Differential involvement of adrenal and gonadal steroids in anterior and intermediate pituitary pro-opiomelanocortin mRNA expression induced by the endogenous benzodiazepine, octadecaneuropeptide, in adult male rats

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Abstract

The involvement of the endogenous benzodiazepine, octadecaneuropeptide (ODN), in the regulation of pro-opiomelanocortin (POMC) mRNA expression at the pituitary level, and the influence of adrenal and gonadal steroids, have been studied using a quantitative *in situ* hybridization technique. I.c.v. injection of ODN (4 μg/kg) in sham-operated rats induced a 17 and 7% decrease in the POMC mRNA expression in anterior and intermediate pituitary lobes respectively. To determine the reciprocal involvement of adrenal and gonadal steroids in this regulation, animals were adrenalectomized and/or castrated. Adrenalectomy significantly increased POMC mRNA expression by 48% at the anterior pituitary level, but induced a 10% decrease of hybridization signal at the intermediate pituitary lobe (vs control sham-operated). Adrenal ablation reversed the effect induced by ODN and increased POMC mRNA expression at the anterior and intermediate pituitary levels by 60 and 10% respectively, compared with control sham-operated. By contrast, castration, which produced a decrease in POMC mRNA in the anterior pituitary and an increase in the intermediate lobe, did not modify the negative influence of ODN observed in sham-operated animals. When rats were adrenalectomized and castrated, the adrenalectomy influence was predominant at the anterior pituitary level, since ODN increased significantly the hybridization signal (+68% vs control sham-operated), while the castration influence was predominant at the intermediate pituitary level, since ODN induced an 11% decrease in POMC mRNA signal compared with control sham-operated. These studies indicate that, *in vivo*, the decrease in POMC mRNA expression in the anterior and intermediate pituitary induced by an endogenous benzodiazepine is differently modulated by adrenal and gonadal steroids, with a predominant influence of adrenal steroids at the anterior pituitary level and gonadal steroids at the intermediate pituitary level.

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Introduction

In the pituitary gland, pro-opiomelanocortin (POMC) is predominantly expressed in corticotropic cells of the pars distalis or anterior pituitary (AP) and in melanotropic cells of the pars intermedia or intermediate pituitary (IP). POMC is also expressed in a discrete population of neurons localized in the arcuate nucleus (AN) of the basal hypothalamus (reviewed in Pelletier (1993)). The hypothalamic peptides, corticotropin-releasing hormone (CRH) and arginine vasopressin, have a major function in stimulating adrenocorticotropic hormone and β-endorphin release from the AP (Vale *et al.* 1981, Assenmacher *et al.* 1995), as well as inducing β-endorphin and α-melanocyte stimulating hormone release from the IP (Saland *et al.* 1988, Muller & Von Werder 1991). These hormones, derived from POMC, are considered as stress-related molecules and are involved in the hypothalamus–pituitary–adrenocortical (HPA) axis response in a variety of circumstances, and thus in the maintenance of homeostasis (reviewed in Whitnall 1993).

The melanotropic cells of the IP are directly innervated by monoaminergic (Björklund *et al.* 1973) and gamma-aminobutyric acid (GABA)ergic neurons located in the central nervous system (Vuillez *et al.* 1987), while the AP corticotropic cells have been shown to contain GABA receptors (Berman *et al.* 1994). These observations have suggested that GABA might be directly involved in the regulation of POMC mRNA expression at both the AP and IP levels. It is well known that GABA can interact with two distinct classes of receptors, termed GABAA and GABAB receptor complexes, which differ in their

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pharmacological, electrophysiological and biochemical properties. The GABAA receptor complex has been shown to contain five major binding sites, for GABA, benzodiazepines (BZD), barbiturates, picrotoxin (specific antagonist) and steroids. GABA, as well as GABAA agonists and other activators of GABAA receptors such as BZD and barbiturates, has been shown to decrease mRNA levels in POMC melanotropic cells (Jegou *et al.* 1991, Garcia-de-Yebenes & Pelletier 1994). The presence of GABAA receptors in melanotropic and corticotropic cells has been reported (Louiset *et al.* 1992, Berman *et al.* 1994).

