Oxytocin receptors on cultured astroglial cells

Kinetic and pharmacological characterization of oxytocin-binding sites on intact hypothalamic and hippocampic cells from foetal rat brain

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The ability of astroglial cells to exhibit oxytocin (OT)-binding sites has been investigated in embryonic hypothalamic and hippocampic astroglial cell cultures. The differential characteristics of binding of OT and [Arg⁸]vasopressin (AVP) agonists and antagonists to the OT-binding sites using the highly selective iodinated OT antagonist d(CH₂)₅-[Tyr(Me)²,Thr⁴,Tyr-NH₂⁸]OVT ([¹²⁵I]OTA) have been evaluated using intact cells maintained for 12 days in culture. The specific binding displayed features of reversibility. Computer analysis of the saturation studies using the LIGAND program indicated that, at 4 °C, the antagonist binds to a homogeneous population of sites with a K_d value of 0.02 nM and a low binding-site density of around 2 fmol/dish for hypothalamic cells and 6 fmol/dish for hippocampic cells. For hypothalamic cells, competition curves using unlabelled OT, AVP or V₂ AVP agonist were characterized by a pseudo-Hill coefficient below unity (0.7), indicating possible heterogeneity among the binding sites. On the other hand, the dose-inhibition curves resulting from competition studies with hippocampic cells had a pseudo-Hill coefficient close to unity, except for OT. Computer analysis (LIGAND) indicated that the OT dose-inhibition curve was significantly better fitted to a two-site model, and this can be explained by two apparent forms of the receptor having high and low affinities for the displacing drug. The relative potencies of the peptides tested for binding to the high-affinity site were: AVP > OT > V₁ AVP antagonist {[d(CH₂)₅-Tyr(Me)²]AVP} = V₂ AVP agonist > AVP-Sar {[d(CH₂)₅-Sar⁷,Arg⁸]VP} in hypothalamic cultures, and OT = AVP > V₁ AVP antagonist > V₂ AVP agonist in hippocampic cultures. In addition, autoradiography allowed visualization of OT-binding sites, which are located on both soma and processes of astrocyte-like type of cells. In conclusion, these data provide evidence that glial cell cultures contain specific OT-binding sites which display pharmacological characteristics different from those already reported in neuronal cultures and in the adult rat brain.

INTRODUCTION

Oxytocin (OT)-containing neurones have been demonstrated in various brain areas in which OT is thought to act as a neurotransmitter or a neuromodulator (Buijs, 1983). Physiological actions of OT, such as facilitation of the milk-ejection reflex (Freund-Mercier & Richard, 1981; Moos & Richard, 1989) or induction of behavioural changes (for references, see De Wied *et al.*, 1989) are mediated via receptors which have been localized in adult rat brain by an autoradiographic technique *in vitro* (for references, see Freund-Mercier *et al.*, 1987, 1988; Tribollet *et al.*, 1988). The pharmacological characteristics of these receptors have been studied in isolated cells (Pliska *et al.*, 1986; Elands *et al.*, 1990) and in cell membranes from peripheral (Elands *et al.*, 1988a,b, 1990; Antoni & Chadio, 1989; Benedetto *et al.*, 1990; Chan *et al.*, 1990; Cantau *et al.*, 1990; Crankshaw *et al.*, 1990; Schmidt *et al.*, 1990) and central (Audigier & Barberis, 1985; Elands *et al.*, 1988a,b; Chadio & Antoni, 1989) structures by binding assays. It was established from both autoradiography and binding assays that OT receptors do not efficiently discriminate between OT and [Arg⁸]vasopressin (AVP) (Audigier & Barberis, 1985; Freund-Mercier *et al.*, 1988), whereas AVP receptors discriminate much more efficiently between OT and AVP.

Until recently, within the central nervous system, the concept that only neurones expressed neuropeptide receptors was com-

monly accepted, but there is now abundant evidence for non-neuronal neuropeptide binding. It is well established that astroglial cells express receptors for many peptides, such as insulin (Raizada *et al.*, 1982), opioid peptides (Rougon *et al.*, 1983; Pearce *et al.*, 1985; Maderspach & Solomon, 1988), angiotensin II (Simonnet *et al.*, 1988) and cholecystokinin (Yu *et al.*, 1990), for glucocorticoids (Chou *et al.*, 1990) and for many classical neurotransmitters (Sumners *et al.*, 1983; Baker *et al.*, 1986; Murphy & Pearce, 1987; Pearce *et al.*, 1988; Richards *et al.*, 1989). In a previous study we reported the presence of OT-binding sites in neurone-enriched cultures (Di Scala-Guenot *et al.*, 1990); however, it could not be excluded that binding also occurred on astroglial cells.

