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Steroid receptor-mediated effects of neuroactive steroids: characterization of structure-activity relationship

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Abstract

Neuroactive steroids rapidly alter neuronal excitability through their action via the cell surface. The 3α-hydroxy ring A-reduced pregnane steroids enhance γ-aminobutyric acid (GABA)-mediated Cl^- currents while pregnenolone sulfate and dehydroepiandrosterone sulfate may exert functional antagonistic properties. Based on our previous findings that the 3α-hydroxy ring A-reduced pregnane steroids allotetrahydroprogesterone and allotetrahydrodeoxycorticosterone may regulate gene expression via the progesterone receptor after intracellular oxidation, we have characterized the effects of a series of natural and synthetic neuroactive steroids at the genomic level using a cotransfection system with various steroid receptor expression vectors and a reporter gene in a human neuroblastoma cell line. Pregnanolone and pregnenolone were able to activate both the chicken and the human progesterone receptor while the synthetic 3α-hydroxylated derivative alphaxalone and dehydroepiandrosterone were active via the chicken progesterone receptor but devoid of transcriptional activity via the human progesterone receptor. Moreover, the antiglucocorticoid activity of dehydroepiandrosterone reported at the systemic level could not be reconstituted in the cellular cotransfection system. None of the neuroactive steroids bound directly to steroid receptors. Thus, their genomic activity appears to be mediated via intracellular metabolization. This study provides evidence for differential genomic effects of neuroactive steroids in a structure-specific and species-specific way that may have impact on the development of these steroids for therapeutic application.

Keywords: Neuroactive steroid; Neurosteroid; Progesterone receptor; Gene expression

1. Introduction

There is now a growing body of evidence that steroids, besides their actions via intracellular receptors as homo- or heterodimers (Evans, 1988; Trapp et al., 1994), may influence brain function via the cell surface (McEwen, 1991). Steroids with these particular properties are called neuroactive steroids (Paul and Purdy, 1992). Certain precursors, e.g. pregnenolone (5-pregnen-3β-ol-20-one) sulfate, or derivatives from progesterone or deoxycorticosterone, e.g. allopregnanolone (THP, 5α-pregnan-3α-ol-20-one) or allotetrahydrodeoxycorticosterone (THDOC, 5α-pregnane-3α,21-diol-20-one), are able to interact with the $GABA_A$ receptor complex (Majewska et al., 1986; Paul and Purdy, 1992). A variety of studies have suggested anxiolytic and potentially anticonvulsant properties of the 3α-reduced neuroactive steroids (Crawley et al., 1986; Wieland et al., 1995). Other systemic studies included effects of neuroactive steroids on sleep electroencephalographic (EEG) recordings. For example, pregnenolone may induce changes in the sleep EEG compatible with an inverse agonistic modulatory activity at the $GABA_A$ receptor complex (Steiger et al., 1993; Lancel et al., 1994). Dehydroepiandrosterone (DHEA, 5-androsten-3β-ol-17-one) has been shown to increase rapid eye movement sleep (Friess et al., 1995) which provides further support for the memory-enhancing properties of DHEA as demonstrated in animal studies (Flood et al., 1988). Moreover, DHEA may exert antiglucocorticoid properties when administered in vivo (Browne et al., 1993; Araneo and Daynes, 1995).

The 3α-reduced neuroactive steroids allopregnanolone (THP), allotetrahydrodeoxycorticosterone (THDOC) and pregnanolone (5β-pregnan-3α-ol-20-one) are potent positive allosteric modulators of the $GABA_A$ receptor (Majewska et al., 1986; Paul and Purdy, 1992). These

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steroids displace *t*-butylbicyclophosphorothionate (TBPS) from the GABA-gated Cl^- channel and enhance the binding of muscimol and benzodiazepines (Majewska et al., 1986; Paul and Purdy, 1992). Expression of recombinant $GABA_A$ receptors and subsequent electrophysiological recordings have enabled the molecular characterization of effects of neuroactive steroids at the receptor subunit level (Puia et al., 1990). Although it has been postulated that neuroactive steroids act through unique recognition sites at the $GABA_A$ receptor (Morrow et al., 1990), a specific steroid binding site has not yet been identified. However, the subunit composition of the $GABA_A$ receptor seems to play a pivotal role for the sensitivity of the receptor to neuroactive steroids (Lan et al., 1991; Hauser et al., 1995). Steroids may also act as negative allosteric modulators of the $GABA_A$ receptor as shown for pregnenolone sulfate and DHEA sulfate (Majewska et al., 1988, 1990). Moreover, also glycine (Wu et al., 1990) and NMDA receptors (Wu et al., 1991) are sensitive to the modulation by neuroactive steroids in a structure-specific way.

