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Increased 5-HT_{2A} receptor expression and function following central glucocorticoid receptor knockdown in vivo

Aminul Islam*, Kevin S.J. Thompson[1], Saghir Akhtar[2], Sheila L. Handley

LHS, Aston University, Aston Triangle, Birmingham B4 7ET, UK; Knoll Pharmaceuticals, Pennyfoot Street, Nottingham NG1 1GF, UK

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Abstract

Central glucocorticoid receptor function may be reduced in depression. In vivo modelling of glucocorticoid receptor underfunctionality would assist in understanding its role in depressive illness. The role of glucocorticoid receptors in modulating 5-HT_{2A} receptor expression and function in the central nervous system (CNS) is presently unclear, but 5-HT_{2A} receptor function also appears altered in depression. With the aid of RNAse H accessibility mapping, we have developed a 21-mer antisense oligodeoxynucleotide (5′-TAAAAACAGGCTTCT-GATCCT-3′, termed GRAS-5) that showed 56% reduction in glucocorticoid receptor mRNA and 80% down-regulation in glucocorticoid receptor protein in rat C6 glioma cells. Sustained delivery to rat cerebral ventricles in slow release biodegradable polymer microspheres produced a marked decrease in glucocorticoid receptor mRNA and protein in hypothalamus (by 39% and 80%, respectively) and frontal cortex (by 26% and 67%, respectively) 5 days after a single injection, with parallel significant up-regulation of 5-HT_{2A} receptor mRNA expression (13%) and binding (21%) in frontal cortex. 5-HT_{2A} receptor function, determined by DOI-head-shakes, showed a 55% increase. These findings suggest that central 5-HT_{2A} receptors are, directly or indirectly, under tonic inhibitory control by glucocorticoid receptor.
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1. Introduction

Hypothalamo-pituitary-adrenal axis dysfunction may play a key role in the pathogenesis of depression (Pariante and Miller, 2001). There is extensive evidence that hypothalamo-pituitary-adrenal axis activity is increased in depression and subsensitive to feedback inhibition, which may be consequent on glucocorticoid receptor underfunctionality (Pariante and Miller, 2001; Holsboer, 2000). The glucocorticoid receptor is widely distributed in the brain and plays a central role in negative feedback regulation of circulating glucocorticoids, particularly their elevation in response to stress (Reul and de Kloet, 1985; Dallman et al., 1987). Thus, in vivo modelling of central glucocorticoid receptor underfunctionality may have considerable heuristic value in understanding its role in depressive illness. Antisense oligodeoxynucleotide (AS-ODN) knockdown would be advantageous in this respect for its ability to produce a selective, temporally regulated reduction in glucocorticoid receptor function in the rat brain. However, selecting effective antisense sequences and ensuring adequate sustained delivery to the central nervous system (CNS) represent a considerable challenge (Hughes et al., 2001; Stone and Vulchanova, 2003) and previous attempts have produced only 15–24% reduction in glucocorticoid receptor protein (Korte et al., 1996; Sakai et al., 1996; Engelmann et al., 1998). In the present work, we have used RNAse H accessibility mapping as a design aid (Ho et al., 1998), to produce an effective AS-ODN termed glucocorticoid receptor antisense sequence 5 (GRAS-5), and its appropriate control sequences. In order to achieve sustained

* Corresponding author. National Institutes of Health, Bethesda, MD 20892-1590, USA. Tel.: +1 301 402 1427; fax: +1 301 435 2883.
E-mail address: islama@nhlbi.nih.gov (A. Islam).
[1] Present address: Akubio Ltd, Cambridge Science Park, Cambridge CB4 0GJ, UK.
[2] Present address: Centre for Genome-based Therapeutics, Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3XF, UK.

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CNS delivery and circumvent the risk of stress-induced changes in glucocorticoid production and/or glucocorticoid receptor density, we administered the chosen sequences to rats as a single sustained-release injection into the cerebral ventricles by pre-loading them into biodegradable polymer microspheres (Lewis et al., 1998).