It is likely that astroglial cells express OT-binding sites, as suggested by various physiological data. During lactation (a condition of high OT demand; Salm *et al.*, 1985), or after continuous intracerebroventricular infusion of OT for several days (Theodosis *et al.*, 1986), an active retraction of astroglial processes occurred within the hypothalamus, leading to increased soma-somatic appositions. Furthermore, this central OT effect required the concomitant action of sex steroids (Montagnese *et al.*, 1990).

We thought that it would be valuable to investigate the presence of OT-binding sites on astroglial cells. Primary brain cell cultures allow examination of the binding properties of different cell types, and it is possible by differential culture

Abbreviations used: OT, oxytocin; AVP, [Arg⁸]vasopressin; OTA, d(CH₂)₅-[Tyr(Me)²,Thr⁴,Tyr-NH₂⁸]OVT; V₁ AVP antagonist, [d(CH₂)₅-Tyr(Me)²]AVP; V₂ AVP agonist, 1-deamino[Val⁴,Arg⁸]vasopressin; Sar, sarcosine; AVP-Sar, [d(CH₂)₅-Sar⁷,Arg⁸]VP; GFAP, glial fibrillary acidic protein; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum.

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techniques to prepare pure astroglial cell cultures. The purpose of the present work was to provide a pharmacological characterization of OT-binding sites on intact astroglial cells and to compare their characteristics with those already described in other tissues and in neurone-enriched cultures (Di Scala-Guenot *et al.*, 1990). Therefore astroglial cells were prepared from the hypothalamus and hippocampus of 16-day-old rat embryos. These structures have been chosen since the hypothalamus is known to exhibit glial plasticity, and the hippocampus is a highly enriched OT-binding-site structure.

MATERIALS AND METHODS

Brain astroglial cell cultures

Pregnant Wistar rats at day 16 of gestation were anaesthetized with urethane; the embryos were aseptically exteriorized and their brains were removed. The hypothalamus and hippocampus were isolated under a stereomicroscope and the tissues, cleaned of their meningeal membranes, were mechanically dispersed by repeated aspirations through a syringe in DMEM (Dulbecco's modified Eagle's medium; Gibco BRL, Les Ullis, France) containing 20% fetal calf serum (FCS; Gibco). Cells were seeded into 35 mm Costar Petri dishes (one-third of a hypothalamus/dish; $1\frac{1}{3}$ hippocampus/dish) and cultivated for 4 days in the above medium at 37 °C in a humid 5% CO₂ atmosphere. Thereafter the medium was replaced with fresh DMEM containing 10% FCS and cells were grown for an additional 8 days before use in binding experiments. This technique produced 95% astroglial cell purity, as determined by staining with anti-(neurone-specific enolase) (marker for neurones) and anti-galactocerebroside (marker for oligodendrocytes).

Binding studies

The radioligand used was an OT antagonist, 9d(CH₂)₅-[Tyr(Me)²,Thr⁴,Tyr-NH₂⁹]ornithine-vasotocin (OTA), radioiodinated in the laboratory according to Elands *et al.* (1988b) with some modifications (Stoeckel & Freund-Mercier, 1989). The specific radioactivity was about 2000 Ci/mmol. This radioligand, with high specific radioactivity, great pharmacological specificity and high affinity for OT receptors, is a very useful tool for the detection and characterization of OT receptors in cultured cells containing relatively low receptor concentrations. The binding of [¹²⁵I]OTA was studied in 12-day-old intact hypothalamic and hippocampic astroglial cells attached to the plastic culture dish as already described (Di Scala-Guenot *et al.*, 1990).

The binding reaction proceeded in 170 mM-Tris/HCl, pH 7.4, 5 mM-MgCl₂ and 0.1% BSA for 90 min at 37 °C, or for 16 h at 4 °C. The binding was stopped by rapid removal of the medium and addition of ice-cold Tris buffer, followed by three 10 min washes. The cells were scraped with a rubber policeman and the suspension was counted for radioactivity. The total binding was determined in the presence of 0.03 nM-[¹²⁵I]OTA; non-specific binding was measured in the presence of 0.03 nM-[¹²⁵I]OTA plus 5 μM unlabelled OT. Specific binding was measured by subtracting the non-specific binding from the total binding. For saturation, kinetic and displacement studies the technical protocols will be given in the Results section, and the data were analysed with the computer programs EBDA, LIGAND (Biosoft, Cambridge, U.K.) and Graphpad (Graphpad Software, San Diego, CA, U.S.A.). LIGAND provides a statistical comparison of one-site versus multiple-site models. For each analysis, estimates of binding parameters were calculated for one class of sites and two classes of sites, and both were corrected for non-specific binding whenever the fit was possible. Both LIGAND and Graphpad gave similar estimates of the binding parameters.