Because of the distinct chemical structure of these steroids (Purdy et al., 1990) and their inability to bind to the rat progesterone receptor (Gee et al., 1988), these steroids have been thought not to possess regulatory properties at the genomic level (Gee et al., 1988; Paul and Purdy, 1992). However, recently we showed that THP and THDOC are capable of regulating gene expression via the progesterone receptor (Rupprecht et al., 1993b).

To study the structural requirements of neuroactive steroids for regulating gene expression we have investigated the effects of a series of naturally occurring and synthetic neuroactive steroids at the genomic level using a cotransfection system in a human neuroblastoma cell line.

2. Materials and methods

2.1. Cell culture and transfection

SK-N-MC cells (human neuroblastoma) were grown in Dulbecco's modified Eagle's medium (DMEM) without phenol red supplemented with 10% charcoal-stripped steroid-free fetal calf serum for reporter gene assays (Arriza et al., 1987; Rupprecht et al., 1993b). Transfections were performed using an electroporation system (Biotechnologies and Experimental Research, San Diego, CA, USA) after determination of the optimal electric field strength (Chu et al., 1987; Rupprecht et al., 1993a). 5 μg reporter gene, the mouse mammary tumor virus promoter upstream of the luciferase gene (MTV-LUC), and 5 μg receptor expression vector were cotransfected with 5 μg pCH110 (Pharmacia LKB, Freiburg, Germany), an SV 40 promoter-driven β-galactosidase expression vector. Electroporated cells were replated in phenol red-free DMEM supplemented with 10% charcoal-stripped steroid-free fetal calf serum and incubated immediately with various concentrations of steroids at a concentration range from 0.01 to 1000 nM. After 24 h, cells were harvested and extracts were assayed for luciferase (DeWet et al., 1987; Rupprecht et al., 1993a) and β-galactosidase (Herbomel et al., 1984; Rupprecht et al., 1993a) activity to control for transfection efficiency.

For determination of hormone-binding parameters and for immunofluorescence studies, COS-1 cells (monkey kidney) were grown and transfected by electroporation with 5 μg receptor expression vector and 10 μg carrier DNA as described previously (Rupprecht et al., 1993b,c).

The construction of the plasmid MTV-LUC (Hollenberg and Evans, 1988) and expression vectors for the human glucocorticoid receptor (hGR α) (Hollenberg et al., 1985), the human mineralocorticoid receptor (hMR) (Arriza et al., 1987), the full length isoform of the chicken (cPR_B) (Conneely et al., 1989) and the human progesterone receptor (hPR_B) (Kastner et al., 1990) and the rat androgen receptor (rAR) (Tan et al., 1988) have been described previously.

2.2. Cell homogenization, cytosol preparation and assessment of steroid binding parameters

Pellets of transfected COS-1 cells were homogenized by 10 strokes at 900 rpm in ice-cold 5 mM Tris-HCl (pH 7.4) containing 0.5 mM phenylmethylsulfonylfluoride, 5 μg/ml antipain, 5 μg/ml leupeptin, 5% glycerol, 10 mM sodium molybdate, 1 mM EDTA and 2 mM β-mercaptoethanol using a glass homogenizer with a teflon pestle milled at a