There is extensive evidence for the involvement of serotonin in the pathophysiology and treatment of depression (Stockmeier, 2003). It has been suggested that altered serotonergic transmission could be a consequence of glucocorticoid receptor dysfunction (Farisse et al., 2000). Platelet studies have consistently demonstrated increased 5-HT_{2A} receptor density in depressed patients (Alda and Hrdina, 2000; Mendelson, 2000; Serres et al., 2000). Post-mortem and imaging studies have resulted in divergent findings concerning 5-HT_{2A} receptor abundance in brain regions in suicide and/or depression, but there is intriguing evidence that increases in central 5-HT_{2A} receptor binding may be identifying suicide victims who had major depression with high levels of pessimism and hopelessness (Stockmeier, 2003). Glucocorticoids, via their interactions with glucocorticoid receptors, modulate expression of many genes but the role of glucocorticoid receptors in modulating 5-HT_{2A} receptor expression and function in the CNS is presently unclear (Chaouloff, 2000). In vitro studies (Garlow and Ciaranello, 1995) indicate that glucocorticoid receptors modulates transcription of the rat 5-HT_{2A} receptor gene but the direction of this effect was cell-type dependent: in CCDL-39 and RS-1 cells, transcription was inhibited, while it was stimulated in Neuro-2A cells (Garlow and Ciaranello, 1995). We have therefore examined the effects of GRAS-5 on expression of glucocorticoid receptors in hypothalamus and frontal cortex and on 5-HT_{2A} receptor expression in frontal cortex. 5-HT_{2A} receptor function was determined from the effects of GRAS-5 on the frequency of 5-HT_{2A} receptor-mediated head-shakes produced by systemic administration of 1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane hydrochloride (DOI) (Lucki et al., 1984; Glennon et al., 1986).

2. Materials and methods

2.1. ODN synthesis

Phosphorothioate ODNs were synthesised on an ABI 392 automated DNA synthesiser (Applied BioSystems, UK) using phosphoramidite chemistry with tetraethyl thuriam disulphide as the sulphurising reagent and desalted by Sephadex G25 gel-filtration (Pharmacia Biotech, UK).

2.2. RNAse H accessibility mapping

A 509 base pair fragment of rat glucocorticoid receptor cDNA, with T7 promoter site attached to the 5′-end, was produced by reverse transcriptase polymerase chain reaction (RT-PCR) from C6 glioma cell total RNA by incorporating a T7-glucocorticoid receptor forward primer (50 μg/ml) in the PCR step as a template for transcription of the corresponding glucocorticoid receptor RNA fragment by T7 RNA polymerase using a SP6/T7 transcription kit (Boehringer Mannheim, UK). The resulting 515 nucleotide T7-glucocorticoid receptor RNA transcript, [^{32}P] radiolabelled at the 5′-end, was purified using the RNeasy Isolation Kit (Qiagen, Germany). Accessible sites (single-stranded regions) for ODN hybridisation to the glucocorticoid receptor mRNA fragment were identified by an RNAse H mediated cleavage reaction (RNAse H (25 units/μl), dithiothreitol (10 mM), random 12-mer phosphorothioate ODN library (500 nmol), 5′-end [^{32}P] radiolabelled glucocorticoid receptor RNA Transcript (0.1 μmol) and RNAse inhibitor (20 units/μl, reaction buffer to 10 μl), mixed and incubated at 37 °C for 30 min) and the resulting mixture assayed using 6% denaturing (7 M Urea) polyacrylamide gel electrophoresis (PAGE) separation at 25 W for 2.5 h and autoradiography.

2.3. In vitro incubation of ODNs

C6 glioma cells expressing glucocorticoid receptors were cultured in Dulbecco's modified Eagle's medium with 10% v/v dialysed foetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine (Gibco-BRL, UK) at 37 °C. Cells (1×10^4 cells/ml) were seeded in 24-well plates and after 12 h were incubated with ODN–lipofectamine complex at 1:1 charge ratio in serum-free medium as previously described (Islam et al., 2000). After 4 h, the original medium was replaced for 20 h and the cycle repeated a further four times over a total treatment period of 5 days.

2.4. Microsphere preparation

An aqueous ODN solution (5 mg in 100 μl 0.4%w/v polyvinyl alcohol (PVA)) was added to 50 mg polylactide-co-glycolide 50:50 copolymer in 5 ml dichloromethane, stirred at 4000 rpm for 5 min, mixed with 160 ml aqueous external phase (saline 0.9%w/v, PVA 4%w/v) and again stirred at 6000 rpm for 5 min. Microspheres (5–15 μm diameter) were formed by solvent evaporation, collected by centrifugation, washed in double distilled water (×3) to remove nonencapsulated ODN and freeze dried (Lewis et al., 1998).

2.5. In vivo experiments

Male Wistar rats (270–310 g, eight animals per group) received one of: sham injection (SH), saline- (V), antisense GRAS-5 (AS)- or mismatched oligonucleotide (MM)-loaded polymer microspheres in left lateral ventricle (3.5 mm below dura, 0.9 mm posterior and 1.4 mm lateral to bregma; Paxinos and Watson, 1986) under nitrous oxide/isoflurane anaesthesia. ODN-loaded microspheres were administered

as 400 μg of microspheres in 2 μl sterile saline. Post-injection, rats were housed in pairs on a 12 h light/dark cycle (lights on at 07:00 h, 21±1 °C; 55% humidity) with free access to food and water, killed on the 5th day and hypothalamus and frontal cortex snap-frozen at −80 °C for analysis. In a separate experiment, head-shakes induced by the 5-HT_{2A} receptor agonist 1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane hydrochloride (DOI) were measured as follows: on the 5th day, (11:00 h) rats received DOI 0.6 mg/kg i.p. in 0.5 ml 0.9% saline, placed singly in an observation chamber (50×50×75 cm) and the number of head-shakes occurring between 10 and 20 min after injection counted from videotape by an observer blind to treatment group. Well-being and body weight determinations were conducted daily. All work was carried out in conformity with the Animals (Scientific Procedures) Act, 1986 of the United Kingdom and the Declaration of Helsinki.

