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Behavioural and physiological effects induced by an infusion of antisense to α_{2D}-adrenoceptors in the rat

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1 The aim of this study was to investigate the behavioural and physiological effects of an i.c.v. infusion of antisense oligonucleotide to the α_{2D}-adrenoceptor subtype. Behavioural and physiological parameters were monitored for 2 days before the infusion, throughout the 3-day infusion period and for 3 days following the end of the infusion.

2 The antisense infusion resulted in a significant increase in behavioural activity characterized by increased locomotion and grooming scores. Behavioural activity scores of rats treated with antisense to α_{2D}-adrenoceptors were significantly higher than those of rats treated with vehicle (H_2O) or the mismatch toxicity control on day 4 and day 5 and, significantly higher than vehicle controls on day 6.

3 Body weight gain was significantly reduced in the antisense-treated rats at the end of the study compared to the vehicle (34%) and mismatch groups (30%), although daily food and water intakes were not significantly different at any time point.

4 Pupil diameters of rats infused with antisense to α_{2D}-adrenoceptors were significantly greater than those of animals treated either with vehicle or mismatch oligonucleotide on day 5 of the study. On day 6, the pupil diameters of these animals were still significantly greater than the mismatch group.

5 In conclusion, an i.c.v. infusion of antisense to the α_{2D}-adrenoceptor induced behavioural activation in rats, increased pupil diameter and reduced total weight gain. These effects were specific to the antisense-treated group and were fully reversed post-infusion.

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Abbreviations: CNS, central nervous system; EWN, Edinger-Westphal nucleus; RX811059, 2-ethoxy idazoxan; RX821002, 2-methoxy idazoxan; UK 14,304, (5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine

Introduction

Pharmacological characterization of α_2-adrenoceptors in the CNS has revealed both pre and postsynaptically located receptors mediating a diverse range of functions (French, 1995). One of the best characterized α_2-adrenoceptor-mediated function is the regulation of neurotransmitter release by α_2-autoreceptors located on noradrenergic neurones and α_2-heteroreceptors located on non-noradrenergic neurones (Langer, 1997). Stimulation of presynaptic α_2-adrenoceptors, by endogenous noradrenaline, activates the negative feedback mechanism, which results in the inhibition of neurotransmitter release. Furthermore, somatodendritic α_2-autoreceptors located on locus coeruleus cell bodies have been shown to regulate neuronal firing (Cederbaum & Aghajanian, 1977). Postsynaptically located α_2-adrenoceptors have been shown to play an important role in a number of physiological functions. For example, α_2-adrenoceptor agonists have been shown to increase pupil diameter (mydriasis) and induce hypothermia in rodents (Heal *et al*., 1995a,b; Lin *et al*., 1981; Unnerstall *et al*., 1984; Bill *et al*., 1989a,b).

Pharmacological and molecular biological studies have shown that four distinct subtypes of α_2-adrenoceptors exist (Bylund, 1988; MacKinnon *et al*., 1994). In the rat the α_{2D}-adrenoceptor subtype is a species homologous of the human α_{2A}-adrenoceptor subtype (Renouard *et al*., 1994) and, in addition the α_{2B}- and α_{2C}-adrenoceptor subtypes are present. Due to the lack of highly selective ligands, the functional roles of the individual subtypes have not been fully elucidated. However, correlation between the functional effects of α_2-adrenoceptor agonists and antagonists and their relative affinities for the different α_2-adrenoceptor subtypes, suggest that pre-synaptic α_{2D}-adrenoceptors mediate their effects on noradrenaline release and motor function, whereas postsynaptically located α_{2D}-adrenoceptors mediate their effects on pupil diameter (Trendelenburg *et al*., 1993; Millan *et al*., 1994; Limberger *et al*., 1995; Heal *et al*., 1995a,b).

