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Atropine acts in the ventral striatum to reduce raclopride-induced catalepsy

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Abstract

While muscarinic receptor antagonists are used to reduce motor side effects associated with the use of antipsychotic drugs, their site of action remains unclear. The study investigated the site of action of the non-selective muscarinic receptor antagonist atropine on catalepsy induced by the selective dopamine D2 receptor antagonist, raclopride. Initially, catalepsy and striatal muscarinic receptor occupancy was assessed 2 h following subcutaneous injection of raclopride and either atropine or vehicle. Catalepsy was significantly reduced by doses of atropine that occupied more than 69% of muscarinic receptors. Next, atropine was injected bilaterally into the ventral striatum, which produced a significant reduction in catalepsy, while injections into the dorsal striatum and substantia nigra had no effect. The site of atropine's action was localised to a discrete area of the ventral striatum through the use of quantitative autoradiographic techniques. These findings provide further evidence for the importance of the ventral striatum in the expression of behaviours.

Keywords: Striatum, ventral; Anticholinergic drug; Antipsychotic drug; Catalepsy; Receptor occupancy

1. Introduction

Antipsychotic drugs have revolutionised the treatment of schizophrenia. Use of the older or typical antipsychotic drugs can be associated with the appearance of severe extrapyramidal side effects, which include Parkinson-like motor side effects such as akinesia, bradykinesia, increased muscle rigidity and tremor (Ayd, 1961). Motor side effects are believed to result from blockade of dopamine D2 receptors located in the striatum (Snyder et al., 1974). This view is supported by human (Farde et al., 1992) and experimental animal studies (Hemsley and Crocker, 1998, 1999), which showed motor side effects occurred when a threshold level of ~70–80% dopamine D2 receptors are occupied by antipsychotic drugs.

Currently, the most effective method of reducing motor side effects is to administer muscarinic receptor antagonists (i.e., anticholinergic medication) such as benztropine, benzhexol and biperiden (Stanilla and Simpson, 1995). These drugs have been used for decades to treat a range of motor disorders including Parkinson's disease (Duvoisin, 1967). Support for the role of the cholinergic system in the reduction of motor side effects is provided by evidence that those antipsychotic drugs associated with few motor side effects have high levels of antimuscarinic activity (Miller and Hiley, 1974). Furthermore, across a range of clinically used antipsychotic drugs, an inverse relationship has been reported between the incidence of motor side effects and the affinity of each drug for muscarinic cholinergic receptors, both in the whole brain and in the striatum in particular (Miller and Hiley, 1974; Snyder et al., 1974).

Several animal models have been developed to determine the motor side effect potential of antipsychotic drugs, including measures of muscle rigidity, tremor, circling, locomotion and catalepsy (Costall et al., 1972; Sayers et al., 1976; Hemsley and Crocker, 1999). Catalepsy is the most widely used behavioural model in rodents for the study of dopamine receptor antagonists. It is defined as a failure to correct an externally imposed posture (Sanberg, 1980), and involves a state of active immobility, whereby the animal exhibits skeletal muscle rigidity, and in which reflexes such as locomotion, orientation and scanning are suppressed (Klemm, 1989).

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It is well established that blockade of dopamine receptors induces catalepsy. This has been demonstrated using a range of antipsychotic drugs and selective D1 and D2 dopamine receptor antagonists (e.g., Sanberg, 1980; Sayers et al., 1976; Klemm, 1989; Wardas et al., 1995). The results of these studies showed there is a correlation between the likelihood a drug produces motor side effects in humans and its ability to induce catalepsy in animals (Sayers et al., 1976). Thus, antipsychotic drugs with a high side effect profile (e.g., haloperidol) produced high levels of catalepsy, whereas atypical antipsychotic drugs (e.g., clozapine), which are associated with minimal motor side effects in humans, have a weaker ability to induce catalepsy (Sayers et al., 1976). This strong relationship between catalepsy and motor side effects in humans has led to the use of catalepsy as a predictive model for motor side effect propensity in humans (Hoffman and Donovan, 1995).