2.6. Assays

Glucocorticoid receptor radioligand binding was performed as described by O'Donnell et al. (1995) using 10 nM [^{3}H] dexamethasone, with 2 μM mifepristone to define nonspecific binding. 5-HT_{2A} receptor density was measured as described by Kettle et al. (1999), with 0.5–50 nM [^{3}H] ketanserin, and 0.5 μM methysergide for nonspecific binding. For quantitative RT-PCR, total RNA samples (20 μl of 25 ng/μl) were incubated in a thermal cycler with Moloney murine leukemia virus (MMLV) reverse transcriptase (200 U/μl), 10× thermophilic buffer (10 mM), $MgCl_2$ (25 mM), RNAse inhibitor (40 U/μl), 10 mM of each deoxynucleotide triphosphate and oligo(dT) random hexamer primer (0.5 μg/μl) for 1 h at 42 °C and 3 min at 94 °C. The resulting samples in a total reaction volume of 120 μl were incubated in a thermal cycler with amplitaq DNA polymerase (5 U/μl), 10× thermophilic buffer (10 mM), $MgCl_2$ (25 mM), glucocorticoid receptor or 5-HT_{2A} receptor amplification primers (50 μg/ml) and control β-actin primers (10 μg/ml) using the following PCR cycles (30×): denaturation at 94 °C for 45 s, primer annealing at 60 °C for 45 s, primer extension at 72 °C for 1.5 min and a final extension step of 10 min at 72 °C. Resulting samples were electrophoresed on a 2.5% agarose gel and stained with ethidium bromide. Quantification of the glucocorticoid receptor or 5-HT_{2A} receptor PCR product bands was normalised to the control β-actin bands using densitometry (NIH image analysis, Scion, USA). Western blot analysis subjected homogenised tissue samples (25 μl, equivalent to 50 μg protein) to denaturing and reducing gel electrophoresis on BioRad 7.5% Tris–HCl (10 well/30 μl) precast Ready Gel cassettes using a BioRad Ready Gel Cell Module at 200 V for 40 min in BioRad Tris glycine/SDS buffer (25 mM Tris, 192 mM glycine and 0.1% SDS) followed by electrophoretic blotting (Towbin et al., 1979) onto BioRad nitrocellulose membrane (0.2 μm). Glucocorticoid receptor protein was detected using a primary mouse monoclonal anti-glucocorticoid receptor IgG_2, (Affinity Bioreagents, USA) diluted 1:1000 and a horseradish peroxidase-conjugated Donkey anti-mouse IgG secondary antibody (Amersham Life Science) diluted 1:1000. Selected blots were reprobed using a β-actin monoclonal antibody diluted 1:500. Quantification of the glucocorticoid receptor protein bands was normalised to the control β-actin bands using densitometry (NIH image analysis, Scion).

2.7. Statistical analyses

Data were analysed by analysis of variance with Duncan's test *post hoc* (SPSS version 11).

2.8. Materials

Radioligands were obtained from Amersham Life Sciences. For in vivo studies, the selected phosphorothioate ODNs were custom-synthesised and purified by Gibco/Life Technologies, UK. All other chemicals were obtained from Sigma (Poole, UK).

3. Results

RNAse H accessibility mapping indicated four accessible sites in the glucocorticoid receptor transcript, the band corresponding to accessible site '4' being the most intense (Fig. 1A, lanes b–d), producing a 5′ fragment of approximately 265±10 nucleotides that corresponds to cleavage of the full-length rat glucocorticoid receptor mRNA at nucleotide 1170±10. A panel of ten 21-mer antisense ODNs spanning 30 nucleotides upstream to 40 nucleotides downstream of the approximate cleavage site (Fig. 1B), screened against 5′-radiolabelled glucocorticoid receptor RNA transcript in vitro, confirmed site '4' as the main cleavage site (Fig. 1C). Densitometric analysis of the cleavage band intensities (data not shown) showed GRAS-5 (5′-TAAAAACAGGCTTCTGATCCT-3′) to be the most effective. Blast searches of GRAS-5, the complementary sense strand 5′-ATTTTTGTCCGAAGACTAGGA-3′ (SS), a mismatched sequence 5′-CGGAAAAGTGCTCACGATAAC-3′ (MM, mismatched nucleotides underlined), and a nonaccessible sequence, 5′-TGGCACCTATTCCAGTTTTCA-3′ (NA, an antisense sequence complementary to a nonaccessible site predicted from the RNAse H accessibility mapping, spanning nucleotides 1211-1231 in the RNA transcript) against the GenBank sequence database produced no significant matches except rat glucocorticoid receptor. Only GRAS-5 produced detectable cleavage product (Fig. 1D) confirming that RNAse H mediated cleavage was dependent upon both complementary sequence specificity and access to single-stranded regions of the RNA transcript.