Recently, antisense technology has emerged as a new approach to explore the functions of the individual α_2-adrenoceptor subtypes. Antisense oligonucleotides target specific mRNAs and inhibit the expression, and thus the function, of specific gene products. They have been used to investigate the physiological roles of a variety of receptors, particularly where highly selective ligands for the receptor are not yet available (for review see Robinson *et al*., 1997). In general, where antisense has been used to target the α_{2D}-adrenoceptors, studies have focused on whether the antisense treatment modifies the behavioural effects of α_2-adrenoceptor agonists. For example, when administered locally over the locus coeruleus, antisense to the α_{2D}-adrenoceptor has been shown to inhibit the loss of righting reflex induced by dexmedetomidine in rats (Mizobe *et al*., 1996). Furthermore, intrathecal administration of antisense to the α_{2D}-subtype has also been shown to inhibit the antinociceptive properties of

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clonidine (Hunter *et al*., 1997). Interestingly, only one previous study by Nunes (1995) investigated the effect of antisense to α_{2D}-adrenoceptors when given alone. This is an important consideration since antisense inhibition of receptor protein expression would be expected to produce similar effects to the use of highly selective antagonists. α_2-Adrenoceptor antagonists are not functionally inert and have been shown to exert opposite effects to α_2-adrenoceptors agonists in some models. For example, α_2-adrenoceptor antagonists, such as idazoxan, RX811059 (2-ethoxy idazoxan) and RX821002 (2-methoxy-idazoxan) have been reported to produce behavioural activation (Dickinson *et al*., 1990) decrease pupil diameter in rats (Heal *et al*., 1995a,b) and increase noradrenaline levels in the brain (Nutt *et al*., 1997).

The aim of the current study was to investigate the effects of antisense to the α_{2D}-adrenoceptor on a number of behavioural and physiological parameters. The effects of antisense treatment were determined using observational analysis, to detect subtle changes in behavioural activity patterns. The physiological parameters monitored included pupil diameter and body temperature which can be modulated by compounds acting at α_2-adrenoceptors. The antisense sequence used in this study has previously been shown to increase systolic blood pressure in the rat (Nunes, 1995) and decrease [^{3}H]-RX821002 binding to specific brain areas when administered i.c.v. (Robinson *et al*., 1999).

Methods

Animals and experimental procedure

Male Wistar rats (Charles River, U.K.) weighing 270–310 g were housed individually on a 12 h light/dark cycle (lights on at 0700 h) at $21 \pm 1^{\circ}$C and 55% humidity. Rats were allowed free access to food (standard rat diet) and tap water. The diet was contained in glass feeding jars (10 cm diameter; 8 cm deep; Solmedia Laboratory Supplies, Romford) to facilitate weighing.

Behavioural and physiological parameters were monitored for 2 days prior to the start of the infusion to obtain baseline readings. On day 3 of the experiment, rats were anaesthetized with isoflurane (5% for induction, 2–3% for maintenance) and stereotaxically implanted with a unilateral i.c.v. cannulae (0.92 mm caudal to bregma, 1.4 mm lateral and 3.5 mm below the surface of the dura; Paxinos & Watson, 1986). Phosphorothioate oligonucleotides were administered using osmotic mini-pumps, combined with a brain infusion kit (Alzet, Charles River, U.K., 1 μl h^{-1}) which were primed overnight with vehicle (H_2O), antisense or mismatch oligonucleotide which was included as a toxicity control (8 μg μl^{-1} h^{-1}, i.c.v.; Schlingensiepen & Heilig, 1997). A naïve group of rats were included to control for any changes induced by the surgical procedures. The mini-pump was located in the midscapular region and the cannulae fixed to the skull using a bone screw and dental cement. Behavioural and physiological parameters were then monitored for the duration of the 3 day infusion and for a further 3 days after the end of the infusion. Correct placement of the cannulae was confirmed using a dye injection at the end of the experiments.

All behavioural and physiological measurements were made in the same animals between 0800 and 1700 h on each day of the study by an experimenter who was unaware of the treatment each animal received. Physiological parameters were performed under low light conditions to enable pupil diameters to be measured. Two rats from each group were tested concurrently and the results were pooled so that each treatment group contained 7–8 animals.

Behavioural observations

Behavioural observations were carried out each day between 1000 and 1300 h. Rats were acclimatized to the test room in their home cages for 1 h and then placed individually into eight spatially adjacent cages containing sawdust bedding. The dimensions of the cages were the same as the home cage ($33 \times 20 \times 19$ cm). Behavioural observations began 5 min after placing the rats in the cages using a time sampling technique (adapted from Reinstein and Isaacson, 1977) where the behaviour of each rat was recorded for 5 s every 1 min for 60 min. The presence of each behaviour was counted only once during each 5-s period to give a maximum response for each behaviour of 60. The following behaviours were scored: locomotion, wall climbing, rearing, grooming, scratching, eating, digging and these individual scores were added together to give a measure of general activity. Animals were also examined for any other overt behaviours which may have been induced by the antisense treatment. The behaviours monitored were: yawning, chewing, forepaw treading, head weaving, ataxia, low body posture, wet dog shakes, hindlimb stretching, tremor and sniffing.