Studies investigating the relationship between dopamine and muscarinic receptor interactions have demonstrated that the addition of a peripherally administered muscarinic receptor antagonist, atropine, resulted in a reduction of haloperidol-induced catalepsy (Costall and Olley, 1971). Further, catalepsy induced by either a selective dopamine D1 (Haraguchi et al., 1997; Ushijima et al., 1997) or dopamine D2 (Haraguchi et al., 1997) receptor antagonist was reduced following administration of a muscarinic receptor antagonist. In a study to investigate levels of dopamine and muscarinic receptor occupancy concurrently with behavioural measures of catalepsy, Haraguchi et al. (1997) reported that the reduction in catalepsy induced by a combination of dopamine D1 and D2 receptor antagonists was dependent on the binding of scopolamine to muscarinic receptors. Although this relationship was not quantified, the findings provide further evidence that muscarinic receptors mediate reductions in catalepsy.

The aim of the current study was to investigate the site of action of a non-selective muscarinic receptor antagonist, atropine, on catalepsy induced by the selective dopamine D2 receptor antagonist, raclopride. Initially, the effect of atropine, administered subcutaneously, was assessed on the magnitude of catalepsy, together with determination of the level of both dopamine D2 and muscarinic cholinergic receptor occupancy using quantitative autoradiographic methods. Next, the site of atropine's action was determined following injections into the substantia nigra and striatum, brain regions shown to be critical in the maintenance of normal motor function (Double and Crocker, 1995; Hemsley and Crocker, 1998). Using quantitative autoradiographic analysis of muscarinic receptor occupancy by atropine, we were able to establish the site of action of atropine in reducing catalepsy produced by the selective dopamine D2 receptor antagonist, raclopride.

Part of this work has been published previously in abstract form (Alcock and Crocker, 1999; Crocker and Hemsley, 2001).

2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley rats (250–350 g) supplied by the Animal House of the Flinders Medical Center, were housed in groups of five, maintained under conditions of constant temperature and humidity on a 12-h light/dark cycle and given free access to food and water. Experiments were carried out in accordance with the guidelines of the Australian National Health and Medical Research Council (NHMRC), and were approved by the Flinders University of South Australia Animal Ethics Committee. Animals were closely monitored during surgery and throughout the course of experiments. None of the procedures appeared to cause pain or distress. The minimum number of animals to provide statistically meaningful data was used.

Raclopride (2.5 mg/ml: Astra) and atropine sulphate (0.5–10 mg/ml: Sigma) were dissolved in isotonic saline (0.9%) and injected subcutaneously in a volume of 1 ml/kg. Atropine (5 mg/ml) or saline was administered intracerebrally in volumes of 40–500 nl as described in the text.

2.2. Implantation of guide cannulae

Rats were anaesthetised to surgical depth with Nembutal (sodium pentobarbitone (Boehringer Ingelheim) 45 mg/kg, i.p.) and the head of the rat was secured in a Stoelting stereotaxic apparatus. Local anaesthetic, Xylocaine (10% lignocaine: Astra) was then applied to the scalp. An incision of approximately 30 mm was made along the midline of the scalp, the membranes covering the skull were cleared and burr holes were drilled bilaterally into the skull using a 0.75-mm wide drill bit (Blackwoods, Adelaide, Australia) in a pin-vice chuck. The cannulae (constructed from flat-ended 23-gauge needles, 1.25-mm diameter) were lowered vertically into the brain to a position 2 mm above the target injection site, then secured using dental cement (GC Fuji II Glass Ionomer Restorative Cement, Halas Dental, Adelaide, Australia). Animals were placed under a warm lamp until they recovered fully from anaesthesia and were returned to their home cages overnight. The coordinates for injections were as follows: dorsolateral striatum (A-P, +1.29; L, ±2.5; D-V, −4), ventrolateral striatum (A-P, +1.29; L, ±2.5; D-V, −6.5), substantia nigra (A-P, −5.2; L, ±2.4; D-V, −7.8) according to the atlas of Paxinos and Watson (1986).