Anxiety and depression, terms used by behavioral scientists and clinicians, can be states induced in part by release of stress hormones. Some anxiolytic agents have been developed to reduce unwanted behavioral traits. Such agents include BZDs, which may act to reduce the anxiety and depressive states by interacting within the central nervous system to modulate the levels of stress hormones including corticotropin releasing factor (Grigoriadis *et al.* 1989). In rat brain, an endogenous BZD polypeptide with high affinity for diazepam-binding sites, named diazepam-binding inhibitor (DBI) has been described (Guidotti *et al.* 1983). This polypeptide is located in the central nervous system in glial cells and neurons (Alho *et al.* 1985). In some neurons, GABA has been co-localized with DBI (Costa *et al.* 1986) and at least two different processing products, DBI_{35-50} or octadecaneuropeptide (ODN) and DBI_{17-50} or triakontatetraneuropeptide (Alho *et al.* 1990). I.c.v. injection of DBI elicits pro-conflict responses and antagonizes the anti-conflict and anxiolytic properties of the GABA/BZD complex or GABAA receptor (Ferrero *et al.* 1984, Kavaliers & Hirst 1986). ODN markedly reduces the effects of GABA on hormonal release (Tonon *et al.* 1989) and totally abolishes the chloride current induced by GABA (Louiset *et al.* 1993). In man, there is a positive correlation between cerebrospinal fluid concentrations of DBI and CRH in depressed patients, suggesting that DBI and processing products might play a role in the release of CRH and the activation of the HPA axis response to stress, probably via GABAA receptors (Roy *et al.* 1989). Recently, we have clearly demonstrated that, *in vivo*, the endogenous BZD, ODN, via an activation of the BZD sites of GABAA receptor, negatively modulates CRH neuronal activity and that this modulation can be negatively or positively influenced by central and peripheral steroids (Givalois *et al.* 1998*a*). Moreover, ODN modulation of the HPA axis activity seems to involve adrenal and/or gonadal factor(s) other than glucocorticoids (Givalois *et al.* 1998). At the pituitary level, the central injection of ODN produces an inhibition of POMC gene expression at the IP level as well as at the AN level (Garcia-de-Yebenes *et al.* 1997). These effects on the POMC mRNA expression were completely reversed by concomitant administration of the GABAA receptor antagonist, picrotoxin, and seem to be modulated allosterically by neurosteroids (Garcia-de-Yebenes *et al.* 1997).

In this study, to further examine the possible role of peripheral steroids in the modulation of POMC gene expression at the AP and IP levels induced by the endozepine ODN, we investigated the impact of adrenalectomy (ADX) and/or castration (CX) on the POMC mRNA levels in the corticotropic and melanotropic cells following ODN i.c.v. injection.

Materials and Methods

Animals

Fifty adult male Sprague–Dawley rats (Charles Rivers Canada Inc., Montreal, Quebec, Canada) weighing 225–250 g at the beginning of the experiment were housed under constant temperature (21 ± 1 °C) and lighting (lights on from 0600 to 2000 h) regimens. They had free access to standard rat chow, and tap water for sham-operated and CX rats or isotonic water (0·9% NaCl) for ADX rats.

Experimental procedures

The drug used was the endogenous BZD receptor ligand, ODN (Peninsula Laboratories, Belmont, CA, USA), which was stereotaxically injected into the left lateral ventricle at a dose of 4 μg/kg and at a rate of 2 μl/min in a total volume of 20 μl (Li & Pelletier 1996). In order to study the involvement of adrenal and sexual steroids in the variation of POMC mRNA expression induced by ODN, the 50 animals were divided into four groups. Five days before experimentation the animals underwent ADX (n=12), CX (n=12) or ADX and CX (ADX/CX; n=14); sham-operated animals (n=12) served as controls. On the day of the experiment, each group was divided into two, and four groups received an i.c.v. injection of ODN (4 μg/kg), while the other four groups (control groups) received an i.c.v. injection of vehicle (0·9% NaCl).

Four hours after i.c.v. injection of either vehicle or ODN, the animals were deeply anesthetized with an i.m. injection of 0·2 ml of a mixture of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg) and then rapidly perfused transcardially with 4% paraformaldehyde in 0·2 M phosphate buffer. The pituitaries were removed and postfixed in the same fixative for 4 h at 4 °C, and then placed in 15% sucrose in 0·2 M phosphate buffer overnight at 4 °C. Thereafter, the tissues were quickly frozen in isopentane chilled in liquid nitrogen. The frozen pituitaries were mounted on a cryostat (Leitz, Montreal, Quebec, Canada) and serially cut into 10 μm coronal sections. The pituitary sections were mounted on gelatin- and poly-L-lysine-coated glass slides and kept at − 80 °C until use. Hybridization histochemical localization of

POMC transcripts was carried out using a ^{35}S-labeled oligoprobe, as described below.