When lipofectamine-complexed ODNs were incubated with C6 cells, GRAS-5 produced a concentration-dependent

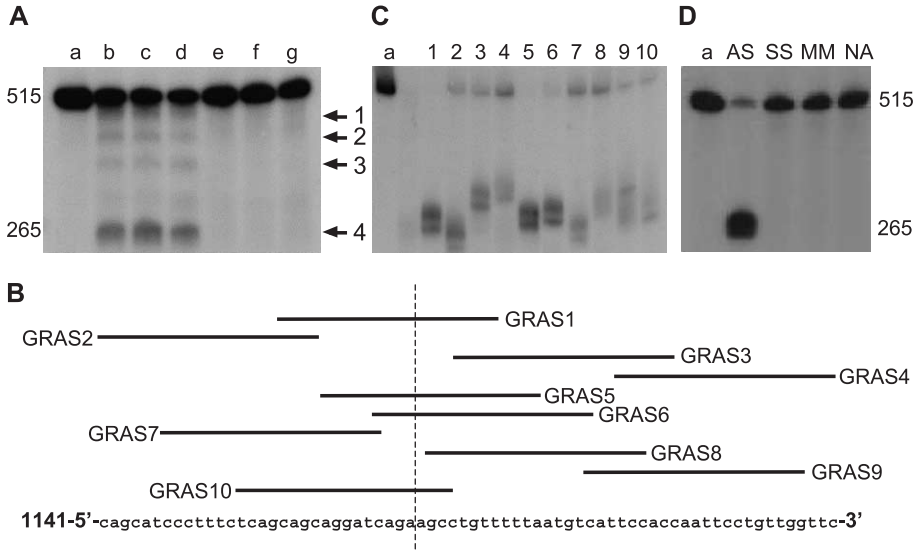

Fig. 1. RNAse H accessibility mapping: (A) autoradiogram of RNA fragment sizes produced by RNAse H mediated cleavage at four accessible sites of a T7-RNA polymerase-generated portion of the rat glucocorticoid receptor. Lane a: 5′-radiolabelled 515 bp glucocorticoid receptor mRNA transcript; b–d: transcript with random ODN library at concentrations of 0.5 nM (b), 1 nM (c) and 2 nM (d) with RNAse H enzyme; e: transcript with 0.5 nM random ODN library only; f: transcript with RNAse H enzyme only; g: transcript with reaction buffer only. (B) Schematic representation of 21-mer antisense ODN sequences spanning the predicted cleavage point 4 (dotted line). (C) Autoradiogram of RNA fragment band densities for cleavage of the rat glucocorticoid receptor RNA fragment (lane a) by the GRAS sequences shown in (B) (lanes 1–10). (D) Autoradiogram of glucocorticoid receptor RNA fragment (a) showing efficacy of antisense GRAS-5 (AS), sense strand (SS), mismatch (MM), and nonaccessible site (NA) control sequences to mediate RNAse H cleavage.

reduction in glucocorticoid receptor mRNA expression ($F_{(6,20)}$=323, p<0.001), resulting in a significant 56% decrease in glucocorticoid receptor mRNA expression at 1 μM compared with a nonsignificant 9–15% decrease in expression obtained with the MM, SS and NA sequences at the same concentration (Fig. 2A, B). [^{3}H] dexamethasone binding to glucocorticoid receptor was also concentration-dependently reduced following GRAS-5 treatment ($F_{(6,20)}$=36.53, p<0.001), resulting in a significant 80% decrease in dexamethasone binding by glucocorticoid receptor at 1 μM, compared to the nonsignificant 4%, 12% and 24% decreases for MM, SS and NA sequences, respectively (Fig. 2C).