Injection needles were constructed from 30-gauge dental needles (0.3-mm diameter: Halas Dental), attached to clear polyethylene tubing (#112010, Critchley Electrical, Silverwater, New South Wales, Australia). The tubing was attached to a water-filled 5-μl Hamilton syringe (Scientific Glass Engineering, Australia) held horizontally on a motor-driven pump (Sage Instruments, Thermo Orion, MA, USA). Animals were gently restrained and the injection

needle was lowered through the cannulae to the brain region of interest. Bilateral injections of atropine were made over 30 s and the needle remained in place for a further 30 s to reduce back-flow up the injection track.

2.3. Behavioural analysis

The assessment of catalepsy was made 2-h post-injection of drug/vehicle using a rating scale method modified from Pedigo et al. (1980). Four tests of cataleptic behaviour were used, each receiving a score of 0–3. The tests were: (1) the time taken for a rat placed on a sloping wire grid, at an angle of approximately 50° to the horizontal, to move at least one hind limb; (2) assessment of the behaviour and posture of the rat while performing the grid test; (3) the posture and movement of the animal in response to lateral displacement; and (4) the time taken for the rat to move one or both hind limbs off a 1-cm block on which the hind limbs had been placed. The sum of the scores of the tests was used to obtain a total catalepsy score, out of a maximum possible score of 12. The dose of raclopride (2.5 mg/kg) used in all experiments was selected on the basis of a preliminary study, which established that 2.5 mg/kg had a sub-maximal effect on catalepsy and elicited a median total catalepsy score of 9, enabling the assessment of treatment effects that either decreased or increased catalepsy. In addition, our previous studies showed that this dose occupied more than threshold levels of striatal dopamine D2 receptors (Hemsley and Crocker, 1999).

2.4. Quantitative autoradiography

Following the analysis of behaviour, rats were sacrificed by decapitation. Brains were removed, bisected along the sagittal plane and both hemispheres embedded in OCT™ compound and snap-frozen in isopentane-quenched liquid nitrogen. The left hemisphere of the brain was cut on a cryostat into 20-μm sections and mounted on gelatin-coated slides. Sections were cut in a sagittal plane to allow assessment of receptor occupancy within the striatum and substantia nigra simultaneously. In some experiments described in the text, coronal sections of the forebrain were processed to determine the site of action of atropine within the striatum. A method based on that described by Schotte et al. (1993) was used to determine the occupancy of receptors by atropine and raclopride.

Briefly, sections were incubated with either the muscarinic cholinergic receptor ligand, [^{3}H] QNB (1 nM, Amersham, Australia) or the dopamine D2 receptor ligand [^{125}I] iodosulpiride (0.3 nM, Amersham) at room temperature. Non-specific binding was determined on adjacent sections using 1 μmol atropine or 1.7 μmol sulpiride for muscarinic cholinergic and dopamine D2 receptors, respectively. Sections were washed (2 × 2 min) in ice-cold Tris buffer (50 mM) to stop the reaction, and were air-dried before being apposed to tritium-sensitive film (Hyperfilm, Amersham) for up to 1 week (dopamine D2) or up to 1 month (muscarinic receptors), together with appropriate standards (Amersham). Schotte et al. (1993) acknowledged that the calculated receptor occupancy using the ex vivo method is likely to be lower than that formed in vivo due to diffusion of drug during the assay. Therefore, pre-incubation washes were not performed, and the incubation time was reduced to 10 min to limit diffusion of drug receptor complexes. Autoradiographs were assessed using a computerized densitometry system (MD20, Flinders Imaging, Flinders University, Australia). Optical density values of selected brain regions, determined using the averaged optical density of three to four sections per rat, were converted to nCi/mg of tissue by reference to ^{3}H or ^{125}I standards (Amersham). Receptor occupancy by the drugs was calculated as a percentage of the average receptor concentration in control rats. Ex vivo receptor binding labelled all receptors in saline injected rats, but only receptors not occupied by drug in the experimental groups (Schotte et al., 1993). Thus, receptor ligand binding was inversely proportional to the receptor occupancy of the test drug administered in vivo. The data presented for receptor occupancy in dorsal and ventral striatal areas were obtained by averaging occupancies in the whole striatal area either dorsal or ventral to D-V coordinate, 5.5 mm, respectively (Paxinos and Watson, 1986).