Preparation of the POMC probe

The probe used for *in situ* hybridization was a [^{35}S]dATP-labeled 30 bases oligonucleotide synthesized in our laboratory, complementary to the POMC-coding region of the rat complementary DNA (bases 297–324) (Garcia-de-Yebenes *et al.* 1997). The sequence corresponding to the probe is: 5′-CTT-GCC-CCA-GCG-GAA-GTG-CTC-CAT-GGA-GTA-3′. Radioactive oligoprobe was obtained using the terminal deoxynucleotidyl transferase (TdT, Pharmacia, Biotech Inc., Baie d'Urfé, Quebec, Canada) technique, by incubation of 300 ng oligonucleotide template in 5 μl buffer 'One for all' 10 × (Pharmacia), in 2 μl TdT (25 U/μl), 4 μl [α-^{35}S]ATP (50 μCi; Dupont NEN, Boston, MA, USA), 37·5 μl Diethyl Pyrocarbonate sterile water for 90 min at 37 °C. In order to reduce background, unincorporated nucleotides were removed using a Microspin column (Pharmacia).

In situ *hybridization with oligoprobe*

Some serial sections from each group were treated with ribonuclease A (RNAse A; 100 μg/ml; Pharmacia) for 45 min at 37 °C before hybridization and served as negative controls. Pituitary sections mounted onto poly-L-lysine-coated slides were rinsed in 2 × standard saline citrate (2 × SSC; 0·15 M NaCl, 0·015 M sodium citrate, pH 7·0) for 10 min at room temperature and treated for 15 min with 0·1% Triton (BDH Inc., Toronto, Ontario, Canada) at room temperature. Thereafter, the pituitary sections were rinsed in 2 × SSC and prehybridized at room temperature in a humid chamber for 2 h in 450 μl/slide of prehybridization buffer containing 50% formamide, 5 × SSPE (1 × SSPE being 0·1 M NaCl, 10 mM NaH_2PO_4, pH 7·4, 1 mM EDTA), 5 × Denhart's buffer, 200 mg/ml denatured salmon testis DNA (Sigma Chemical Co., St Louis, MO, USA), 200 μg/ml yeast tRNA, 2 μg/ml poly A (Boehringer-Mannheim Canada, Dorval, Quebec, Canada) and 4% dextran sulfate. After prehybridization treatment, 100 μl hybridization mixture (prehybridization buffer containing in addition 10 mM dithiothreitol and ^{35}S-oligoprobe at a concentration of 10×10^6 c.p.m./ml) were spotted on each slide, sealed under a coverslip, and incubated at 37 °C overnight (15–20 h) in a humid chamber. After hybridization, coverslips were removed and slides rinsed in descending concentrations of SSC (2 ×, 1 × and 0·5 ×) for 30 min each at room temperature, washed in 0·5 × SSC for 1 h at 37 °C and finally for 30 min at room temperature in 0·5 × SSC. After the posthybridization treatments, the sections were dehydrated and exposed to Kodak X-Omat films (Eastman Kodak, Rochester, NY, USA) for 5 h for the IP lobe and overnight for the AP lobe, before being coated with liquid photographic emulsion (Kodak-NTB2; diluted 1:1 with water). Slides were exposed for 1 day, developed in Dektol developer (Kodak) for 2 min and fixed in rapid fixer (Kodak) for 4 min. Thereafter, tissues were rinsed in running water for 30 min, counterstained with thionin (0·25%) and rapidly dehydrated through graded concentrations of ethanol, cleared in toluene and coverslipped with Permount (Fisher Scientific Co. Ltd, Ottawa, Ontario, Canada).

Quantitative analysis

Semiquantitative analysis of hybridization signals for POMC mRNA was carried out on autoradiographic films differentially exposed to show differences between IP and AP lobes, using a Sony CCD XC-77 video camera with high resolution (570(H) × 485(V) TV lines) coupled to a Macintosh computer (Power PC 7500/100) and Image Software (version 1·60 non-FPU, W Rasband, NIH, Bethesda, MA, USA). The optical density (OD) of the hybridization signal was measured under brightfield illumination. The images of the pituitaries were digitized and subjected to densitometric analysis, yielding measurements of integrated OD (area of different lobes × average OD).

Statistical analysis

Quantitative data are presented as mean ± S.E.M. The mean and S.E.M. were calculated from six to eight measurements per animals performed in serial sections of respective pituitaries. Comparison of the mRNA levels between treatment groups was performed by an ANOVA (Statview 4·5 ANOVA) (Rivest & Laflamme 1995, Givalois *et al.* 1997); $P<0·05$ was considered as statistically significant.

Bioethics

Each rat was used for experimentation only once, and all protocols were approved by the Laval University's Animal Welfare Committee.