Body weight, food and water intake

Body weights were measured daily between 0800 and 0900 h and the weight gain for each day and total weight gain for the duration of the study calculated. Food and water intakes were measured daily. Food and water intakes are expressed as g kg^{-1} rat weight to account for any variations in individual body weight.

Pupil diameter

Pupil diameter was measured using a Wild M1 binocular microscope containing a graticule scale in one eyepiece. A Swift light box illuminated the microscope with the voltage set at 6 V (light intensity 450 lux). The procedure was carried out in an artificially lit room of light intensity of 20 lux. Animals were acclimatized to the lighting conditions in the test room for at least 30 min before the first reading. Each rat was then carefully restrained and held under the light source and its pupil diameter was read off the graticule scale in eyepiece units. This value was then converted into millimetres. Pupil diameter readings were made daily between 0800 and 0900 h and 1600 and 1700 h during the 9 days of the study.

Body temperature

Body temperature was measured using a rat rectal probe (inserted 2 cm) and digital thermometer (Model Bat-12, Sensortek; both obtained from CP Instruments, Bishop's Stortford, U.K.). Ambient temperature was maintained at $21 \pm 1^{\circ}$C and 55% humidity. Rectal temperature readings were made daily between 0800 h and 0900 h and 1600 and 1700 h during the 9 days of the study.

Drugs

The oligonucleotide sequences used were previously described by Nunes (1995), and target the initiating coding region of the RG20 gene mRNA (antisense 5′-ATCCGGCTGCAGGGAGCC-3′, mismatch 5′-ATCCAGCGGCTGGGAGCC-3′).

The oligonucleotides were fully modified phosphorothioate oligonucleotides kindly synthesized by Prof L. Hall, Biochemistry Department, University of Bristol.

Statistics

Behavioural observation data are expressed as treatment group median scores + upper range for the 60 min observational period each day and the results analysed using a non-parametric approach, the Cochran-Manzel-Haenszel test (Koch & Edwards, 1988), to test the association between behaviour and treatment. Body weight, food and water intake, pupil diameters and rectal temperature data are shown as mean values ± s.e.mean for each treatment group. The results for each reading were compared statistically using a one-way analysis of variance with treatment as factor. *Post hoc* comparisons were then made using the Dunnett's test. In all statistical analysis a value of $P<0.05$ was considered significant.

Results

Behavioural activity scores for rats treated with antisense to α_{2D}-adrenoceptors were significantly higher than those of rats treated with vehicle or mismatch control during the 0–60 min period, 24 h after the start of the antisense infusion (day 4). Similar results were seen during the 0–60 min observational period the following day. On day 6, the last day of the infusion, the activity scores of the antisense-treated rats were significantly higher than the vehicle-control group but were not significantly different from rats given the mismatch control. Over the 3 day period following the end of the i.c.v. infusion, the activity levels of the antisense-treated group returned to the level of the control rats. Activity scores between the vehicle, mismatch and naïve group of rats did not differ significantly at any time during the study. The activity scores for the 0–60 min observational period on each day are shown in Figure 1.

The increase in total activity observed during the 60 min behavioural observation period was primarily due to increase in locomotion (Figure 2) and grooming behaviour (Figure 3). The locomotion scores for the antisense-treated group were significantly higher than those for the vehicle and mismatch controls on days 4, 5 and 6 whereas, on day 7, locomotion scores for the antisense-treated group were significantly higher than the mismatch control group only. Grooming scores in the antisense-treated rats were significantly higher than both the vehicle and the mismatch control groups on day 4 and day 6 of