Four groups of eight rats received either sham injection (SH), saline-loaded polymer microspheres as vehicle control (V), GRAS-5 (AS) loaded polymer microspheres or mismatch ODN (MM) loaded polymer microspheres. No changes in well-being were seen over 5 days. At the end of this time, GRAS-5 induced significant knockdown of glucocorticoid receptor mRNA and protein in both hypothalamus and frontal cortex. In the hypothalamus (Fig. 3), GRAS-5 reduced glucocorticoid receptor mRNA by 39% ($F_{3,28}$=69.36, p<0.05), and glucocorticoid receptor protein by 80% ($F_{3,28}$=913.08, p<0.05) measured by Western blot or by 75% ($F_{3,28}$=97.55, p<0.05) measured by [^{3}H] dexamethasone binding. In frontal cortex (Fig. 4), glucocorticoid receptor mRNA was reduced by 26% ($F_{3,28}$=28.15, p<0.05) and [^{3}H] dexamethasone binding by 67% ($F_{3,28}$=97.55, p<0.05). The presence of saline- or mismatch-loaded polymer microspheres had no significant effect on protein expression (measured by dexamethasone binding) in either area (Figs. 3 and 4). Minor but statistically significant (p<0.05) changes in mRNA in these control groups were not consistent between cortex and hypothalamus: the group receiving vehicle-loaded microspheres exhibited an apparent 11% increase in expression in frontal cortex only, while mismatch-loaded microspheres resulted in a 14% increase in frontal cortex and a 9% decrease in hypothalamus. Weight-gain ($F_{3,28}$=0.13, p>0.05) and adrenal weights ($F_{3,28}$=0.07, p>0.05) showed no significant differences between groups.

In the same animals (Fig. 4), GRAS-5 increased 5-HT$_{2A}$ receptor mRNA in frontal cortex by 13% ($F_{3,28}$=28.15, p<0.05). 5-HT$_{2A}$ receptor density, as measured by radioligand binding ($F_{3,28}$=14.39, p<0.05), was increased by 21% while K_D was unchanged. There were no other significant between-group differences. A strong correlation was observed between glucocorticoid receptor and 5-HT$_{2A}$ receptor expression, for both mRNA (r=−0.563, p<0.001) and ligand binding (r=−0.755, p<0.0001).

In a separate experiment, groups of eight rats were treated as above with SH, V, AS or MM, and DOI-induced head-shakes observed on the 5th day. GRAS-5 ($F_{3,28}$=4.07, p<0.05) increased head-shakes by 55% (Fig. 5) whereas no other treatment produced statistically significant differences.

4. Discussion

RNAse H accessibility mapping identified accessible regions on the glucocorticoid receptor RNA transcript which, combined with in vitro selection, resulted in a