Immunocytochemistry

An antibody to glial fibrillary acidic protein (GFAP), a marker specific for the astroglial cell type, was used to determine the morphology of the cultured cells. Astroglial cells (12 days old) grown on glass cover slides were rinsed three times with 0.1 M-phosphate-buffered saline (pH 7.4) and then fixed for 10 s with cold methanol (−20 °C) and incubated for 1 h at room temperature with the anti-GFAP antibody (Dakopatts, Copenhagen, Denmark) at a dilution of 1:500. The immunoreactivity was revealed using the indirect horseradish peroxidase-labelled Fab technique (Biosys, Compiègne, France). Antibodies to neurone-specific enolase (Polyscience) and galactocerebroside (kindly donated by M. Sensenbrenner, UPR 0417 CNRS, Strasbourg, France), were used at a dilution of 1:2000 and 1:200 respectively.

Autoradiography

Binding was performed with cells grown on glass cover slides. After the binding reaction was stopped, cells were fixed for 1 h with 4% paraformaldehyde, rinsed with distilled water and allowed to dry before coating with nuclear emulsion (K5, Ilford, St Priest, France). The preparation was kept for 4 weeks at 4 °C, then the emulsion was developed and the cultures were examined by light microscopy.

Chemicals

OTA and the AVP antagonist [d(CH₂)₅-Sar⁷,Arg⁸]VP (AVP-Sar) were generously supplied by Dr. M. Manning (Medical College of Ohio, Toledo, OH, U.S.A.) and Dr. G. Grzonka

Fig. 1. Photomicrographs showing 12-day-old astroglial cells from cultured embryonic rat hypothalamus

Cells were immunocytochemically stained for GFAP (a) or labelled with [¹²⁵I]OTA (b). The latter have a comparable morphology to the GFAP-positive cells. Arrows indicate cells stained for GFAP (a) and labelled with [¹²⁵I]OTA (b) with comparable morphology. Magnification × 144.

(Institute of Chemistry, Gdansk, Poland) respectively. OT and AVP were purchased from Bachem (Torrance, CA, U.S.A.). $[d(CH_2)_5-Tyr(Me)^2]AVP$ (V_1 AVP antagonist) was purchased from Peninsula Laboratories (Belmont, CA, U.S.A.), and 1-deamino $[Val^4, D-Arg^8]$ vasopressin (V_2 AVP agonist) was from Ferring A.B. (Malmö, Sweden).

RESULTS

Immunocytochemical and autoradiography studies

Phase-contrast examination of cultures on day 12 showed monolayers consisting of confluent large flat cells. The majority of the cells were stained for GFAP and showed variable morphology: flat and polygonal or elongated shape (Fig. 1a). Screening of these cultures with markers for neurones and oligodendrocytes showed very few stained cells. Therefore the cultures consisted mainly of astrocytic glial cells. Autoradiography studies after binding revealed silver grains on cells that were morphologically comparable with those stained for GFAP (Figs. 1a and 1b). Binding sites were distributed all over the soma and processes of these cells, and this binding was specific, since no binding could be observed in the presence of an excess of unlabelled OT ($5 \mu M$; results not shown).

Kinetic studies

The time course of $[^{125}I]OTA$ association with intact glial cells

was temperature-dependent, and reached equilibrium after 90 min at $37^\circ C$ and after 16 h at $4^\circ C$ for both hypothalamic and hippocampic cells (Fig. 2). The experimental data could be fitted to a single exponential, allowing us to calculate the apparent rate constant, k_{app} , (see Table 1). Dissociation of bound $[^{125}I]OTA$, initiated after 90 min of incubation by addition of an excess of unlabelled OT ($5 \mu M$), was determined at various time intervals (Fig. 3). Bound $[^{125}I]OTA$ dissociated slowly for both types of cells and the first-order rate constant, k_{-1} (see Table 1), was calculated by linear regression analysis (Fig. 3, inset). The dissociation reaction was not performed at $4^\circ C$ because of a loss of cells over 24 h of incubation. Knowledge of both k_{app} and k_{-1} at $37^\circ C$ allowed us to calculate the second-order rate of association, k_{+1} , which was 0.67 and $0.65 \text{ nM}^{-1} \cdot \text{min}^{-1}$ for hypothalamic and hippocampic cells respectively (Table 1).