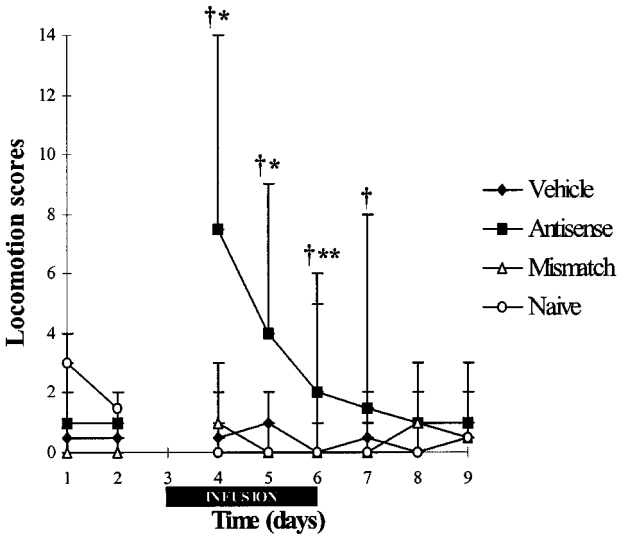

Figure 2 Locomotion scores of rats during the 60-min observational period each day before, during and after a 3 day i.c.v. infusion of antisense to α_{2D}-adrenoceptor, oligonucleotide or vehicle (H_2O). Scores are also given for naïve rats, which were not given any treatment, or subject to any surgical procedures. Results are shown as median scores + upper ranges for 7–8 animals per group, $*P<0.05$, $**P<0.01$ compared to vehicle-infused controls, $\dagger P<0.05$ compared to mismatch-infused controls.

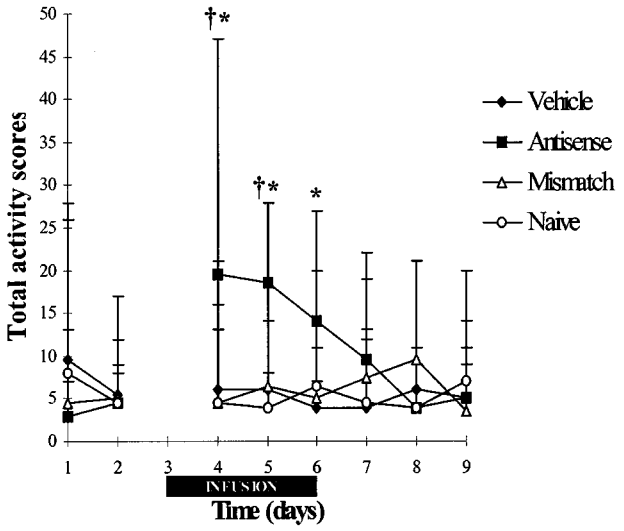

Figure 1 Total activity scores of rats during the 60-min observational period each day before, during and after a 3 day i.c.v. infusion of antisense to α_{2D}-adrenoceptor, mismatch oligonucleotide or vehicle (H_2O). Scores are also given for naïve rats, which were not given any treatment, or subject to any surgical procedures. Results are shown as median scores + upper ranges for 7–8 animals per group, $*P<0.05$, compared to vehicle-infused controls, $\dagger P<0.05$ compared to mismatch-infused controls.

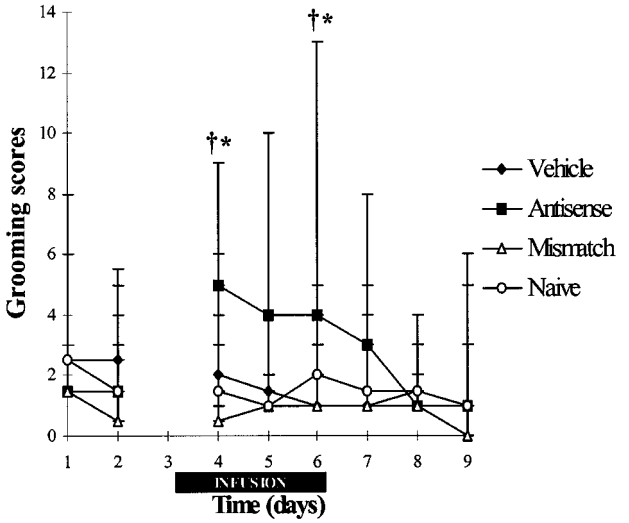

Figure 3 Grooming scores of rats during the 60-min observational period each day before, during and after a 3 day i.c.v. infusion of antisense to α_{2D}-adrenoceptor, oligonucleotide or vehicle (H_2O). Scores are also given for naïve rats, which were not given any treatment, or subject to any surgical procedures. Results are shown as median frequency scores + upper range for 7–8 animals per group, $*P<0.05$ compared to vehicle, $\dagger P<0.05$ compared to mismatch.

the time course study. The grooming scores were similarly elevated on day 5 but this result was not significantly different from either the vehicle or the mismatch controls. During the 3-day period after the end of the i.c.v. infusion, both locomotion and grooming scores for the antisense-treated rats returned to control values.

