibians, elasmobranchs and teleosts, sodium balance can be regulated via glucocorticoids [10,48]. Although birds do secrete ALDO, the ratio of basal circulating ALDO to CORT is ~1:100 in mature birds [18], and at least some species of birds also use CORT as a mineralocorticoid [49,50]. Interestingly, house sparrow GR also demonstrated a much higher affinity for ALDO than has been shown in mammalian GR - we found complete displacement of CORT by ALDO in house sparrow brain and skin at a 1 μM concentration, as was also found in a previous study [8]. However, ALDO binding could not displace CORT binding to human GR until a 10 μM concentration of ALDO was used [44]. It is possible that house sparrows may use both CORT and ALDO to regulate mineral balance, and that these effects could occur via both GR and MR.

Another unexpected result was the low ratio of MR to GR found in house sparrow brain in this study. Specifically, we typically found 3–6 times more GR in brain cytosol than MR, whereas previous work found approximately equal concentrations of the two receptors [7,8]. This could be due to population differences – our study used birds from Massachusetts, rather than the southwestern United States. Indeed, plasma CORT titers between house sparrows in Massachusetts and New Mexico showed few differences, but there were much higher CBG capacities in New Mexico birds compared to Massachusetts birds [42]. Differences in relative concentrations of MR to GR could be another difference between these two populations, although the effects this might have on Massachusetts birds compared to New Mexico birds are not known.

In conclusion, characterizing CORT receptors in a variety of peripheral tissues in a commonly-studied avian species expands our ability to investigate CORT's role in helping animals cope with environmental challenges. This will doubtless prove useful for understanding the physiological mechanisms of a hormone shown to have so many tissue-specific effects. It also provides new data showing similarities and differences between CORT receptor presence and possible function in birds compared to other vertebrates.