2.5. Statistical analysis

Catalepsy data was analysed using two non-parametric tests due to its ordinal nature. Kruskall–Wallis one-way analysis of variance (ANOVA) was first performed to determine the statistical difference between all groups in an experiment. In the case that a significant result was obtained, the Mann–Whitney *U*-test was then used to assess the statistical difference between any two groups in that experiment. Dopamine and muscarinic receptor occupancy was first analysed using a one-way ANOVA to establish a significant difference across all groups within an experiment. A *t*-test was then used to assess differences between any two groups. A probability (P) value of less than 0.05 was taken as significantly different corrected for multiple comparisons using a Bonferroni correction.

3. Results

3.1. Effect of atropine on catalepsy induced by the selective D2 receptor antagonist, raclopride

Rats were injected subcutaneously with either raclopride (2.5 mg/kg) + saline (1 ml/kg), atropine (5 mg/kg) + saline, saline + saline, or raclopride (2.5 mg/kg) + atropine (0.5, 1, 5 or 10 mg/kg), and behaviour was assessed at 2-h post-injection (Fig. 1). Rats treated

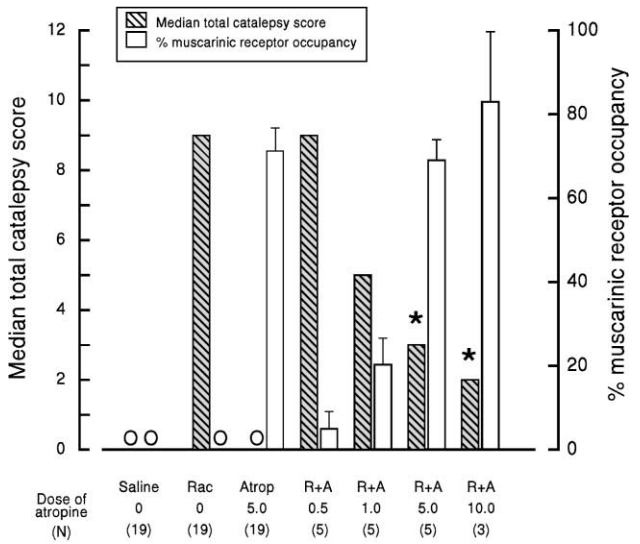

Fig. 1. Median total catalepsy score and occupancy of muscarinic receptors at 2 h following injection of saline (s.c.), raclopride (Rac) (2.5 mg/kg), atropine (Atrop) (5 mg/kg) or raclopride (2.5 mg/kg) + various doses of atropine (R + A) (*N* for each treatment shown). * $P < 0.03$ compared with raclopride group, ♦ $P < 0.001$ compared with saline group. O = total catalepsy score of 0 or zero receptor occupancy.

with either saline + saline, or saline + atropine (5 mg/kg), did not exhibit catalepsy, whereas those treated with raclopride + saline exhibited a significant level of catalepsy, median total catalepsy score = 9; $P < 0.001$, compared with the saline group. Administration of atropine (0.5, 1, 5 or 10 mg/kg) together with raclopride (2.5 mg/kg) resulted in dose-dependent reductions in total catalepsy score compared with the raclopride + saline group. Concurrent administration of raclopride with atropine at a dose of 10 or 5 mg/kg resulted in significant reductions in catalepsy from a score of 9 to a median score of 2 and 3 ($P < 0.03$), respectively. However, doses of 1 and 0.5 mg/kg atropine, co-administered with raclopride, failed to significantly reduce raclopride-induced catalepsy (Fig. 1).