The results for the other individual behaviours scored during the 0–60 min observational period are not shown. Antisense-treatment did not significantly alter wall climbing or rearing scores compared to the vehicle and mismatch controls. Increases in scores for scratching were seen on days 4, 5 and 6 compared to either the vehicle or the mismatch control groups and a transient increase in eating scores was seen in the antisense-treated group compared to vehicle on day 6. Digging scores were significantly increased in the antisense-treated group compared to both the vehicle and the mismatch control groups on day 4 of the study and increased significantly compared to the mismatch on day 5.

Implantation of the osmotic minipumps did not appear to have any affect on the behaviour of the animals as shown in Figures 1, 2 and 3. The behavioural profiles of rats receiving i.c.v. infusions of vehicle were similar to those of naïve animals throughout the study. Furthermore, the behaviours of the four different groups of rats were not significantly different during the two day baseline period as shown in Figures 1–3. The antisense treatment did not induce any other overt behavioural effects or signs of neurotoxicity throughout the study (data not shown).

Body weight, food and water intake

Total weight gain for the period of the study was significantly lower in the antisense-treated rats compared to the vehicle (34%) and mismatch (30%) control groups (Figure 4). There was no significant difference in total weight gain between the control groups i.e. vehicle, mismatch and naïve groups. Daily food and water intakes for the different treatment groups were not significantly different throughout the study (Figure 5). Comparison between the naïve group of rats and the vehicle controls did not reveal any significant differences in weight gain or in food and water intake on any day of the study (Figures 4 and 5).

Pupil diameter

The results from the study of pupil diameter in antisense-infused rats were compared statistically to vehicle and mismatch-infused controls at each time point throughout the study. The pupil diameters of rats infused with antisense to α_{2D}-adrenoceptors were significantly greater than those animals treated either with vehicle or mismatch oligonucleotide during the morning (+0.10–0.15 mm) and afternoon (+0.08–0.10 mm) readings on the fifth day of the study (i.e. 48 h after the start of the i.c.v. infusion). On day 6, the pupil diameters of these animals were still significantly greater than those of the mismatch control group (+0.11 mm) but were not significantly different from the vehicle-treated controls. The increase in pupil diameter observed in the antisense-treated animals gradually decreased after day 5, returning to baseline levels during the recovery period (Figure 6). The results for vehicle and mismatch controls did not differ significantly from the naïve treatment group at any time point during the study.

Body temperature

Rectal temperatures of rats given antisense to α_{2D}-adrenoceptors were significantly greater than the vehicle-treated controls on the morning of the fourth day of the study, (i.e. 24 h after the start of the infusion). The body temperatures of these animals were not significantly different to the mismatch control group at this time point (Figure 6). Furthermore, the body temperatures of the different groups of rats were not significantly different at any other time point before, during or after i.c.v. infusion.

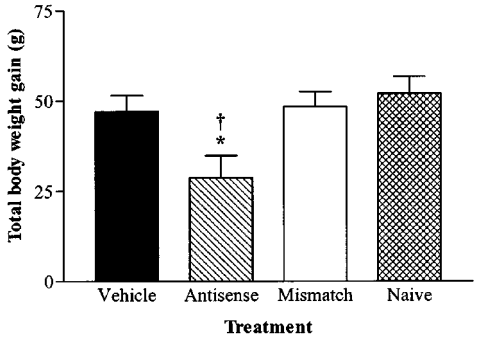

Figure 4 Weight gain of rats given a 3 day i.c.v. infusion of antisense to the α_{2D}-adrenoceptor, oligonucleotide or vehicle (H_2O). Results are also given for the naïve animals, which were not given any treatment, or subject to any surgical procedures. Total weight gain was recorded for the duration of the 9 day time course study. Values represent means+s.e.mean for 7–8 animals per group, $*P<0.05$ compared to vehicle, $\dagger P<0.05$ compared to mismatch.