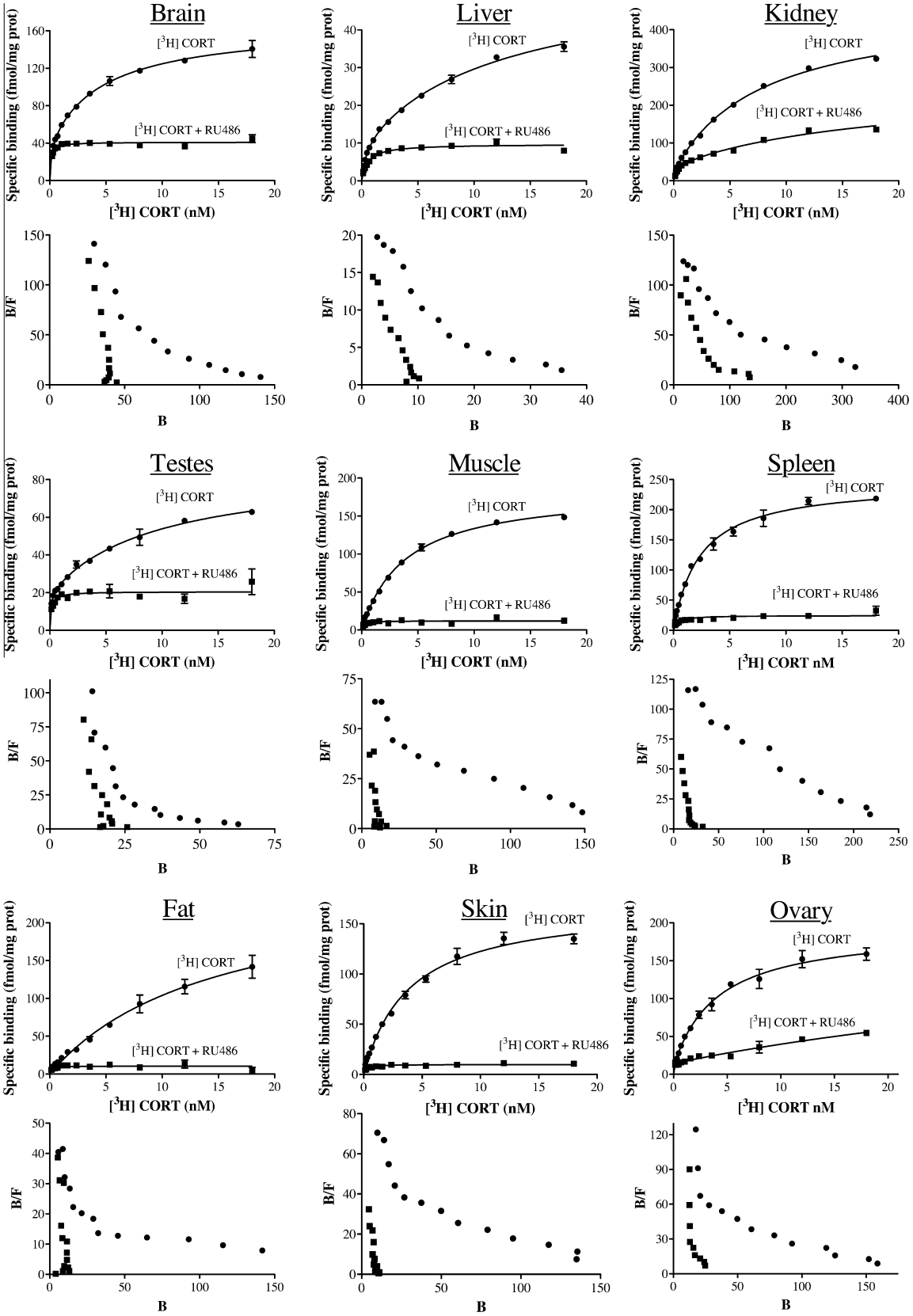

Fig. 1. Equilibrium saturation binding of [^{3}H]CORT to cytosol prepared from nine different house sparrow tissues. Tissue was pooled from several different individuals. The top graph in each panel shows specific binding data from a representative experiment (means ± SEM at each concentration) depicting [^{3}H]CORT in the presence (squares) or absence (circles) of 1 μM RU486. See Table 2 for binding parameters (K_d and B_{max}) specific to each tissue type. The bottom graph in each panel depicts a Scatchard–Rosenthal replot of data.