In these conditions, at equilibrium, the average non-specific binding was about 10% and the maximum bound $[^{125}I]OTA$ was not more than 10% of the total labelled OTA. It is assumed that the concentrations of the free ligand and of the unlabelled peptides in the incubation medium remained constant with time.

Saturation studies

The effect of increasing the $[^{125}I]OTA$ concentration on $[^{125}I]OTA$ binding was investigated at $4^\circ C$ and $37^\circ C$. When the saturation experiment was performed at $4^\circ C$ with ligand concentrations ranging from 3.25 pM to 0.2 nM , the corresponding

Fig. 2. Time- and temperature-dependence of specific binding of $[^{125}I]OTA$ to hypothalamic ($\blacklozenge$) and hippocampic ($\blacklozenge$) astroglial cells at $37^\circ C$ (a) and $4^\circ C$ (b)

The lower panels show semilogarithmic plots of the association curves. Values of k_{app} are given in Table 1. B_{eq} , $[^{125}I]OTA$ bound at equilibrium; B_t , $[^{125}I]OTA$ bound at a given time (both d.p.m.).

Fig. 3. Time course of dissociation of [¹²⁵I]OTA at 37 °C
Dissociation was measured in hypothalamic cells (◆) and hippocampic cells (◇). Inset: semilogarithmic plots of the data. Values of *k*₋₁ and *k*₊₁ are given in Table 1.

isotherms (Fig. 4a, inset) indicated that [¹²⁵I]OTA specific binding at equilibrium was saturable for both culture types, and the corresponding Scatchard plot (Fig. 4a) was linear with a pseudo-Hill coefficient (*h*) close to unity. LIGAND analysis yielded a best fit with a one-site model of high affinity (*K*_d 0.02 nM for both hypothalamic and hippocampic cells) and low capacity (*B*_{max} 2.2 fmol/dish for hypothalamic cells and 5.6 fmol/dish for hippocampic cells). These values were close to those estimated

Table 1. Characteristics of binding of [¹²⁵I]OTA to cultured astroglial cells

*k*_{app.} and *k*₋₁ are the linear regression slopes in association and dissociation experiments, and correspond to the apparent association and dissociation rate constants respectively. *k*₊₁ corresponds to the second-order rate constant of association, and is equal to (*k*_{app.} - *k*₋₁)/*F*, where *F* is the free labelled ligand concentration (0.03 nM). *K*_d from kinetic data is equal to *k*₋₁/*k*₊₁. The dissociation reaction at 4 °C was not performed.

	Hypothalamus		Hippocampus	
	37 °C	4 °C	37 °C	4 °C
<i>k</i> _{app.} (min ⁻¹)	0.0272	0.49	0.0267	0.315
<i>k</i> ₋₁ (min ⁻¹)	0.007	—	0.0072	—
<i>k</i> ₊₁ (nM ⁻¹ ·min ⁻¹)	0.67	—	0.65	—
<i>K</i> _d from kinetic studies (nM)	0.010	—	0.011	—
<i>K</i> _d from saturation study (nM)	0.022	0.020	0.020	0.020
<i>B</i> _{max.} (fmol/dish)	2.2	2.2	7.5	5.6

from the kinetic studies. It should be noticed that the *K*_d and *B*_{max.} were identical at both 37 °C and 4 °C. However, at 37 °C the Scatchard plot of the data was curvilinear (Fig. 4b) and computer analysis of the data by non-linear regression indicated that the pseudo-Hill coefficient was different from unity (*h* = 0.67). This result was suggestive of more than one class of binding site; however, statistical analysis with LIGAND produced a better fit with a one-site model (*P* = 0.71). Thus [¹²⁵I]OTA appears to react with high affinity to a homogeneous population of binding sites.

Competition studies

These experiments were carried out at 4 °C. The ligand specificity was estimated from competition studies using the unlabelled peptides OT, AVP, V₁ AVP antagonist and V₂ AVP

Fig. 4. Binding of [¹²⁵I]OTA to astroglial OT-binding sites with increasing concentrations of the labelled ligand
Scatchard plots are given of saturation studies performed at 4 °C (a) and 37 °C (b) for hypothalamic (◆) and hippocampic cells (◇). Insets: saturation isotherms of specific binding at 4 °C (a) and 37 °C (b). *K*_d and *B*_{max.} values are given in Table 1.

Fig. 5. Competitive displacement of specifically bound [¹²⁵I]OTA by increasing concentrations of unlabelled OT (○), AVP (◆), V₁ AVP antagonist (■), AVP-Sar (□) and V₂ AVP agonist (△) in hypothalamic (a) and hippocampic cells (b) at 4 °C

The lines are the computer fitting (Graphpad) of the experimental points. Values of *K_d*, calculated using the LIGAND program, and the pseudo-Hill coefficients are given in Table 2.