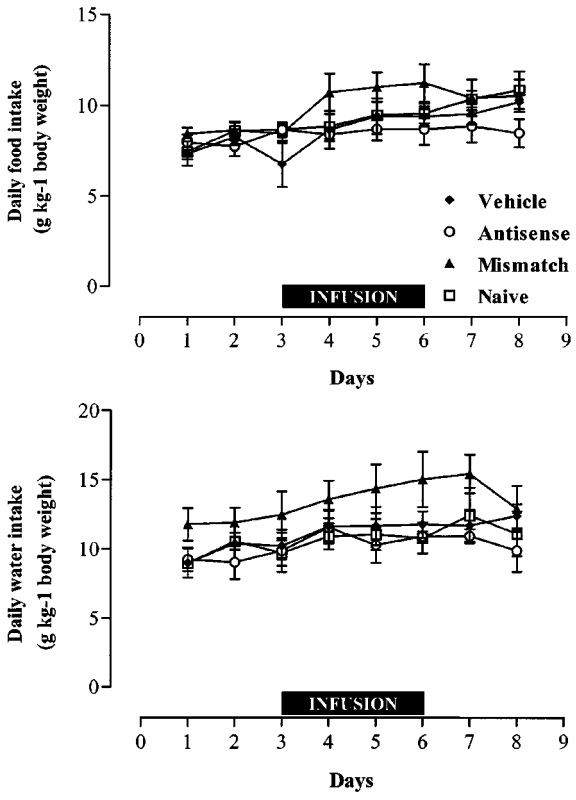

Figure 5 Daily food (top) and water (bottom) intakes of rats before, during and after a 3-day i.c.v. infusion of antisense to α_{2D}-adrenoceptor, mismatch oligonucleotide or vehicle (H_2O). Results are also given for the naïve animals, which were not given any treatment, or subject to any surgical procedures. Values represent means±s.e.mean for 7–8 animals per group, $*P<0.05$ compared to vehicle, $\dagger P<0.05$ compared to mismatch.

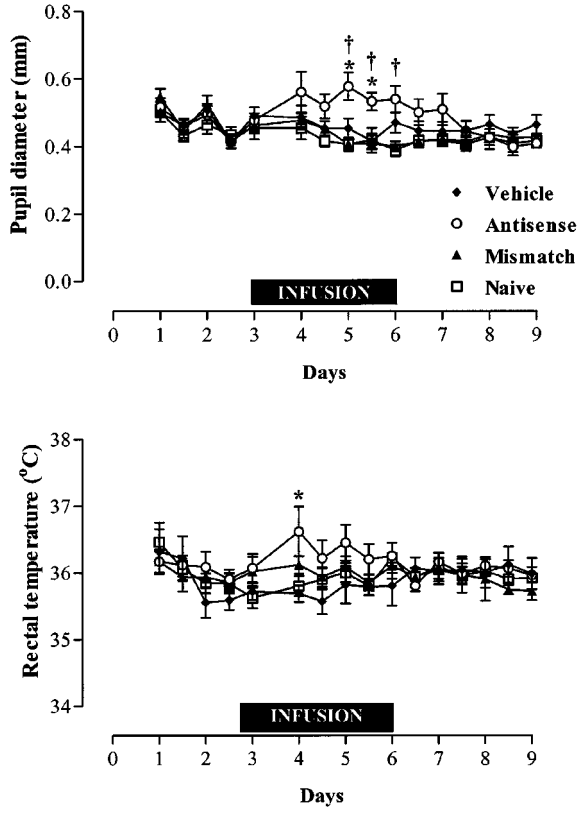

Figure 6 Pupil diameters (top) and rectal temperatures (bottom) of rats before, during and after a 3-day i.c.v. infusion of antisense to α_{2D}-adrenoceptor, mismatch oligonucleotide or vehicle (H_2O). Results are also given for the naïve animals, which were not given any treatment, or subject to any surgical procedures. Values represent means ± s.e.mean for 7–8 animals per group, $^*P<0.05$ compared to vehicle, $\dagger P<0.05$ compared to mismatch.