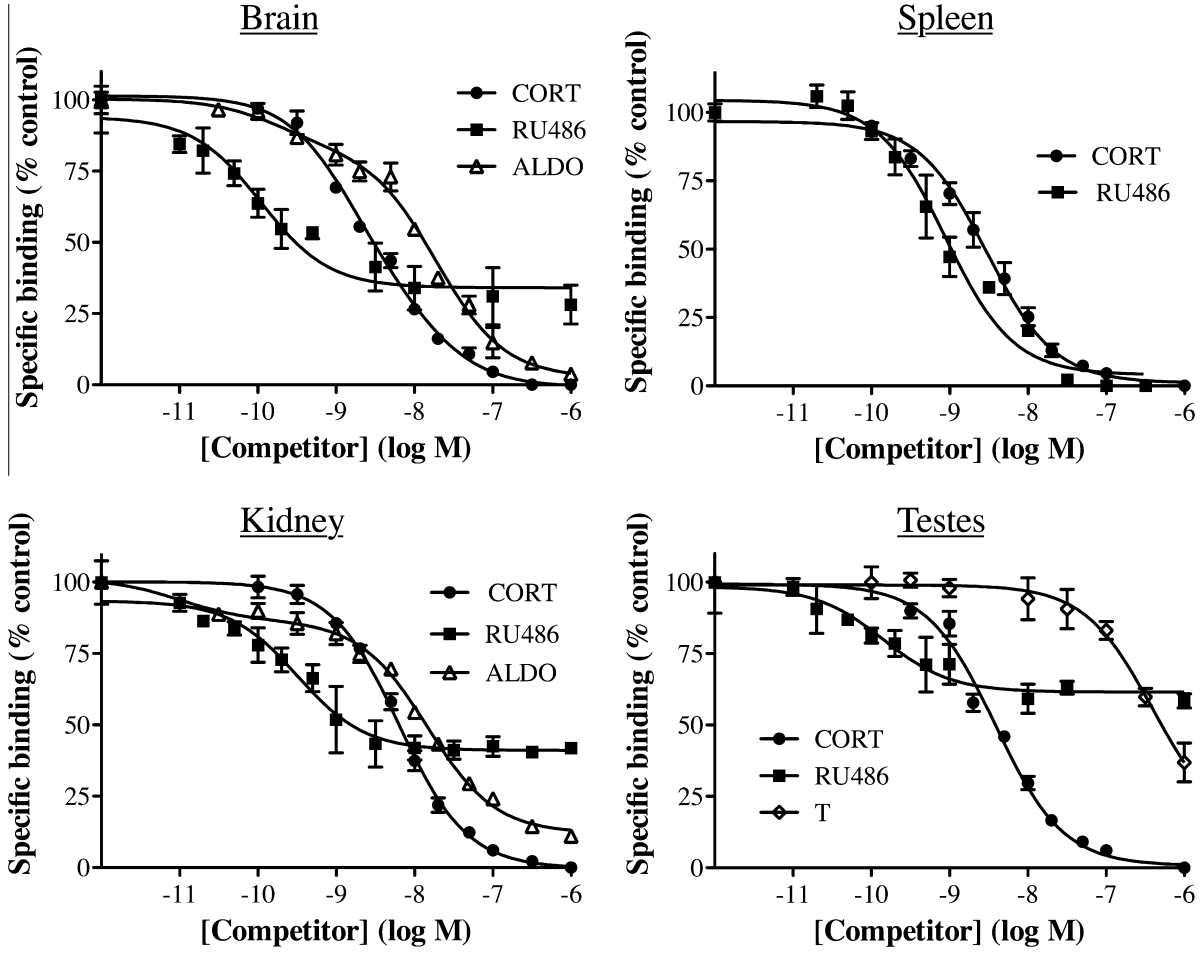

Fig. 2. Inhibition of [^{3}H]CORT binding by unlabeled corticosterone (CORT, circles), RU486 (squares), aldosterone (ALDO, triangles), and testosterone (*T*, diamonds) in cytosol from four different house sparrow tissues. Tissue was pooled from several different individuals. Shown are specific binding data (means ± SEM at each concentration), expressed as the percentage of [^{3}H]CORT binding in the absence of competitor, from representative experiments. IC_{50} values for each tissue type are given in Table 3.

Table 3
Inhibition of [^{3}H]CORT specific binding by competing steroids in eight different house sparrow tissues. Competitors used were non-radiolabeled corticosterone (CORT), RU486, aldosterone (ALDO) and testosterone (T), depending on tissue type. Affinity is presented as IC_{50}, an estimate of K_d, because not all data described simple bimolecular reactions. All competition assays were run twice to ensure the repeatability of results.

Tissue	Ligand	Number of sites	IC_{50} (nM)	% of sites	% Inhibition at 1 μM
Brain	CORT	2 sites	*site 1:* 13.6 ± 2.6	60	100
			site 2: 1.4 ± 1.6	40	
	RU486	1 site	0.11 ± 0.17		60
	ALDO	2 sites	*site 1:* 19.3 ± 1.2	82	100
			site 2: 0.16 ± 0.29	18	
Liver	CORT	2 sites	*site 1:* 7.8 ± 1.8	53	100
			site 2: 1.1 ± 2.0	47	
	RU486	1 site	0.24 ± 0.18		50
Skeletal muscle	CORT	1 site	1.1 ± 1.2		100
	RU486	1 site	0.99 ± 1.1		90
Kidney	CORT	1 site	4.9 ± 1.6		100
	RU486	1 site	0.32 ± 0.10		50
	ALDO	1 site	12.4 ± 1.1		85
Spleen	CORT	1 site	3.0 ± 0.41		100
	RU486	1 site	0.82 ± 0.08		95
Fat	CORT	1 site	9.7 ± 0.2		100
	RU486	1 site	0.10 ± 0.11		88
Skin	CORT	1 site	7.7 ± 1.9		100
	RU486	1 site	0.04 ± 0.03		100
	ALDO	1 site	15.3 ± 3.2		100
Testes	CORT	1 site	3.4 ± 0.4		100
	RU486	1 site	0.09 ± 0.06		50
	T	1 site	389.3 ± 207.1		60

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