Results

As shown by light microscope darkfield micrographs in Fig. 1, a very intense labeling obtained after *in situ* hybridization with the ^{35}S-labeled POMC oligoprobe was observed in the IP lobe, while a much weaker hybridization signal corresponding to the labeling of the corticotropic cells occurred in the AP. The posterior pituitary (PP) lobe remained completely unlabeled. Pretreatment with RNAse A, as negative control, completely prevented

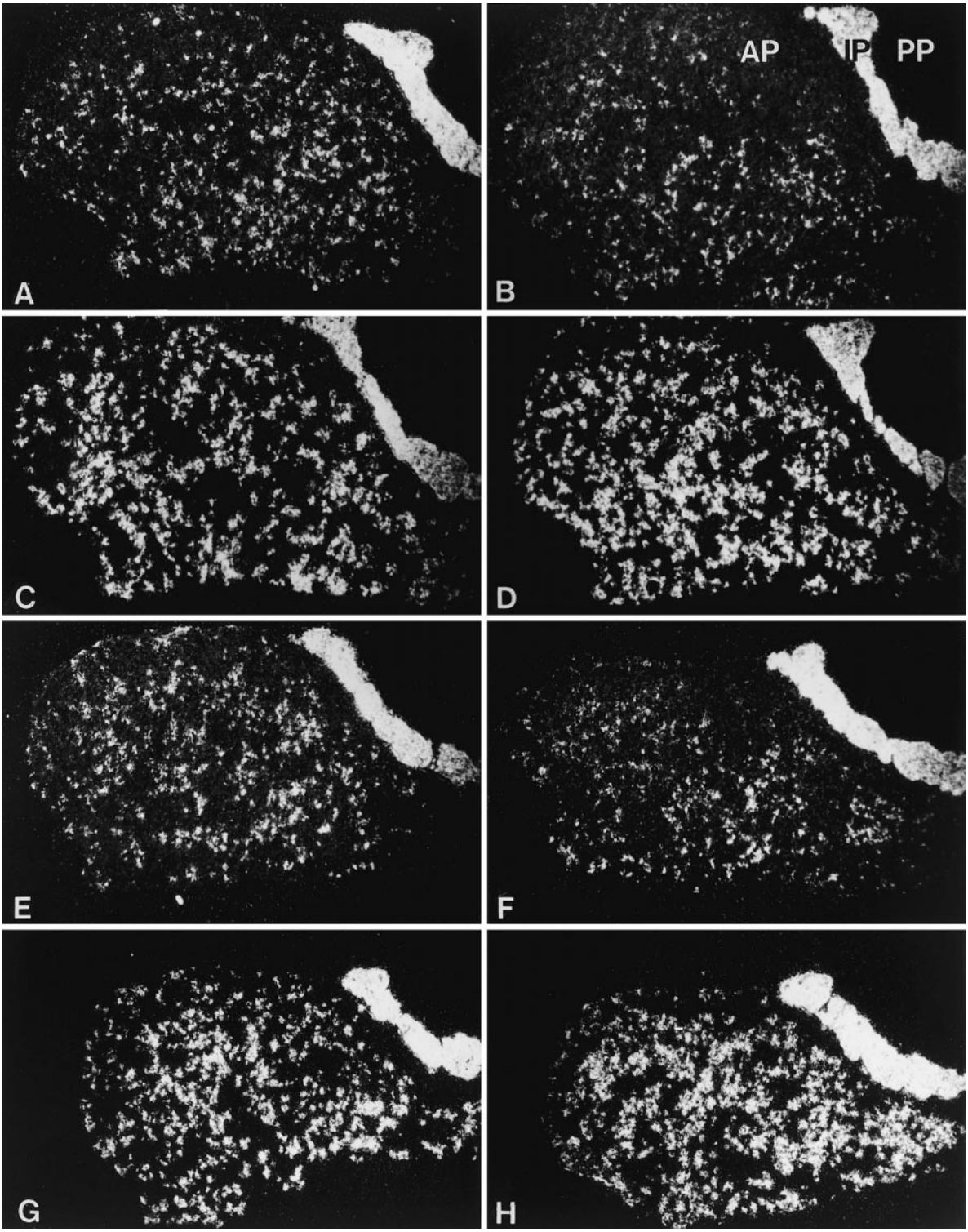

Figure 1 Representative examples illustrating POMC mRNA expression in the IP and AP lobes, 4 h after i.c.v. administration of vehicle (control rats; left panels) or ODN (right panels), in sham-operated (panels A and B), ADX (C and D), CX (E and F) and ADX/CX (G and H) rats. These depict darkfield photomicrographs of dipped autoradiographs of hybridized 10 µm pituitary sections with the ^{35}S-labeled specific oligoprobe to detect POMC mRNA. Note the absence of hybridization signal in the PP lobe. (Magnification × 40.)