Discussion

The present study demonstrates both behavioural and physiological changes induced by an infusion of antisense to α_{2D}-adrenoceptors. Animals treated with antisense to α_{2D}-adrenoceptors are more active than controls, a response that appears to be largely due to increases in locomotion and grooming scores. The reduced weight gain seen in the antisense-treated group would appear to reflect this behavioural activation. Paradoxically, the antisense treatment increased pupil diameter, a response consistent with α_2-adrenoceptor agonism rather than the decrease in pupil diameter produced by α_2-adrenoceptor antagonists (Heal *et al*., 1995a). The most likely explanation for these discrepant results is the distribution of the oligonucleotide in the CNS in relation to the location of the receptor mediating the response. Although the antisense was administered i.c.v. it is unlikely to be fully distributed throughout the CNS (Yaida & Nowak, 1995).

One of the important observations in the current study was that the increases in activity and pupil diameter induced by antisense to α_{2D}-adrenoceptors were specific to the antisense treatment. In a previous study, antisense to the α_{2D}-adrenoceptor selectively decreased the binding of the selective α_2-adrenoceptor antagonist [^{3}H]-RX821002 (Robinson *et al*., 1999). Using quantitative receptor autoradiography following a 3-day infusion with antisense to the α_{2D}-adrenoceptor, reductions in binding were observed in the lateral septal nuclei and anterior hypothalamic areas. The sequence used in both the binding studies and the present functional investigations did not cause any obvious neurological or behavioural toxicity and the mismatch sequence had no effect on receptor expression or function. Non-specific toxic side effects of antisense oligonucleotides have been reported in the literature but appear to vary with the sequence used and certain toxic motifs within the sequence (Crooke, 1992; Bourson *et al*., 1995; Le Corre *et al*., 1997).

Interestingly, the rate of onset of the behavioural and physiological changes produced by the antisense treatment does not correlate with the half-life of the receptor involved. The α_2-adrenoceptor has a half-life of approximately 4 days as reported by Adler *et al*. (1985) whereas antisense-induced functional changes were seen in the current study on day 4, i.e. only 24 h after the start of the infusion. One possible explanation for this discrepancy, proposed by Qin *et al*. (1995), is that receptor function is mediated *via* an active pool of newly synthesised receptors which are rapidly turned over and thus, affected by the antisense treatment (Qin *et al*., 1995; Weiss *et al*., 1997). The increase in behavioural activity and pupil diameter were only apparent during the infusion of antisense and these parameters returned to control levels at the end of the infusion. Binding studies were not performed at the end of the experiment to determine α_2-adrenoceptor density but the results suggest antisense-mediated inhibition of receptor expression during the infusion and recovery following the end of the infusion. Alternatively, compensatory mechanisms may have occurred to counteract the effects of the antisense treatment, although on recovery, rebound decreases in activity and pupil diameter were not apparent.

The behavioural activation induced by the antisense infusion is consistent with a previous report that the α_2-adrenoceptor antagonists, idazoxan, efaroxan and RX811059 (2-ethoxy idazoxan) increase locomotor activity in rats (Dickinson *et al*., 1990). Moreover, in our own behavioural observation studies in rats, acute administration of RX821002 (2-methoxy idazoxan), induced an increase in activity scores with associated increases in locomotion and grooming behaviour (unpublished observation). The pharmacological mechanisms underlying the stimulatory behavioural effects of the antisense treatment were not directly investigated during the present study. However, the antisense treatment may be expected to increase noradrenaline levels in the CNS in a similar manner to an α_2-adrenoceptor antagonist (Nutt *et al*., 1997). This elevation in noradrenaline may subsequently activate postsynaptic α_1-adrenoceptors to increase locomotor activity (Clineschmidt *et al*., 1979; Heal, 1984). This theory could be confirmed by using *in vivo* dialysis to measure noradrenaline levels following antisense treatment.

The role of other neurotransmitters, such as 5-HT and dopamine, in the behavioural effects of antisense to the α_{2D}-adrenoceptor should also be considered. α_2-Adrenoceptors have been shown to regulate both 5-HT and dopamine levels in the CNS (Göthert & Schlicker, 1991; Xu *et al*., 1993; Nutt *et al*, 1994) therefore, inhibition of α_{2D}-adrenoceptors may also affect central levels of these monoamines. Interestingly, activation of dopamine receptors is associated with specific behavioural changes namely, increased locomotion and stereotypic behaviour (Watchel *et al*., 1992). Since antisense to α_{2D}-adrenoceptors increased both locomotion and grooming scores in the current study, it cannot be precluded that it may have increased dopamine levels in the CNS and that this may have contributed to some of the behavioural effects observed. *In vivo* dialysis or the use of monoamine receptor antagonists could be used to identify the neurotransmitters involved in the increase in activity observed.