Table 2. Potency of OT, AVP and AVP analogues in competing with [¹²⁵I]OTA binding on hypothalamic and hippocampic glial cells

K_H and *K_L* are the dissociation constants of the high- and low-affinity binding sites respectively, as determined by analysis with LIGAND. *h* is the pseudo-Hill coefficient.

Peptide	Hypothalamus			Hippocampus		
	<i>K_d</i> (nM)	<i>B_{max}</i> (fmol/dish)	<i>h</i>	<i>K_d</i> (nM)	<i>B_{max}</i> (fmol/dish)	<i>h</i>
OT <i>K_H</i>	2.0	1.5	0.76	0.3	1.12	0.78
OT <i>K_L</i>	500	0.42		200	0.17	
AVP <i>K_H</i>	0.1	14	0.73	0.7	5.0	0.93
AVP <i>K_L</i>	2.0	1.1				
V ₂ agonist <i>K_H</i>	6.0	5.5	0.71	43	1.0	0.94
V ₂ agonist <i>K_L</i>	700	0.7				
V ₁ antagonist	2.0	9.1	0.92	1.9	7.7	1.10
AVP-Sar	200	5.8	0.98	—	—	—

agonist tested at concentrations ranging from 0.01 nM to 5 μM. The competition curves are shown in Fig. 5.

For hypothalamic astroglial cells, OT, AVP and V₂ AVP agonist competition experiments produced multiphasic shallow curves (Fig. 5a), and regression analysis indicated a Hill coefficient as low as 0.7, suggesting the existence of multiple binding sites. Computer analysis of the data with LIGAND confirmed a better fit with a two-site model. Values for the high- and low-affinity sites and for the binding capacities are given in Table 2. Competition curves obtained with V₁ AVP and AVP-Sar antagonists were monophasic, with a *h* close to unity, and LIGAND analysis indicated a single class of binding sites, with high affinity (*K_d* 2 nM) for the V₁ antagonist and low affinity (*K_d* 200 nM) for the AVP-Sar antagonist.

For hippocampic astroglial cells (Fig. 5b), computer analysis of the data indicated that AVP, V₁ AVP antagonist and V₂ AVP agonist competed for a single class of sites, and displaced [¹²⁵I]OTA binding with *K_d* values of 0.7 nM, 1.9 nM and 43 nM respectively. However, for OT, the dose-inhibition curve was found to be multiphasic, with the pseudo-Hill coefficient being below unity (Table 2). Computer analysis of the OT competition

curve indicated a better fit with a two-site model (*P* = 0.07 for one-site model), with OT discriminating between two affinities of the binding sites labelled by [¹²⁵I]OTA, with *K_d* values of 0.3 nM and 200 nM for the high- and low-affinity sites respectively. The high-affinity form appeared to be predominant, despite the low binding capacity for both states (< 2 fmol/dish). The displacement experiments were repeated four times for hypothalamic cells and twice for hippocampic cells, and gave similar results.

DISCUSSION

The major finding of this paper is that astroglial cells express OT-binding sites. The data clearly demonstrate the ability of a highly specific radiolabelled OT antagonist to identify OT-binding sites in astroglial cell cultures. Visualization of OT-binding sites on individual cells by autoradiography proved that these cells, labelled on soma and processes, have a comparable morphology to the astroglial cells stained by immunocytochemistry for GFAP.

The OT-binding sites fulfilled the classical receptor criteria of high affinity, saturability, reversibility and pharmacological

specificity. Analysis of saturation experiments performed at 4 °C with hypothalamic and hippocampic glial cells indicated that the [¹²⁵I]OTA binds with high affinity to a homogeneous population of binding sites. The calculated affinity of 0.03 nM is very close to that already reported for this ligand with membrane preparations from the central nervous system (Elands *et al.*, 1988a) and the periphery (Elands *et al.*, 1990; Antoni & Chadio, 1989), and in cell cultures (Di Scala-Guenot *et al.*, 1990; Cantau *et al.*, 1990). However, with respect to OT receptor characteristics and ligand specificity, differences between this study and others were noted. In our system, the shallow OT competition curves are compatible with at least two states of the receptor, and computer analysis of the dose-inhibition curves with LIGAND suggested that OT receptors are present in high- and low-affinity forms in both the hypothalamus and the hippocampus. In contrast, except for the work of Crankshaw *et al.* (1990), who identified three populations of binding sites on myometrium cell membranes, in all the remaining studies only one population of high-affinity binding sites was identified (Elands *et al.*, 1988b, 1990; Chadio & Antoni, 1989; Cantau *et al.*, 1990). However, it should be mentioned that some binding studies performed with [³H]OT or [³H]AVP (Audigier & Barberis, 1985; Junig *et al.*, 1985; Stassen *et al.*, 1987; Ayad *et al.*, 1990) also predicted two classes of binding sites, but this may have resulted from a cross-reactivity of the tritiated ligands on OT- and AVP-binding sites. The presence of two binding sites on glial cells, whereas cultured neuronal cells only express one high-affinity binding site, could result from alteration of the cell receptor characteristics by isolation of the cells from their regulatory signals; another explanation for this discrepancy, as already stated by Crankshaw *et al.* (1990), could be that the previous competition studies with neuronal cultures did not use a sufficient range of concentrations, and the two-site model could not be statistically proven.