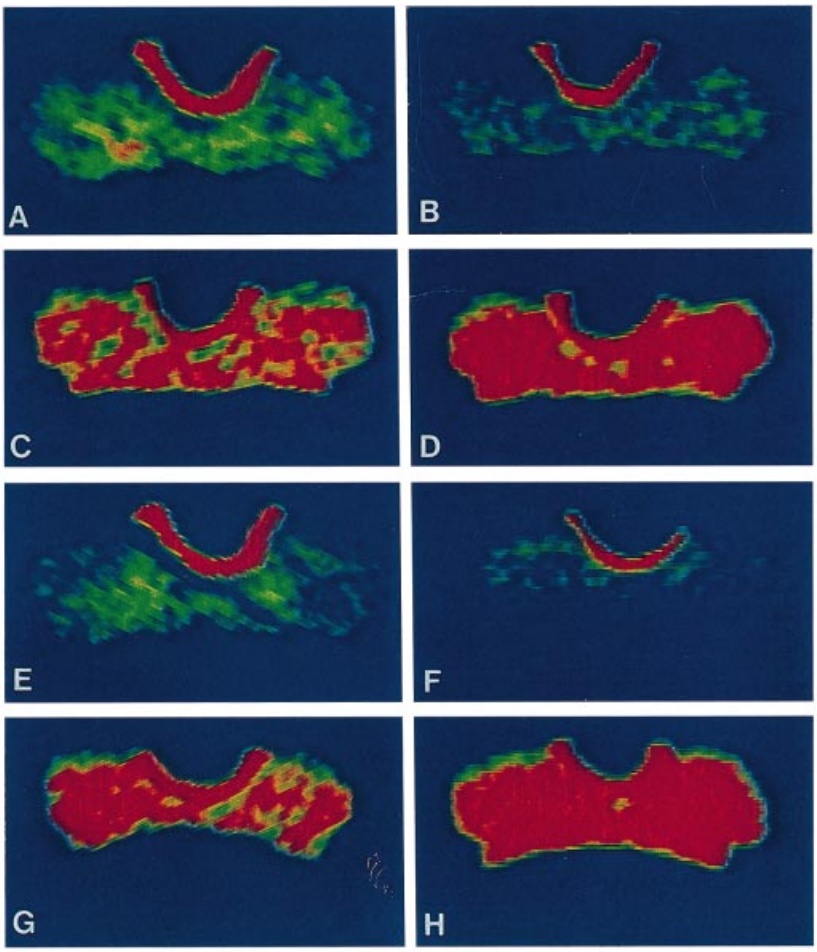

Figure 2 Digitized autoradiographs of representative POMC mRNA expression in sham-operated (panels A and B), ADX (C and D), CX (E and F) and ADX/CX (G and H) rats, 4 h after i.c.v. injection of vehicle (left panels) or ODN (4 µg/kg; right panels) at the AP level. Autoradiographic film was exposed overnight to detect POMC variations at the AP level. The intensity of the *in situ* hybridization signal is represented by rainbow colors with blue corresponding to low, green to medium, and red to high OD. (Magnification × 25.)

any labeling following hybridization with the labeled oligoprobe (data not shown).

AP lobe

To accurately analyze POMC mRNA levels in the AP and IP in the same sections, autoradiographic films were exposed overnight and for 5 h respectively. The respective involvement of adrenal and testis in the variations of POMC mRNA expression in response to ODN are presented in Figs 1–5. At the AP level (Figs 2 and 3), the i.c.v. injection of ODN in sham-operated rats decreased (− 17%; $P<0{\cdot}01$ vs control sham-operated) the *in situ* hybridization signal measured 4 h later (Fig. 2A and B). ADX significantly increased by 48% ($P<0{\cdot}01$ vs control sham-operated) the basal POMC mRNA levels (Fig. 2C). In these animals, the effect of ODN was reversed, since a 60% ($P<0{\cdot}01$) increase compared with control sham-operated and an 8% ($P<0{\cdot}01$) increase compared with control ADX rats were observed (Fig. 2D). In contrast to ADX, CX decreased basal POMC mRNA expression (− 15%; $P<0{\cdot}01$ vs control sham-operated) and did not modify the negative response to ODN which appeared very similar to that observed in sham-operated animals (Fig. 2E and F). Finally, ADX and CX induced a significant increase of basal POMC gene expression at the AP level (+53%; $P<0{\cdot}01$ vs control sham-operated), which was