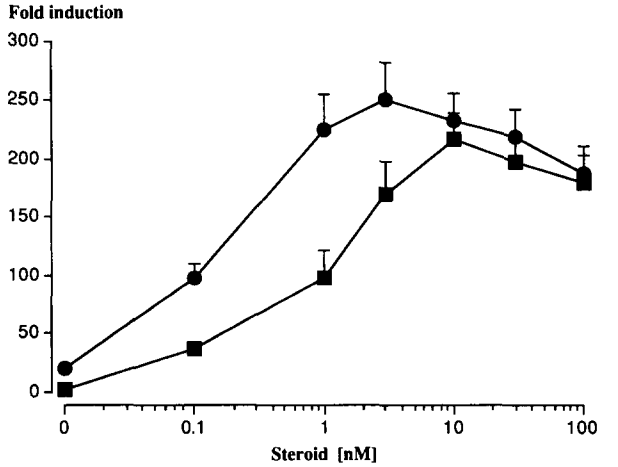

Fig. 1. Transactivation properties obtained in the cotransfection assay for the rat androgen receptor (rAR) in response to dihydrotestosterone (DHT) (filled squares) and R 1881 (filled circles). Individual plates of SK-N-MC cells were cotransfected with 5 μg ß-galactosidase expression plasmid, 5 μg of the reporter plasmid MTV-LUC and 5 μg of rAR expression plasmid. Transfected cells were incubated with steroids at the indicated concentrations. The induction of the MTV promoter was calculated from the relative light units (RLU) measured in the individual cell extracts and the baseline activity of the MTV promoter, equivalent to approximately 2000 RLU, was set at 1. The results are presented as the means ± S.D. of three independent experiments.

clearance of 0.25 mm on the radius. The homogenate was centrifuged (100000 × g, 0–2°C) for 60 min to obtain cytosol. The cytosol was incubated in a total volume of 150 μl in duplicate with 2.5–3 nM [^{3}H]R 5020 (86 Ci/mmol, NEN DuPont, Dreieich, Germany) to label the chicken (cPR_B) or human progesterone receptor (hPR_B), with 2.5–3 nM [^{3}H]R1881 (86 Ci/mmol, NEN DuPont, Dreieich, Germany) to label the rat androgen receptor (rAR) and with 3–5 nM [^{3}H]dexamethasone (87 Ci/mmol, NEN DuPont, Dreieich, Germany) to label the human glucocorticoid receptor (hGR α). The respective unlabeled steroids were included in these incubations to compete for receptor binding. The concentrations used for displacement were 0.1, 1, 10, 100, 1000 and 10000 nM. After incubation for 20–24 h at 0°C, bound and free ^{3}H steroid were separated by Sephadex LH-20 (Pharmacia, Sweden) gel filtration, radioactivity was measured in a liquid scintillation counter. Data are expressed as ^{3}H steroid receptor binding in mol/l (M).

Apart from displacement binding analysis, a typical experiment also included saturation binding of [^{3}H]R 5020 (0.1–5 nM), [^{3}H]R1881, or [^{3}H]dexamethasone, respectively, to determine by Scatchard analysis the K_d values of the respective ^{3}H ligands for the cPR_B, hPR_B, rAR or hGR α. The parameter K_d was required to calculate the inhibition constant (K_i) by a combined method of Hill plot analysis and the Cheng-Prusoff equation (Cheng and Prusoff, 1973). Slope index and K_i were calculated by fitting the experimental data to the expected linear relationship $\log [(B_0/B) - 1][([^3H]/K_d\,^3H) + 1] = \log [I] - \log (K_i)$, in which B_0 represents the specific binding in the absence and B the specific binding in the presence of unlabeled competitor. [^{3}H] is the concentration of ^{3}H ligand. K_d ^{3}H is the dissociation constant (apparent affinity constant) of the ^{3}H ligand, which was determined by Scatchard analysis, as described earlier, in the same experiment. [I] is the concentration of the unlabeled competitor.

2.3. Immunolabeling of transfected COS-1 cells

After transfection with the hPR_B expression vector, cells were cultured in phenol red-free DMEM containing 10% charcoal-stripped fetal calf serum for 20–24 h in the presence or absence of hormone in coverslips on 25-well polystyrene plates (Greiner, Alphen a/d Rijn, Netherlands). Coverslips were pretreated with 1 mg/ml Alcian Blue 8 GX (Sigma) and sterilized with ethanol.