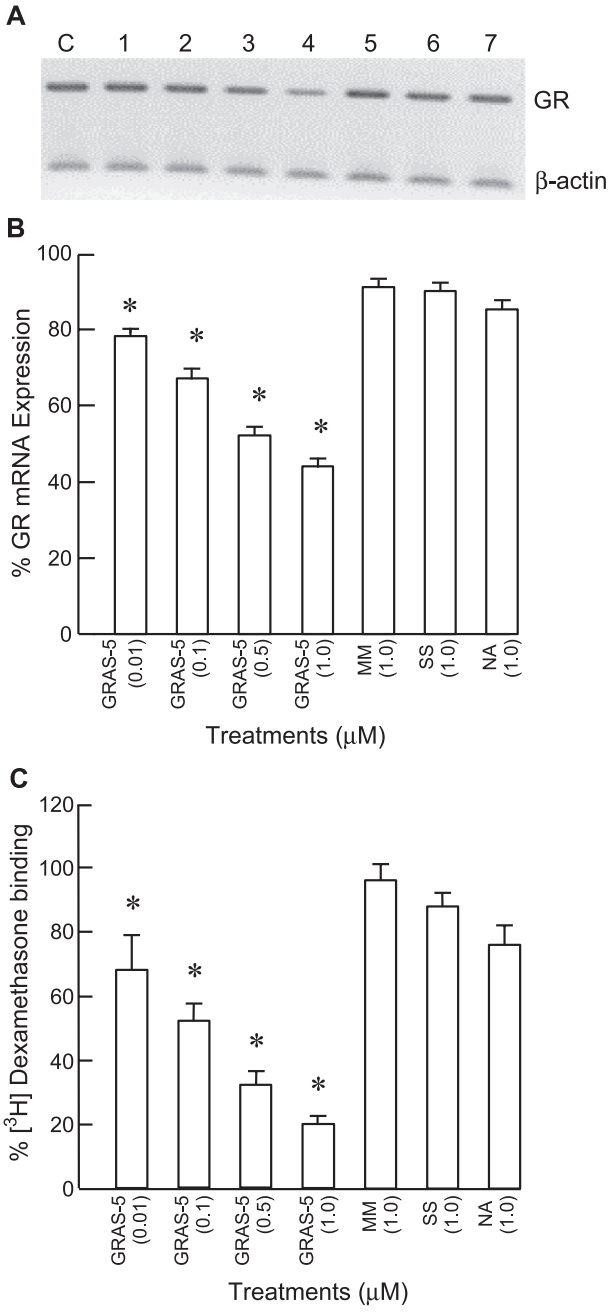

Fig. 2. Effect of GRAS-5 on glucocorticoid receptor (GR) mRNA and protein expression in rat C6 glioma cells following 5-day exposure in vitro. (A) Illustrative gel image of a quantitative RT-PCR analysis of glucocorticoid receptor mRNA expression. Lane c is the untreated control; remaining lanes are as denoted for the corresponding columns in part B immediately below. (B) Densitometric analysis of glucocorticoid receptor mRNA expression (mean±S.E.M., n=3) for increasing concentrations of GRAS-5 and control mismatch (MM), sense (SS), and nonaccessible site (NA) sequences, expressed as a percentage of the untreated controls. (C) [^{3}H] dexamethasone radioligand binding (mean±S.E.M. n=3) for increasing concentrations of GRAS-5 and control mismatch (MM), sense (SS), and nonaccessible site (NA) sequences, expressed as a percentage of the untreated controls. *Significantly different from controls, p<0.01.

glucocorticoid receptor antisense design (GRAS-5) with high efficacy. GRAS-5 was nontoxic and its effects were sequence specific, since mismatch and sense sequences, as well as a nonaccessible site sequence, produced negligible changes in glucocorticoid receptor expression in vitro and/or in vivo, indicating a mechanism typical of antisense ODN technology (Weiss et al., 1997; Petch et al., 2003). We did not attempt to investigate the selectivity of GRAS-5 to down-regulate glucocorticoid receptor expression versus that of another gene, because it was not possible to identify a control gene that is unequivocally resistant to modulation of expression by altered availability of glucocorticoid receptor. However, the up-regulation of 5-HT$_{2A}$ receptors indicates that GRAS-5 has no general inhibitory effect on gene expression.

The cell toxicity of sustained/repeated lipofectamine exposure (Akhtar et al., 2000), used to enhance cell

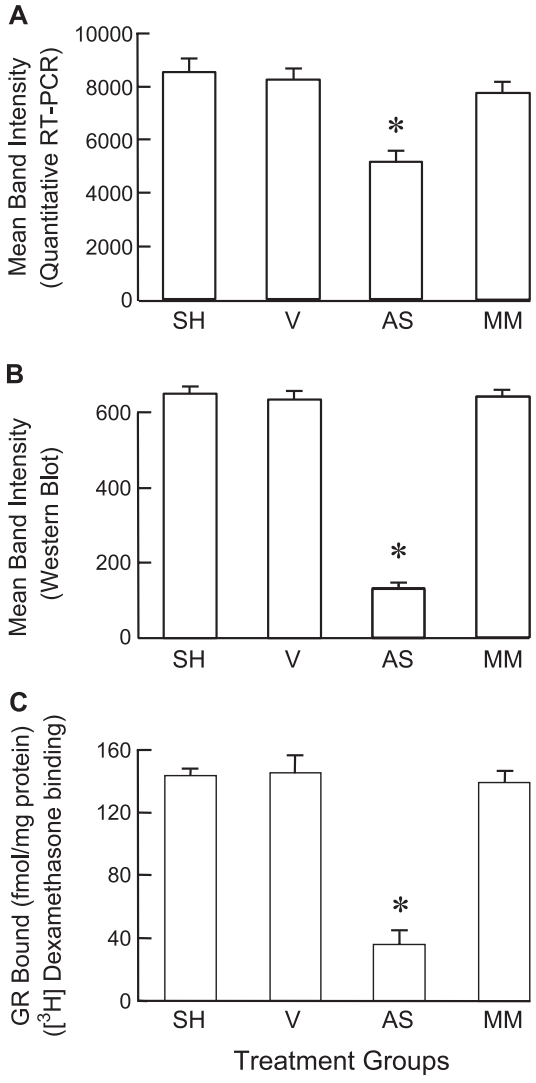

Fig. 3. Hypothalamic glucocorticoid receptor mRNA and protein expression 5 days after sham treatment (SH) or a single intraventricular injection of polymer microspheres containing: vehicle (V), antisense GRAS-5 (AS), or antisense mismatch (MM). (A) Densitometric analysis of quantitative RT-PCR band intensity normalised to β-actin. (B) Western blot analysis of glucocorticoid receptor (GR) protein expression band intensity. (C) [^{3}H] dexamethasone radioligand binding to glucocorticoid receptor. *AS group significantly different from all other groups, p<0.05.