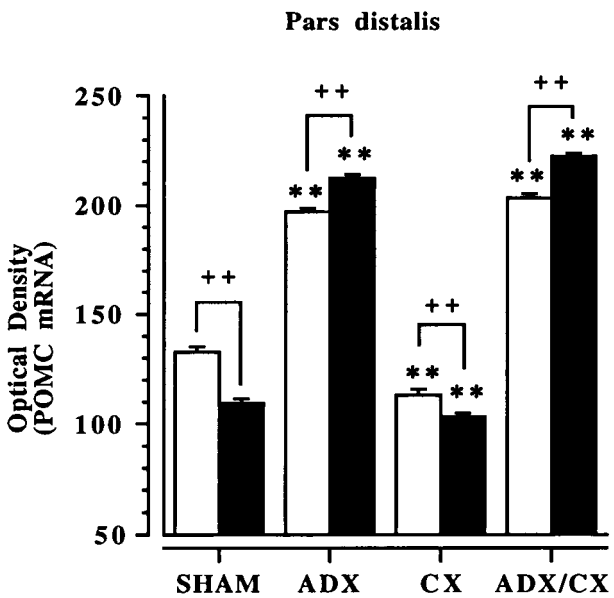

Figure 3 Effects of i.c.v. vehicle (open bars) or ODN (filled bars) injection on POMC mRNA expression in the AP cells of the sham-operated (SHAM), ADX, CX and ADX/CX rats. Autoradiographic film was exposed overnight at 4 °C. Results are expressed as the means ± S.E.M. with $n=5$ in each groups of rats. $^{**}P<0{\cdot}01$ vs control sham-operated rats and $^{++}P<0{\cdot}01$ vs control rats in each group.

more marked, but not significantly different from that observed after ADX alone (Fig. 2G). ODN injection into ADX/CX rats induced an increase of POMC mRNA levels in the AP (+68%; $P<0{\cdot}01$ vs control sham-operated, and +9%; $P<0{\cdot}01$ vs control ADX/CX), which was comparable to the increase observed in animals which were only ADX (Fig. 2D and H).

IP lobe

At the IP level (Figs 4 and 5), as already reported (Garcia-de-Yebenes & Pelletier 1994), the acute i.c.v. administration of ODN (4 h before killing) induced a significant decrease of POMC mRNA expression (− 7%; $P<0{\cdot}05$ vs control sham-operated) (Fig. 4A and B). ADX performed 5 days before the experiment produced a 10% ($P<0{\cdot}01$ vs control sham-operated) decrease in the hybridization signal (Fig. 4C). The injection of ODN to ADX animals not only did not produce a decrease in POMC mRNA levels, but rather resulted in a significant increase of 10% ($P<0{\cdot}01$) over the levels measured in control ADX animals (Fig. 4D). In contrast, CX that increased basal POMC mRNA expression (+6%; $P<0{\cdot}05$ vs control sham-operated) did not modify the response to ODN, which produced an inhibitory influence on POMC mRNA levels similar to that observed in sham-operated animals (the decrease being 13% compared with 7% for the sham-operated animals) (Fig. 4E and F). Finally, the combination of ADX and CX aimed at eliminating the production of steroid hormones resulted in a significant increase in the POMC mRNA hybridization signal (Fig. 4G) which was not different from the increase obtained after CX. In ADX/CX rats, ODN administration (Fig. 4H) was shown to induce an 11% decrease ($P<0{\cdot}01$ vs control ADX/CX) in the POMC mRNA level, which was intermediate between levels obtained after CX (Fig. 4D) and which was not different from that observed in control sham-operated rats (Fig. 4B).

Discussion

The negative modulation of POMC mRNA in both melanotropic and corticotropic cells by GABAA receptor complex ligands such BZD and barbiturates has already been demonstrated. The present results obtained in intact rats confirm these different studies (Loeffer *et al.* 1986, Jegou *et al.* 1991, Garcia-de-Yebenes & Pelletier 1994, Garcia-de-Yebenes *et al.* 1997). However, on the basis of results obtained on the behavior of rats, it has been suggested that ODN could act as an inverse agonist of central type BZD receptors or GABAA receptor complex (Ferrero *et al.* 1986). In the present study, in agreement with previous works (Li & Pelletier 1995, Garcia-de-Yebenes *et al.* 1997, Li *et al.* 1997*a*,*b*, Givalois *et al.* 1998*a*), we clearly demonstrate that ODN exerts an agonistic influence on GABAA receptors. This apparent discrepancy might be explained by the differences in the experimental conditions or in the doses used, as well as in the parameters which have been measured.

The present results did not provide precise information about the site(s) of action of ODN on POMC mRNA expression. DBI and DBI-related peptides have been shown to be produced by glial cells (Tonon *et al.* 1990, Tong *et al.* 1991, Malagon *et al.* 1993). Thus it might be suggested that DBI-related peptides, including ODN released from glial cells, can reach CRH neurons at the hypothalamic level in a paracrine way and directly interact with these neurons, which have been shown to contain GABAA receptors (Fenelon & Herbison 1995), to modulate POMC mRNA expression at the pituitary level. We have already shown that ODN negatively modulates CRH neuronal activity (Givalois *et al.* 1998*a*), but since GABAA receptors are present at the pituitary level (Louiset *et al.* 1992, Berman *et al.* 1994), a direct action of ODN, which could reach the pituitary via a diffusion of the peptide into the cerebrospinal fluid or via the short portal veins, should also be considered. On the other hand, the possibility that the effect of ODN on the IP might be mediated by modification of hypothalamic neuronal activity such as dopaminergic neurons which reach the melanotropic cells through the tuberohypophyseal dopaminergic tract, and which negatively control POMC mRNA expression (Hollt *et al.* 1982, Chen *et al.* 1983, Saland *et al.* 1988) cannot be disregarded.