3.2. Dopamine D2 and muscarinic receptor occupancy

Following the assessment of catalepsy, brains were removed and quantitative autoradiography was performed to determine the occupancy of muscarinic and dopamine D2 receptors by atropine and raclopride, respectively.

Raclopride (2.5 mg/kg) occupied 80 ± 3.0% and 73 ± 6.1% of dopamine D2 receptors in the striatum and substantia nigra, respectively. Co-administration of atropine with raclopride had no effect on dopamine D2 occupancy levels by raclopride, which were 84 ± 2.2% in the striatum and 70 ± 7.7% in the substantia nigra. However, dose-dependent occupancy of striatal muscarinic cholinergic receptors was observed at 2-h post-injection (Fig. 1). Thus, following doses of 10, 5, 1 and 0.5 mg/kg atropine, striatal muscarinic receptor occupancies of 83 ± 16.7%, 69 ± 4.9%, 20 ± 6.3% and 5.0 ± 4.1%, respectively, were determined. Fig. 1 shows that significant reductions in total catalepsy score were observed at doses of 5 and 10 mg/kg atropine, associated with muscarinic receptor occupancies of 69% or greater.

3.3. Investigation of the site of action of atropine in mediating raclopride-induced catalepsy

To identify the location of the muscarinic receptors involved in the reduction of catalepsy, intracerebral injections of atropine were made via cannulae into either the ventral or dorsal striatum, or the substantia nigra. Atropine (5 mg/ml) was administered bilaterally in volumes of 40 nl into the substantia nigra, and either 50, 100 or 500 nl into the striatum. Raclopride was administered concurrently at a dose of 2.5 mg/kg (s.c.). The effect on behaviour and muscarinic receptor occupancy was assessed at 2-h post-injection. The effects of these treatments on catalepsy are shown in Fig. 2.

3.3.1. Ventral striatum

Administration of 50, 100 or 500 nl saline + raclopride (2.5 mg/kg, s.c.) resulted in significant increases in total catalepsy score at 2-h post-injection. These increases were significantly reduced when atropine (50, 100 or 500 nl) was injected into the ventral striatum. This reduction is comparable to that observed following peripheral injections of atropine at a dose of 5 mg/kg. Injection of saline or atropine alone into the ventral striatum had no significant effect on total catalepsy score.

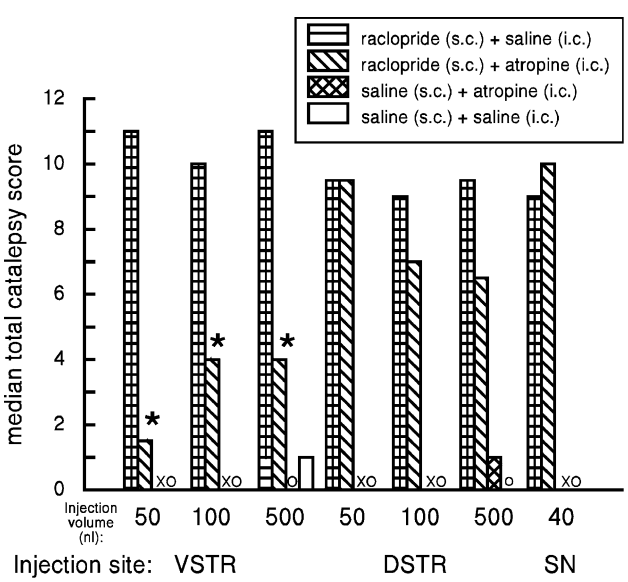

Fig. 2. Median total catalepsy score at 2 h following intracerebral (i.c.) injection of saline or 50–500 nl atropine (5 mg/ml) into the ventral striatum (VSTR), dorsal striatum (DSTR) or substantia nigra (SN) of rats injected subcutaneously (s.c.) with either saline or raclopride (2.5 mg/kg). * $P < 0.05$ compared with raclopride group, O = total catalepsy score of zero, X = not determined.