Another difference between previous studies and the present report was observed for AVP and the V₂ AVP agonist which, like OT, displaced the radiolabelled ligand with two distinct affinities on hypothalamic cells. In hippocampic cells, both analogues bound to a single class of high-affinity sites; however, differences between specificities of the OT receptors on the two tissues would need more detailed pharmacological characterization to confirm tissue specificity.

Ligand specificity studies with cultured glial cells highlighted the equal ability of OT, V₁ AVP antagonist and V₂ AVP agonist to compete with high affinity for [¹²⁵I]OTA binding on hypothalamic glial cells, whereas on central and peripheral tissue sections, membrane preparations and cultured neuronal cells the V₂ AVP agonist always displaced the bound ligand with a low affinity (10⁻⁷ M range) (Di Scala-Guenot *et al.*, 1990; Elands *et al.*, 1990; Schmidt *et al.*, 1990). It is well established that V₁ AVP receptors are coupled to phosphoinositide metabolism (Michell *et al.*, 1979) and V₂ AVP receptors to adenylate cyclase activation (Jard, 1983). On the other hand, several studies have demonstrated a coupling of the central and peripheral OT receptors with phosphoinositide metabolism only (Marc *et al.*, 1986; Schrey *et al.*, 1986; Moore *et al.*, 1988; Soloff *et al.*, 1989; Pettibone *et al.*, 1990), with the cyclic AMP pathway generally being excluded, except for electrophysiological and biochemical studies performed by Funase (1990) and Meeker *et al.* (1990) respectively. Therefore, given the equal ability of OT, V₁ AVP antagonist and V₂ AVP agonist to displace the bound ligand on hypothalamic glial cells, coupling of the OT receptors with activation of adenylate cyclase should also be considered.

In summary, the pharmacological characterization of the OT-binding sites on cultured glial cells, different from the neuronal ones described previously, suggests distinct populations of OT receptors on these two cell types.

Other studies have also demonstrated expression of membrane-associated or intracellular receptors for a variety of peptides and steroids in both astroglial cells and neurones (Rougon *et al.*, 1983; Evans *et al.*, 1984; Laribi *et al.*, 1985; Chou *et al.*, 1990). Although binding affinity was almost identical between the two types of cells, differences could be found in site density and/or in the pharmacological properties of the ligand-binding sites.

As far as the physiological significance of astroglial OT-binding sites is concerned, they are thought to be involved in plasticity at least within the hypothalamus. Indeed, direct apposition of the neuronal membrane, due to an active retraction of the glial processes interposed between neurones under basal conditions, has been observed in response to physiological stimuli such as lactation, parturition and prolonged dehydration (Theodosis & Poulain, 1987). These morphological modifications are fully reversible on return to conditions of low hormone secretion. Although no direct effect of OT on glial hippocampic cells has been reported so far, electrophysiological data have demonstrated that OT enhances the firing rate of spontaneously active neurones, and this effect is mediated mainly by uterine-like OT receptors on non-pyramidal cells in field CA1 of the hippocampus (Mühlethaler *et al.*, 1983). Moreover, a hyper-osmotic stimulus elicits a local release of OT in the hippocampus (Landgraf *et al.*, 1988). On the other hand, OT has been shown to inhibit morphine tolerance in several parts of the limbic system (Sarnyai *et al.*, 1988) and to be involved in sexual behaviour (Melis *et al.*, 1986).