Cells were briefly washed with phosphate-buffered saline (pH 7.4) and fixed by adding 2% formaldehyde in phosphate buffered saline to the coverslips. After incubation for 10 min, cells were washed twice with phosphate buffered saline and made permeable with 0.5% Nonidet P-40 (USB, Cleveland, OH, USA) in PBS for 10 min, followed by two brief washing steps. Then cells were incubated as follows: 5 min with 100 mM glycine in phosphate-buffered saline to block free aldehyde groups; two times 15 min with 0.5% bovine serum albumin, 0.2% gelatin (Sigma) in phosphate-buffered saline (PBG); 1 h or overnight with a mouse monoclonal antibody mPRI against the hPR_B (Dianova, Hamburg, Germany) (Perrot-Applanat et al., 1987) at a concentration of 50 μg/ml in PBG. After six washing steps cells were incubated for 5 min in PBG and 1 h with a fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit antibody (Nordic, Breda, Nether-

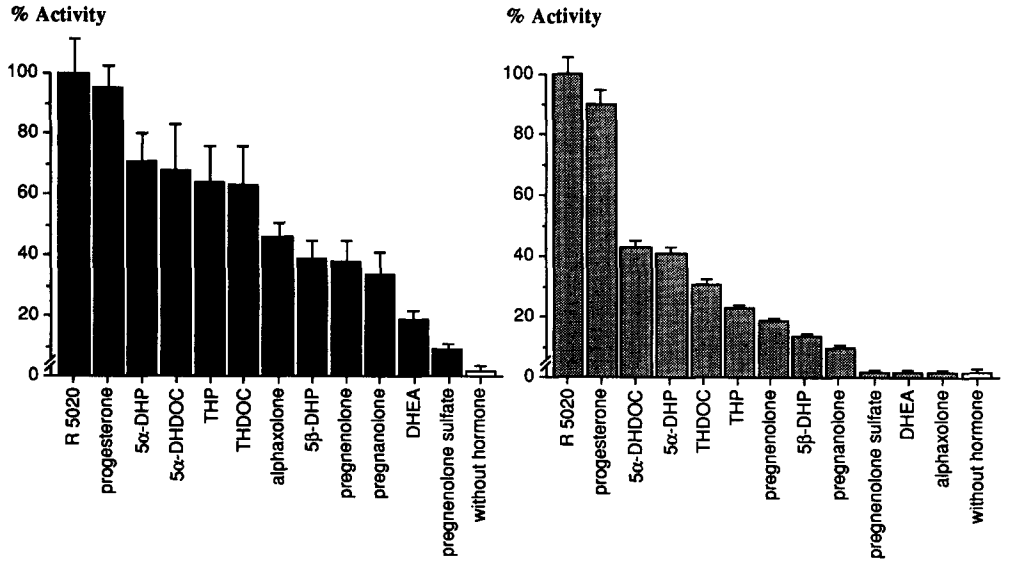

Fig. 2. Maximal biological activity (means ± S.D.) of progestins and neuroactive steroids at the level of gene expression measured by the induction of the MTV promoter for the chicken (cPR_B) (left panel) and the human progesterone receptor (hPR_B) (right panel). The concentrations used were those eliciting the maximal activity in the respective dose response curves. The results are presented as the means ± S.D. of three to six independent experiments. cPR_B: R 5020, progesterone, 5α-DHP, 5α-DHDOC 10 nM; THP, THDOC, 5ß-DHP 100 nM; pregnanolone, pregnenolone, pregnenolone sulfate, DHEA, alphaxalone 1000 nM. hPR_B: R 5020, progesterone 10 nM; 5α-DHP, 5α-DHDOC, THP, THDOC, 5ß-DHP, pregnanolone, pregnenolone, pregnenolone sulfate, DHEA, alphaxalone 1000 nM.

lands) diluted 1:200 in PBG followed by four washing steps in PBG and four washing steps in phosphate buffered saline. Labeled samples were mounted with 90% glycerol in phosphate-buffered saline containing 1 mg/ml *p*-phenylenediaminine (Sigma) to prevent bleaching and photographed on Kodak Tri-X films at 400 ASA using a Zeiss Axiophot microscope with a Planapo 63/1.25 phase-contrast objective. Immunofluorescence studies were performed as three independent blind experiments.