Antisense treatment also resulted in a significant decrease in the total weight gain during the 9-day period of the time course study. This effect was specific to the antisense-treated group and was not as a result of toxicity as the mismatch controls showed a similar weight gain, during the study, as the vehicle and naïve-treated groups. A reduction in body weight gain was also observed following a chronic infusion with the α_2-adrenoceptor antagonist, idazoxan (Dickinson *et al*., 1989). In both these studies, the reduction in weight gain appears to reflect a behavioural activation and thus, increased energy expenditure, rather than inhibition of feeding. As seen in the present time course study, antisense-treatment had no significant effect on food and water intake. Although, idazoxan has previously been reported to increase food and water intake in rats this appears to be due to its high affinity for imidazoline I_2 sites as more selective α_2-adrenoceptor antagonists such as RX811059 and RX821002 do not produce hyperphagia (Jackson *et al*., 1991). Finally, idazoxan and selective α_2-adrenoceptor antagonists have been reported to increase water intake in rats *via* a peripherally mediated mechanism (Jackson *et al*., 1991) which would explain why increased water intake was not observed in the current study.

The antisense infusion resulted in an increase in pupil diameter (mydriasis), similar to that seen with an α_2-adrenoceptor agonist (Heal *et al*., 1995a). These findings were unexpected because antisense treatment would be expected to produce similar effects to conventional α_2-adrenoceptor antagonists i.e. decrease pupil diameter (miosis) (Heal *et al*., 1995a). One explanation for these findings may be that the antisense did not penetrate to the Edinger-Westphal nucleus (EWN) which mediates the effects of α_2-adrenoceptor agonists and antagonists on pupil diameter (Koss, 1986). Previous studies combining functional and binding data have shown that the α_{2D}-adrenoceptor is the subtype most likely to be mediating this response (Heal *et al*., 1995b) *via* modulation of parasympathetic tone to the iris (Hey *et al*., 1985). The results observed in the present study may reflect an elevation in brain levels of noradrenaline induced by the antisense treatment affecting the pathway from the EWN to the iris. Alternatively, the elevated levels of noradrenaline in the brain may feedback to the EWN and thus have produced an agonist-like increase in pupil diameter. Previous studies using antisense to α_{2D}-adrenoceptors, i.c.v. also resulted in an elevation in baseline pupil diameters but did not significantly attenuate mydriasis induced by the α_2-adrenoceptor agonist, UK 14,304 (Robinson *et al*., 1999). Therefore, the function of α_{2D}-adrenoceptors in the EWN does not appear to be inhibited by antisense treatment.

The antisense treatment did not significantly affect rectal temperature compared to both the vehicle and mismatch controls at any time point. A significant but transient increase in temperature was observed on day 4 in the antisense group compared to the vehicle only. Interestingly, in our previous studies using antisense to α_{2D}-adrenoceptors, a significant attenuation in the hypothermic response to the α_2-adrenoceptor agonist UK 14,304 was observed in the antisense treated group (Robinson *et al*., 1999). Furthermore, this effect was fully reversed 4 days after the end of the infusion suggesting a specific role for the α_{2D}-adrenoceptor subtype in mediating the hypothermic effect of an α_2-adrenoceptor agonist. In agreement with the present study, the specific α_2-adrenoceptor antagonist, RX821002, did not affect rectal temperature following acute administration (unpublished observation), suggesting α_{2D}-adrenoceptors are not involved in tonic control of temperature but do mediate the hypothermic effects observed with α_2-adrenoceptor agonists (Bill *et al*., 1989a,b).

In conclusion, the present study has shown that an antisense sequence targeting the α_{2D}-adrenoceptor induces specific behavioural and physiological changes during the infusion period, which are fully reversed after the end of the infusion. Further studies are required to provide insight into the neurochemical changes underlying these responses. In conjunction with our previous findings using antisense to the α_{2D}-adrenoceptor, the sequence described in the present study has been shown to specifically inhibit binding to α_2-adrenoceptors, attenuate α_2-adrenoceptor agonist-induced functions and induce behavioural activation similar to that reported following α_2-adrenoceptor blockade (Robinson *et al*., 1999). These experiments have shown that an antisense-based strategy provides a novel approach to investigating the function of a receptor subtype in the absence of highly selective ligands.

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