In conclusion, we have demonstrated in the present paper that OT-binding sites are present on hypothalamic and hippocampic astroglial cells in culture. Saturation studies with the antagonist [¹²⁵I]OTA demonstrated a single class of high-affinity, low-capacity, binding sites with a low dissociation rate of [¹²⁵I]OTA, whereas competition studies with agonists displayed competition curves with Hill coefficients below unity, arguing for receptor heterogeneity or multiple affinities of a single receptor. Regulation of cell membrane receptors by G-proteins appears to be a common feature of receptor-agonist interactions (Rodbell, 1980; Spiegel, 1987; Litosch, 1987), and since most receptor-ligand interactions are modulated by bivalent cations such as Mg²⁺ (Birnbaumer *et al.*, 1985; Antoni & Chadio, 1989), the differential binding properties of agonists and antagonists to OT-binding sites in the presence and the absence of guanine nucleotides and Mg²⁺ will be investigated in the accompanying paper (Di Scala-Guenot & Strosser, 1992).

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REFERENCES

- Antoni, F. A. & Chadio, S. E. (1989) *Biochem. J.* **257**, 611-614
- Audigier, S. & Barberis, C. (1985) *EMBO J.* **4**, 1407-1412
- Ayad, V. J., McGoff, S. A. & Wathes, D. C. (1990) *J. Endocrinol.* **124**, 353-359
- Baker, S. P., Sumners, C., Pitha, J. & Raizada, M. K. (1986) *J. Neurochem.* **47**, 1318-1326
- Benedetto, M. T., De Cicco, F., Rossiello, F., Nicosia, A. L., Lupi, G. & Dell'Acqua, S. (1990) *J. Steroid Biochem.* **35**, 205-208
- Birnbaumer, L., Codina, J., Mattera, R., Cerione, R. A., Hildebrandt, J. D., Sunyer, T., Rojas, F. J., Caron, M. G., Lefkowitz, R. J. & Iyengar, R. (1985) *Recent Prog. Horm. Res.* **41**, 41-100
- Buijs, R. M. (1983) *Pharmacol. Ther.* **22**, 127-141
- Cantau, B., Barjon, J. N., Chicot, D., Baskevitch, P. P. & Jard, S. (1990) *Am. J. Physiol.* **258**, F963-F972
- Chadio, S. E. & Antoni, F. A. (1989) *J. Endocrinol.* **122**, 465-470