3. Results

3.1. Transcriptional activity of neuroactive steroids

The genomic effects of a variety of naturally occurring (THP, THDOC, pregnanolone, pregnenolone, pregnenolone sulfate, DHEA) and synthetic (alphaxalone, 5α-pregnane-3α-ol-11,20-one) neuroactive steroids were determined by assessing the potency of the steroids to induce transcription through the hormone-responsive MTV promoter. Various steroid receptor expression vectors were cotransfected with the MTV-LUC reporter gene in the human neuroblastoma cell line SK-N-MC. In this system, the agonistic activity of steroids is proportional to the induction of the MTV promoter as measured by the extent of luciferase production in the cells. Using expression vectors for the human glucocorticoid receptor (hGRα), the human mineralocorticoid receptor (hMR), the chicken (cPR_B) or the human progesterone receptor (hPR_B) and the rat androgen receptor (rAR), we obtained dose response curves for all the neuroactive steroids investigated in relation to standard natural and synthetic steroid receptor ligands. A representative example of dose response

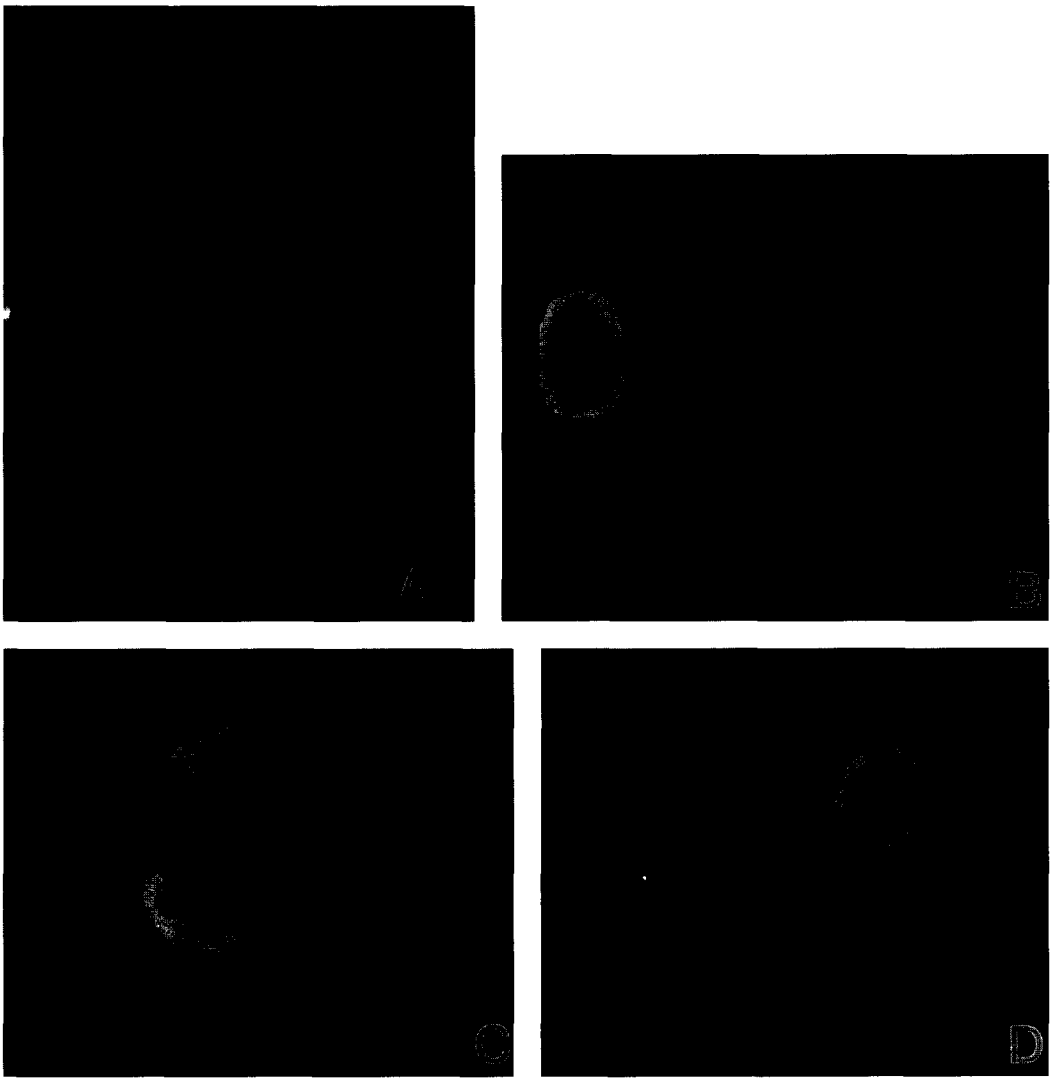

Fig. 3. Effect of neuroactive steroids and progesterone on the intracellular localization of human progesterone receptor (hPR_B). COS-1 cells transfected with hPR_B were either left untreated (A) or treated with 10 nM progesterone (B), 1 μM pregnenolone (C) or 1 μM pregnanolone (D) immediately after the transfection for 24 h. The concentrations used were those eliciting the maximal activity in the respective dose response curves in the transfection assay. Immunofluorescence studies were performed as three separate blind experiments.