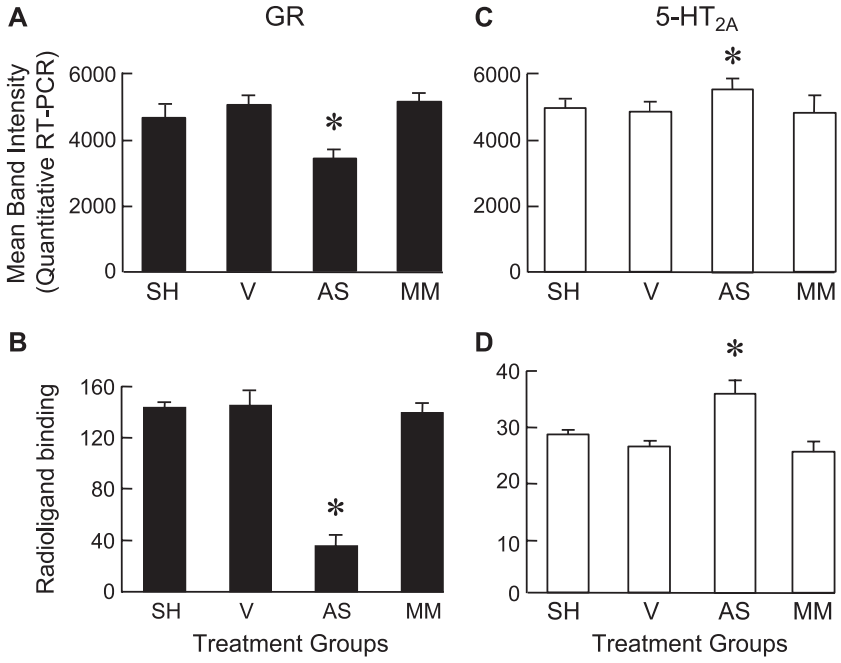

Fig. 4. Frontal cortex glucocorticoid receptor (GR) and 5-HT_{2A} mRNA and protein expression 5 days following sham treatment (SH) or a single intraventricular injection of polymer microspheres containing: vehicle (V), antisense GRAS-5 (AS), or antisense mismatch (MM) in the same animals as Fig. 3. (A, C) Densitometric analysis of Quantitative RT-PCR band intensity normalised to β-actin. (B, D) Radioligand binding measures of protein expression: glucocorticoid receptor: [^{3}H] dexamethasone binding (fmol/mg protein); 5-HT_{2A} B_{MAX} ([^{3}H] Ketanserin). *AS group significantly different from all other groups, $p<0.05$.

penetration in vitro, precluded its use in vivo. However, intracerebroventricular (i.c.v.) injection of biodegradable polymer microspheres proved a safe and successful means of delivering GRAS-5 to the brain. Vehicle-loaded microspheres did not result in any alteration in well-being or weight gain, measures of 5-hydroxytryptamine (5-HT) receptor expression and function, or glucocorticoid receptor protein measures. The apparent minor increase in glucocorticoid receptor mRNA in frontal cortex would require confirmation, since it did not appear in the hypothalamus. The efficacy of polymer delivery in vivo may be due at least partly to protection from nuclease digestion (Lewis et al., 1998; Szklarczyk and Kaczmarek, 1999) over the 5-day treatment period, whereas single i.c.v. injections of free ODN are degraded and cleared from the CNS within 24 h of administration (Cleek et al., 1997). However, unpublished studies from this laboratory indicate microspheres of the size and composition used here do not migrate from the cerebral ventricles, suggesting that they are acting purely as a depot for sustained release.

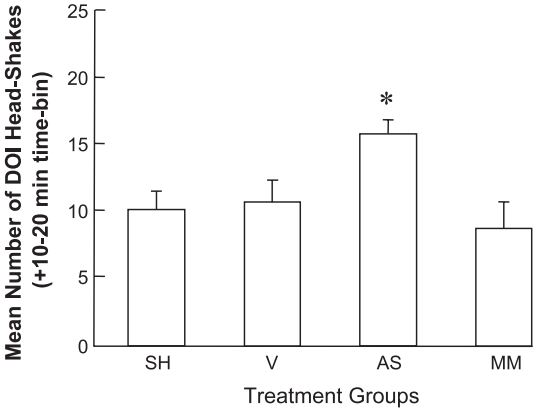

Fig. 5. DOI-induced head-shakes 5 days following sham treatment (SH) or a single intraventricular injection of polymer microspheres containing: vehicle (V), antisense GRAS-5 (AS), or antisense mismatch (MM). Head-shake frequency was measured for 10 min, between 10 and 20 min after DOI 0.6 mg/kg i.p. *AS group significantly different from all other groups, $p<0.05$.

GRAS-5 reduced glucocorticoid receptor expression extensively in both the brain areas examined, the hypothalamus and frontal cortex. This reduction was detected at the level of protein (up to 80%) and mRNA (up to 40%). Previous attempts to knockdown glucocorticoid receptor with antisense ODNs, using conventional selection and delivery techniques, have met with limited success, producing only 15–24% reduction in protein measures (Korte et al., 1996; Engelmann et al., 1998; Sakai et al., 1996). We evaluated the effectiveness of GRAS-5 at both levels to reduce the possibility of false-negatives (Stone and Vulchanova, 2003). The knockdown in glucocorticoid receptor induced by GRAS-5, together with the concomitant increase in 5-HT_{2A} receptors, was more intense at the protein than the mRNA level. The detection of a significant reduction in glucocorticoid receptor mRNA indicates that knockdown was probably occurring through an RNase-H-dependent mechanism, but the relationship between mRNA and protein

is often nonlinear (Griffin et al., 2002; Stone and Vulchanova, 2003) and the abundance of dynamically regulated functional proteins, such as receptors, is likely to be subject to complex post-transcriptional processes.