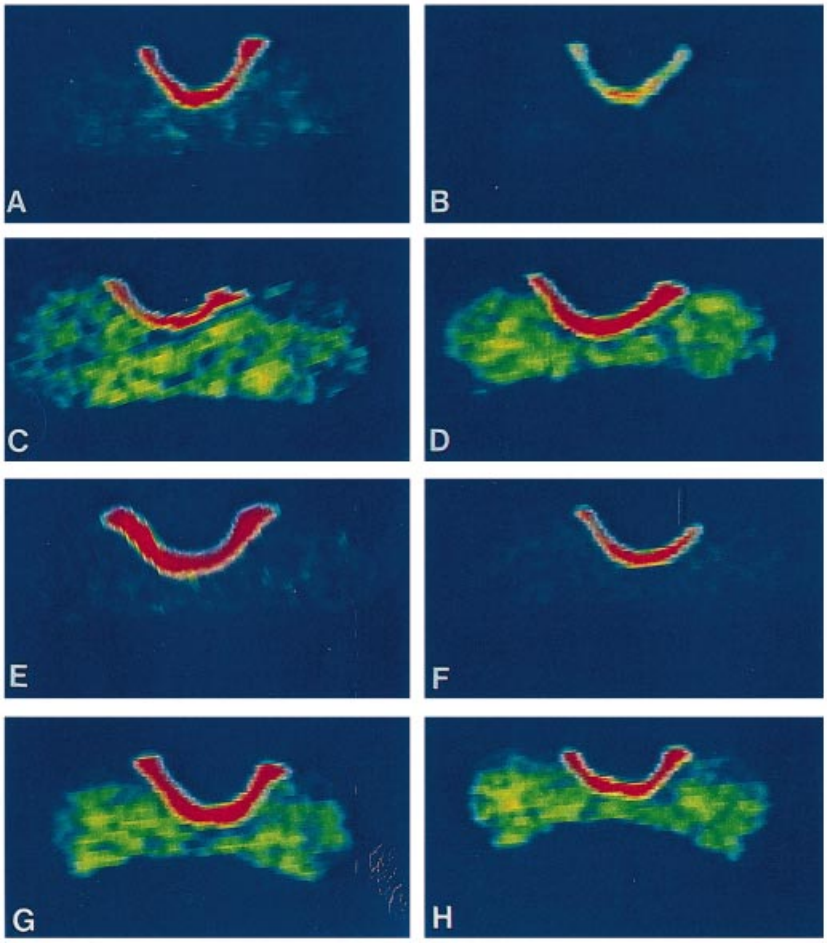

Figure 4 Digitized autoradiographs of representative POMC mRNA expression in sham-operated (panels A and B), ADX (C and D), CX (E and F) and ADX/CX (G and H) rats, 4 h after i.c.v. injection of vehicle (left panels) or ODN (4 µg/kg; right panels) at the IP level. Autoradiographic film was exposed overnight to detect POMC variations at the IP level. The intensity of the *in situ* hybridization signal is represented by rainbow colors with blue corresponding to low, green to medium, and red to high OD. (Magnification × 25.)

In order to study the role of circulating steroids and the respective involvement of the adrenals and testis in the effect of ODN on the POMC mRNA expression, we have evaluated the influence of ADX, CX and the combination of both treatments on the ODN-induced response of POMC mRNA expression at the IP and AP levels. We demonstrated that the direct and/or indirect effect induced by the endogenous BZD on the POMC mRNA expression was influenced by adrenal and gonadal factors at the AP and IP levels respectively.

ADX, which increases the HPA axis activity at the central level (Sawchenko 1987, Spinedi *et al.* 1991, Givalois *et al.* 1998*a*), reversed the effects of ODN on POMC mRNA expression, since the administration of ODN to ADX animals slightly increased mRNA levels instead of inducing the expected decrease in the hybridization signal observed at the AP and IP levels in control sham-operated rats. In fact, in ADX animals, which are deprived of adrenal hormones, we find, as previously reported with CRH mRNA expression (Givalois *et al.* 1998*a*), that ODN is acting as an inverse agonist on POMC mRNA at the AP and also at the IP lobe. By contrast, CX, which decreased at the AP level and increased at the IP level the basal POMC mRNA expression, did not modify the negative influence of ODN on the POMC mRNA hybridization signal. In fact, this endozepine amplified the response to ODN in the IP. Moreover, in accordance with our results on CRH mRNA expression (Givalois *et al.* 1998*a*), the involvement of the adrenal in the action of ODN seems to be predominant at the AP level on corticotropic cells, since in ADX/CX animals ODN induced a significant increase in