curves obtained with dihydrotestosterone and R 1881 via the rat androgen receptor is given in Fig. 1; dose response curves for corticosteroid receptors (Rupprecht et al., 1993a) and progesterone receptors (Rupprecht et al., 1993b) for their standard ligands in SK-N-MC cells have been published previously. None of the neuroactive steroids studied bound to either the human mineralocorticoid receptor, the human glucocorticoid receptor or the rat androgen receptor nor induced any significant increase in transcriptional activity via these steroid receptors (data not shown).

As we have shown recently that THP and THDOC may regulate gene expression via progesterone receptors, we characterized the genomic effects of a series of other neuroactive steroids via the full length isoform (B-form) of both the chicken (cPR_B) and the human progesterone receptor (hPR_B) in the cotransfection system. The B-form was used because of its greater transactivation potential when compared with the truncated A-form. The rank order of the maximal transcriptional response elicited by the various steroids is shown in Fig. 2. The progesterone receptor ligands R 5020 and progesterone displayed the most pronounced agonistic activity via the progesterone receptor of both species. The chicken progesterone receptor (cPR_B) was activated by all neuroactive steroids used in this study. Surprisingly, also pregnanolone and 5β-dihydroprogesterone (5β-DHP, 5β-pregnane-3,20-dione) with an A-ring in a 5β position were potent agonists via the chicken progesterone receptor. The genomic effects of THDOC, THP, pregnenolone and pregnanolone were also demonstrable via the human progesterone receptor (hPR_B) though less pronounced when compared with the chicken progesterone receptor. However, in contrast to the chicken progesterone receptor, the human progesterone receptor was not responsive to pregnenolone sulfate, DHEA and alphaxalone.

Table 1
Binding affinity (K_i values determined from the respective displacement curves) versus functional sensitivity (ED_{50}) in the cotransfection assay for the chicken (cPR_B) and the human progesterone receptor (hPR_B)

	cPR_B		hPR_B	
	ED_{50} (nM)	K_i (nM)	ED_{50} (nM)	K_i (nM)
R 5020	0.3 ± 0.1	0.04 ± 0.01	0.2 ± 0.05	0.01 ± 0.01
Progesterone	0.7 ± 0.2	0.09 ± 0.02	6.0 ± 1.5	0.35 ± 0.1
5α-DHP	0.3 ± 0.1	0.9 ± 0.15	105 ± 25	22 ± 7
5α-DHDOC	0.2 ± 0.05	1.0 ± 0.3	116 ± 29	20 ± 6
THP	2.0 ± 0.6	n.m.	122 ± 31	n.m.
THDOC	1.0 ± 0.6	n.m.	153 ± 39	n.m.
5ß-DHP	15 ± 4.5	1.1 ± 0.3	212 ± 65	35 ± 12
Pregnanolone	203 ± 68	n.m.	305 ± 89	n.m.
Pregnenolone	205 ± 70	n.m.	307 ± 86	n.m.
Pregnenolone sulfate	200 ± 71	n.m.	n.m.	n.m.
DHEA	175 ± 49	n.m.	n.m.	n.m.
Alphaxalone	102 ± 32	n.m.	n.m.	n.m.

n.m. = not measurable. ED_{50} values were obtained from dose response curves as shown in Fig. 1 and K_i values from displacement curves as described previously (Rupprecht et al., 1993b,c). Results are presented as the mean ± S.D. from three to six independent experiments.

3.2. Effects of neuroactive steroids on the intracellular localization of the human progesterone receptor

In our previous study we showed that in immunofluorescence studies the neuroactive steroids THP and THDOC induced a nuclear localization of the human progesterone receptor (hPR_B) indistinguishable from that obtained with progesterone after expression of the hPR_B in COS-1 cells. Therefore we questioned, whether pregnenolone and pregnanolone may also influence the intracellular localization of the human progesterone receptor. Without hormone, immunostaining of the human progesterone receptor was evident in the cytoplasm, the perinuclear region and the nucleus. Administration of progesterone, however, induced an exclusively nuclear localization. The same shift in receptor localization was obtained after treatment of the cells with pregnenolone and pregnanolone (Fig. 3).