In the study of the roles and functions of glucocorticoid receptors, conventional antagonists have been regarded as insufficiently specific (Guo et al., 1995) to maintain selective antagonism during subchronic administration. Transgenic mice yield valuable information but the lifelong nature of the genetic change is a potential disadvantage: the glucocorticoid receptor antisense-expressing mouse (Pepin et al., 1992) has been studied extensively (e.g. Barden, 1996; Karanth et al., 1997; Farisse et al., 1999; 2000) and a glucocorticoid receptor-null mouse has been developed (Cole et al., 1995) in which 5-HT_{1A} receptor expression was shown to be unchanged (Meijer et al., 1997). It would be interesting to examine serotonergic function in transgenic mice with failure to express glucocorticoid receptors only in the CNS (Tronche et al., 1999). The antisense ODN, GRAS-5, directed against the rat glucocorticoid receptor sequence, represents a useful tool with which to study the consequences of reduced glucocorticoid receptor availability in a different species; it can be used post-developmentally and is potentially time-limited in its effects.

Here, we found no effect of GRAS-5 on adrenal weight, although the 5-day exposure period was likely to be too short for significant changes to occur. We also examined the effects of GRAS-5 on 5-HT_{2A} receptor expression and function. Glucocorticoid receptor knockdown was accompanied by a significant up-regulation of 5-HT_{2A} receptors in frontal cortex, an area where 5-HT_{2A} receptor density is high (Chaouloff, 1995). This was seen as an increase in both mRNA and B_{MAX} and was accompanied by a significant increase in 5-HT_{2A} receptor mediated (Lucki et al., 1984; Glennon et al., 1986) DOI head-shakes, indicating an increase in net 5-HT_{2A} receptor function. In addition, there was a strong inverse correlation between glucocorticoid receptor and 5-HT_{2A} mRNA abundance, and between glucocorticoid receptor and 5-HT_{2A} receptor ligand binding. Increased 5-HT_{2A} receptor binding has previously been observed in mice bearing a transgene coding for a glucocorticoid receptor antisense mRNA (Farisse et al., 2000); however, this increase was expressed only in the hippocampus, whereas it appeared to be more generalised in the present study. Surprisingly, 5-HT_{2A} receptor mRNA was unchanged in these mice compared to wild-type controls, except for a decrease in certain subregions of the hippocampus (Cyr et al., 2001).

The present findings suggest that glucocorticoid receptors have a repressive role with respect to 5-HT_{2A} receptors but provide no information whether this is a direct action of glucocorticoid receptor on the 5-HT_{2A} receptor gene. Effects of glucocorticoid receptor activation on 5-HT_{2A} receptor expression observed in cell lines are likely to be indirect and mediated through c-Fos and c-Jun (Garlow and Ciaranello, 1995). In vivo, indirect effects via altered secretion of corticotrophin releasing factor (CRF), adrenocorticotrophic hormone (ACTH) or corticosterone (Chaouloff, 1995; 2000) cannot yet be excluded. Farisse et al. (2000) speculated that the increase they observed in 5-HT_{2A} receptor expression could have been due to up-regulation consequent on decreased 5-HT turnover, which has been observed in these glucocorticoid receptor antisense-expressing mice (Farisse et al., 1999). The action of GRAS-5 on 5-HT turnover has not yet been measured but it is worth noting that 5-HT_{2A} receptor regulation does not follow a classical pattern and serotonergic denervation does not modify its density (for review see Eison and Mullins, 1996). Alternatively, a small reduction in 5-HT_{1A} receptor expression was also detected in the hippocampus of the glucocorticoid receptor-antisense expressing mice and it was suggested that this could have reduced tonic 5-HT_{1A} receptor mediated inhibition of 5-HT_{2A} receptor responsiveness (Farisse et al., 2000). However, although extensive interactions between 5-HT_{1A} and 5-HT_{2A} receptors have been recorded at a functional level (e.g. Dursun and Handley, 1993; 1996), it is not yet known whether this also extends to alterations in receptor expression.

In conclusion, a rational design and selection strategy has produced an antisense ODN, GRAS-5 that, when delivered to rat cerebral ventricles in polymer microspheres, substantially reduced the expression of glucocorticoid receptors in the absence of overt toxicity. This molecule may prove helpful in evaluating the role(s) of the glucocorticoid receptor. The ability of glucocorticoid receptor knockdown to enhance 5-HT_{2A} receptor expression and function has implications for understanding the consequences of corticosteroid dysregulation in depression.

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