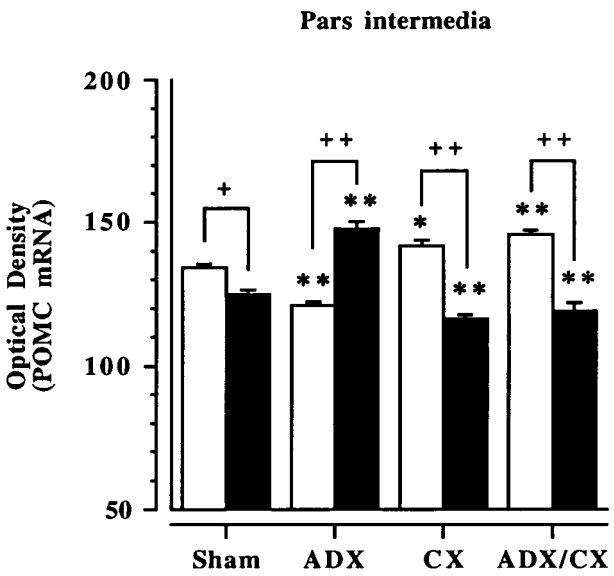

Figure 5 Effects of i.c.v. vehicle (open bars) or ODN (filled bars) injection on POMC mRNA expression in the IP lobe of sham-operated (Sham), ADX, CX and ADX/CX rats. Autoradiographic film was exposed for 5 h at 4 °C. Results are expressed as the means ± S.E.M. with n=5 in each groups of rats. *P<0·05 and **P<0·01 vs control sham-operated rats; +P<0·05 and ++P<0·01 vs control rats in each group.

POMC mRNA levels. At the IP level, the involvement of the testis in the ODN POMC mRNA modulation is predominant in ADX/CX rats, since ODN induced a decrease of the hybridization signal very similar to that observed in animals which were only CX.

GABAA receptors, which are oligomeric structures composed of multiple subunits that form chloride channels (MacDonald & Olsen 1994), present some regional and functional heterogeneity (Sapp *et al.* 1992) that depend on the subunit composition of the receptor complex (MacDonald & Angelotti 1993). Alterations in the gene expression of selective subunits seem to lead to changes in the density of GABAA receptor protein and the receptor subunit composition (Orchinik *et al.* 1994, 1995, Weiland & Orchinik 1995). These might alter receptor sensitivity to activation by GABA or modulators such as BZD and thereby the pharmacological properties of the receptors (Orchinik *et al.* 1994, 1995, Weiland & Orchinik 1995). Thus, several reports have shown some modifications in the GABAA receptor subunit composition after chronic stress or corticosterone exposure (Orchinik *et al.* 1995 and after progesterone injections (Weiland & Orchinik 1995). Moreover ADX, by suppressing circulating glucocorticoids and progesterone (which in male rats originate from the adrenal cortex), might induce changes in receptor subunit composition of the GABAA receptor complex (Orchinik *et al.* 1994) and might then modify the ODN influence on POMC mRNA expression, at the hypothalamic level by decreasing CRH mRNA expression (Givalois *et al.* 1998*a*) and/or directly at the pituitary level (Louiset *et al.* 1992, Berman *et al.* 1994). In the IP, testicular influence on POMC mRNA expression induced by ODN does not seem to involve the tuberoinfundibular and tuberohypophyseal dopamine neurons, since gonadectomy did not alter dopamine synthesis and dopamine metabolism at these levels (Manzanares *et al.* 1992). A direct influence on the GABAA receptor complex is easier to envisage.

In conclusion, our data indicate that modifications in POMC mRNA expression in both the IP and AP induced by the endogenous BZD receptor ligand ODN are influenced by peripheral steroids, with a predominant influence of adrenals at the AP level and of the testis at the IP level. The mechanism(s) of action of these steroids appears more complex than a simple allosteric modulation of GABAA receptor, since some variations in the subunit composition of this receptor complex that alter its pharmacological properties have been described following variations in steroid concentrations. Other studies, such as the determination of the subunit composition of GABAA receptor after ADX and/or CX and evaluation of the effects of glucocorticoids, progesterone and testosterone, are required to establish the mechanism(s) of action of adrenal and gonadal factors involved in the regulation of POMC mRNA expression by ODN at the AP and IP levels. Such data should eventually contribute to a better understanding of the role of endozepines in the increased activity of the HPA axis observed in depressed patients (Roy *et al.* 1989).

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