- Chan, W. Y., Cao, L., Hill, P. S. & Hruby, V. J. (1990) *Endocrinology* (Baltimore) **126**, 2095–2101
- Chou, Y. C., Luttge, W. G. & Sumners, C. (1990) *J. Neuroendocrinol.* **2**, 29–38
- Crankshaw, D., Gaspar, V. & Pliska, V. (1990) *J. Recept. Res.* **10**, 269–285
- De Wied, D., Gispén, W. H. & van Wimersma Greidanus, Tj. B. (1989) *Neuropeptides and Behavior: vol. 2, The Neurohypophyseal Hormones*, Pergamon, Oxford
- Di Scala-Guenot, D. & Strosser, M.-T. (1992) *Biochem. J.* **284**, 499–505
- Di Scala-Guenot, D., Strosser, M.-T., Freund-Mercier, M. J. & Richard, Ph. (1990) *Brain Res.* **524**, 10–16
- Elands, J., Barberis, C. & Jard, S. (1988a) *Am. J. Physiol.* **254**, E31–E38
- Elands, J., Barberis, C., Jard, S., Tribollet, E., Dreifuss, J. J., Bankowski, K., Manning, M. & Sawyer, W. H. (1988b) *Eur. J. Pharmacol.* **147**, 197–207
- Elands, J., Resink, A. & De Kloet, E. R. (1990) *Endocrinology* (Baltimore) **126**, 2703–2710
- Evans, T., McCarty, K. D. & Harden, K. T. (1984) *J. Neurochem.* **43**, 131–138
- Freund-Mercier, M. J. & Richard, Ph. (1981) *Neurosci. Lett.* **23**, 193–198
- Freund-Mercier, M. J., Stoeckel, M. E., Palacios, J. M., Pazos, A., Reichhart, J. M., Porte, A. & Richard, Ph. (1987) *Neuroscience* **20**, 599–614
- Freund-Mercier, M. J., Dietl, M. M., Stoeckel, M. E., Palacios, J. M. & Richard, Ph. (1988) *Neuroscience* **26**, 261–272
- Funase, K. (1990) *Brain Res.* **517**, 263–268
- Jard, S. (1983) *Curr. Top. Membr. Transp.* **18**, 255–285
- Junig, J. T., Abood, L. G. & Skrobala, A. M. (1985) *Neurochem. Res.* **10**, 1187–1202
- Landgraf, R., Neumann, I. & Schwarzbarg, H. (1988) *Brain Res.* **457**, 219–225
- Laribi, C., Legendre, P., Dupouy, B., Vincent, J. D. & Simonnet, G. (1985) *Brain Res.* **347**, 94–103
- Litosch, I. (1987) *Life Sci.* **41**, 251–258
- Maderspach, K. & Solomon, R. (1988) *Brain Res.* **441**, 41–47
- Marc, S., Leiber, D. & Harbon, S. (1986) *FEBS Lett.* **201**, 9–14
- Meeker, R. B., Michels, K. M. & Hayward, J. N. (1990) *Neurosci. Lett.* **114**, 225–230
- Melis, M. R., Argiolas, A. & Gessa, G. L. (1986) *Brain Res.* **398**, 259–265
- Michell, R. H., Kirk, C. J. & Billah, M. M. (1979) *Biochem. Soc. Trans.* **7**, 861–865
- Montagnese, C., Poulain, D. A. & Theodosis, D. T. (1990) *J. Neuroendocrinol.* **2**, 225–231
- Moore, J. J., Dubyak, G. R., Moore, R. M. & Vander Kooy, D. (1988) *Endocrinology* (Baltimore) **123**, 1771–1777
- Moos, F. & Richard, Ph. (1989) *J. Physiol. (London)* **408**, 1–18
- Mühlethaler, M., Sawyer, W. H., Manning, M. & Dreifuss, J. J. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6713–6717
- Murphy, S. & Pearce, B. R. (1987) *Neuroscience* **22**, 381–394
- Pearce, B. R. & Murphy, S. (1988) in *Glial Cell Receptors* (Kimelberg, H., ed.), pp. 197–223, Raven Press, New York
- Pearce, B. R., Cambray-Deakin, M. & Murphy, S. (1985) *Neurosci. Lett.* **55**, 157–160
- Pettibone, D. J., Woyden, C. J. & Totaro, J. A. (1990) *Eur. J. Pharmacol.* **188**, 235–242
- Pliska, V., Heiniger, J., Müller-Lhotsky, A., Pliska, P. & Ekberg, B. (1986) *J. Biol. Chem.* **261**, 16984–16989
- Raizada, M. K., Stamler, J. F., Quinlan, J., Landas, S. & Philipps, M. I. (1982) *Cell Mol. Neurobiol.* **2**, 47–52
- Richards, E. M., Sumners, C., Chou, Y. C., Raizada, M. K. & Phillips, M. I. (1989) *J. Neurochem.* **53**, 287–296
- Rodbell, M. (1980) *Nature (London)* **284**, 17–22
- Rougon, G., Noble, M. & Munge, A. W. (1983) *Nature (London)* **305**, 715–717
- Salm, A. K., Smithson, K. G. & Hatton, G. I. (1985) *Cell Tissue Res.* **242**, 9–15
- Sarnyai, Z., Viski, S., Krivan, M., Szabo, G., Kovacs, G. L. & Telegdy, G. (1988) *Brain Res.* **463**, 284–288
- Schmidt, A., Jard, S., Dreifuss, J. J. & Tribollet, E. (1990) *Am. J. Physiol.* **259**, F872–F881
- Schrey, M. P., Read, A. M. & Steer, P. J. (1986) *Biosci. Rep.* **6**, 613–619
- Simonnet, G., Legendre, P., Laribi, C., Allard, M. & Vincent, J. D. (1988) *Brain Res.* **443**, 403–406
- Soloff, M. S., Fernstrom, M. A. & Fernstrom, M. (1989) *Biochem. Cell Biol.* **57**, 152–162
- Spiegel, A. M. (1987) *Mol. Cell Endocrinol.* **49**, 1–16
- Stassen, F. L., Heckman, D., Schmidt, D., Aiyar, N., Nambi, P. & Crooke, S. T. (1987) *Mol. Pharmacol.* **31**, 259–266
- Stoeckel, M. E. & Freund-Mercier, M. J. (1989) *Am. J. Physiol.* **257**, F310–F314
- Sumners, C., Phillips, M. I. & Raizada, M. K. (1983) *Brain Res.* **264**, 267–275
- Theodosis, D. T. & Poulain, D. A. (1987) *Trends NeuroSci.* **10**, 426–430
- Theodosis, D. T., Montagnese, C., Rodriguez, F., Vincent, J. D. & Poulain, D. A. (1986) *Nature (London)* **322**, 738–740
- Tribollet, E., Barberis, C., Jard, S., Dubois-Dauphin, M. & Dreifuss, J. J. (1988) *Brain Res.* **442**, 105–118
- Yu, D. H., Huang, S. C., Wank, S. A., Mantey, S., Gardner, J. D. & Jensen, R. T. (1990) *Am. J. Physiol.* **258**, G86–G95

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