3.3. Binding characteristics of neuroactive steroids to progesterone receptors

The binding properties of neuroactive steroids to either the chicken or the human progesterone receptor were assessed after reconstitution of the respective expression vectors in COS-1 cells. Table 1 shows the K_i values derived from the binding studies and the ED_{50} values obtained in the transfection experiments. Like the 5α-pregnane steroids 5α-DHP and 5α-dihydrodeoxycorticosterone (5α-DHDOC, 5α-pregnan-21-ol-3,20-dione) the 5β-pregnane steroid 5β-DHP bound to the chicken progesterone receptor with considerable affinity while its binding to the human progesterone receptor was less pronounced (Table 1). As shown previously for THDOC and THP (Rupprecht et al., 1993b), also the other neuroactive steroids did not bind directly to the progesterone receptor of either species. However, in contrast to THP and THDOC, which activate both the chicken and the human progesterone receptor, the synthetic neuroactive steroid alphaxalone induced gene expression only via the chicken progesterone receptor and was completely inactive via the human progesterone receptor. In a similar way, DHEA displayed a pronounced agonistic activity only via the chicken progesterone receptor in the transactivation assay without being a ligand of the progesterone receptor of either species. Moreover, the genomic effects of pregnenolone in the cotransfection system were not mediated by direct binding of the molecule to the progesterone receptors.

3.4. Functional properties of dehydroepiandrosterone via the human glucocorticoid receptor

To address potential modulatory properties of DHEA via the glucocorticoid receptor, we investigated whether an

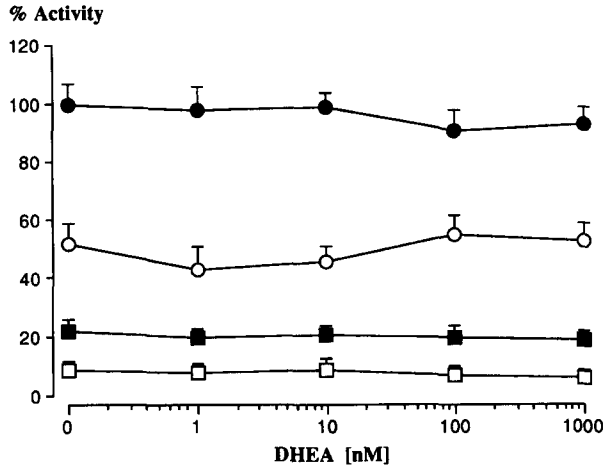

Fig. 4. Lack of competition of glucocorticoid action by DHEA in the cotransfection assay using the human glucocorticoid receptor (hGRα) expression plasmid. The value obtained with 10 nM dexamethasone without DHEA was set as 100%. Results are expressed in terms of percentage activity following coincubation of 10 nM dexamethasone (filled circles), 1 nM dexamethasone (open circles), 10 nM cortisol (filled squares) and 1 nM cortisol (open squares) with increasing doses of DHEA as indicated and represent the means ± S.D. of three independent experiments.

antiglucocorticoid activity of DHEA as suggested by in vivo studies may be reconstituted in the cotransfection system. The activation of the human glucocorticoid receptor (hGRα) obtained by dexamethasone or cortisol at a concentration of 1 nM or 10 nM, respectively, was not antagonized by DHEA, not even at a micromolar concentration (Fig. 4). Moreover, DHEA did not displace [^{3}H]dexamethasone from the human glucocorticoid receptor (hGRα) expressed in COS-1 cells (data not shown).

4. Discussion

In line with previous concepts attributing the effects of neuroactive steroids solely to the cell surface (Paul and Purdy, 1992) these steroids are devoid of transcriptional activity via several members of the steroid receptor superfamily. However, our data show that a variety of neuroactive steroids besides their modulatory activity at the $GABA_A$ receptor may regulate also gene expression via the progesterone receptor of the chicken and the human species. While the chicken progesterone receptor is activated by nearly all of the steroids investigated, the human progesterone receptor displays a more distinct transactivation pattern in view of its greater selectivity and the higher concentrations required for transactivation. In our previous study we demonstrated that the genomic effects of the neuroactive steroids THP and THDOC are not mediated by direct binding to the progesterone receptor but via intracellular metabolization (Rupprecht et al., 1993b). As also the other neuroactive steroids investigated in the present study do not bind to the progesterone receptor of either species, similar mechanisms seem to play a role for their genomic activity. This is also supported by the immunofluorescence studies which show that neuroactive steroids may induce a nuclear localization of the progesterone receptor when added to living cells containing the necessary cofactors for the respective enzymes. This contrasts with the experimental conditions during the in vitro binding assay where those cofactors are lacking.

Neuroactive steroids carrying a 3α-hydroxy group, which are related to the chemical structure of THP, e.g. pregnanolone and alphaxalone, are potential substrates of the 3α-hydroxysteroid oxidoreductase (Krause and Karavolas, 1980). Pregnanolone may be converted by the 3α-hydroxysteroid oxidoreductase (Krause and Karavolas, 1980) into the 5β-pregnane steroid 5β-dihydroprogesterone (5β-DHP). This steroid bound to the chicken progesterone receptor with considerable affinity while its binding to the human progesterone receptor was less pronounced (Table 1). Although the A-ring of steroid hormones usually is in a 5α-position, the present observations suggest that a steroid with a *cis*-standing A-ring, leading to an alternate three-dimensional structure of the molecule, can also interact with progesterone receptors. These data also emphasize the physiological role of 5β-reduced steroids for adaptive genomic processes within the central nervous system (Kubli-Garfias, 1984).

The synthetic steroid alphaxalone itself was not a ligand of the progesterone receptor of either species. However, intracellular oxidation by the 3α-hydroxysteroid oxidoreductase (Krause and Karavolas, 1980) would enable the formation of a respective 5α-pregnane steroid. In contrast to THP and THDOC, which activate both the chicken and the human progesterone receptor, alphaxalone induced gene expression only via the chicken progesterone receptor and was completely inactive via the human progesterone receptor. Thus, the introduction of a ketone group within the C-ring at the 11-position into the THP molecule is sufficient to avoid the agonistic effect via the human progesterone receptor. Therefore, chemical modifications at the C-ring may lead to neuroactive steroids which maintain the modulatory action at the $GABA_A$ receptor but are devoid of transcriptional activity via human steroid receptors.

The genomic effects of pregnenolone in the cotransfection system were not mediated by direct binding of the molecule to the progesterone receptors but by potential conversion of pregnenolone to progesterone by the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase. The formation of progesterone from pregnenolone is a central step in steroidogenesis and the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase is expressed abundantly in a variety of non-neuronal and neuronal tissues (Akwa et al., 1991).

DHEA was neither a ligand of the chicken nor of the human progesterone receptor. However, in the transactivation assay DHEA displayed a pronounced agonistic activ-

ity only via the chicken progesterone receptor, probably through generation of an androgen-like metabolite (Akwa et al., 1991), which binds to the chicken but not to the human progesterone receptor.

Besides the modulatory actions at ligand-gated ion channels and the genomic activity via the chicken progesterone receptor, DHEA has been suggested to exert an antiglucocorticoid activity when administered in vivo (Browne et al., 1993; Araneo and Daynes, 1995). Therefore, we investigated whether the antiglucocorticoid activity of DHEA may be reconstituted in the cotransfection system. DHEA did not antagonize the glucocorticoid-induced transactivation via the human glucocorticoid receptor and did not displace [^{3}H]dexamethasone from the human glucocorticoid receptor expressed in COS-1 cells as it has been predicted by previous studies in murine T cells (Browne et al., 1993) and AtT20 cells (Vedder et al., 1993). Thus, the proposed antiglucocorticoid activity of systemically administered DHEA appears not to be mediated by a direct interaction of DHEA with the intracellular glucocorticoid receptor molecule.

In conclusion, the neuroactive steroids studied here regulate neuronal function through their concurrent influence on transmitter-gated ion channels and gene expression at physiological concentrations in a structure-specific way. Whether genomic or nongenomic effects of neuroactive steroids predominate depends on the chemical structure of the steroid molecule, the relative expression of progesterone receptors, neurotransmitter receptors, and of metabolizing enzymes in the target tissues. Besides their potential role for physiological adaptive processes in the central nervous system, steroid receptor-mediated effects of neuroactive steroids may be also be important when designing new therapeutic drugs based on the molecular structure of these steroids.

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