3.3.2. Dorsal striatum

As observed above, injection of 50–500 nl saline into the dorsal striatum + raclopride (s.c.) resulted in significant increases in total catalepsy score at 2 h. However, injections of atropine (50, 100 or 500 nl) into this region did not significantly reduce the catalepsy induced by raclopride ($P > 0.05$). Injection of saline or atropine alone into the dorsal striatum had no significant effect on total catalepsy score.

3.3.3. Substantia nigra

Injection of atropine into the substantia nigra did not result in a significant change in the raclopride-induced increase in total catalepsy score. Rats injected with 40 nl saline + raclopride (s.c.) obtained a total catalepsy score of 9, compared with those receiving 40 nl atropine + raclopride (s.c.) which had a total catalepsy score of 10.

3.4. The occupancy of muscarinic receptors by atropine following intracerebral injections

Occupancy of muscarinic receptors by atropine was assessed as the average occupancy in either the dorsal or ventral half of the striatum, following injection of 50, 100 or 500 nl of atropine (5 mg/ml) into the two striatal sites. The results in Fig. 3 show that high levels of occupancy ranging from 74% to 82% were found in the ventral striatum following ventral injections and a similar range of occupancies (59–95%) were determined in the dorsal stratum following dorsal injections. Visual and computer-assisted examination of the autoradiographic films indicated that there was considerable spread of atropine from the ventral injection site following the 500 nl injection which resulted in 74% occupancy throughout ventral and dorsal regions of the striatum. Following dorsal injection of 500 nl atropine into the dorsal site, occupancy levels of 95% were found in the dorsal striatum and 58% in the ventral striatum. There was also considerable spread to surrounding structures including the cortex. Spread from the injection site was also seen to a lesser extent with 100 nl injections, as shown by the occupancy levels in Fig. 3. For example, following 100 nl dorsal injections, 80% of dorsal muscarinic receptors were occupied, while only 18% were occupied in the ventral striatum. Conversely, following ventral injections of 100 nl, 82% of ventral receptors were occupied and 21% of dorsal. However, smaller, discrete areas of occupancy were obtained following injection of 50 nl atropine, as shown in the representative autoradiographics presented in Fig. 4.

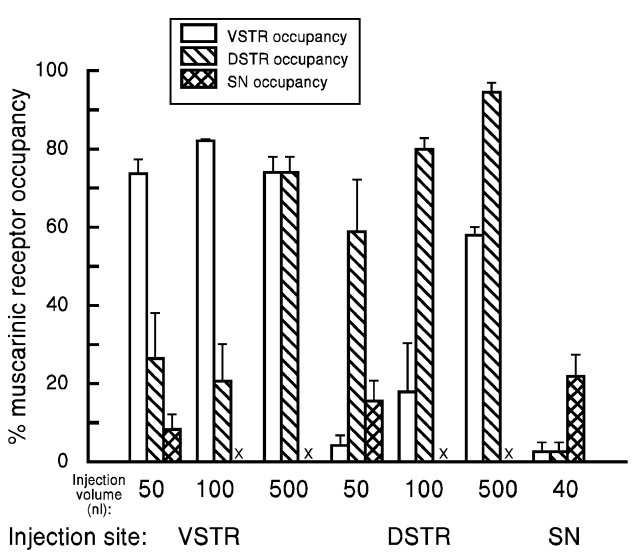

Fig. 3. Percentage of muscarinic cholinergic receptors occupied in the VSTR, DSTR and SN at 2 h following 50 nl atropine (5 mg/ml) injected into the striatum or 40 nl into SN. X = not determined.

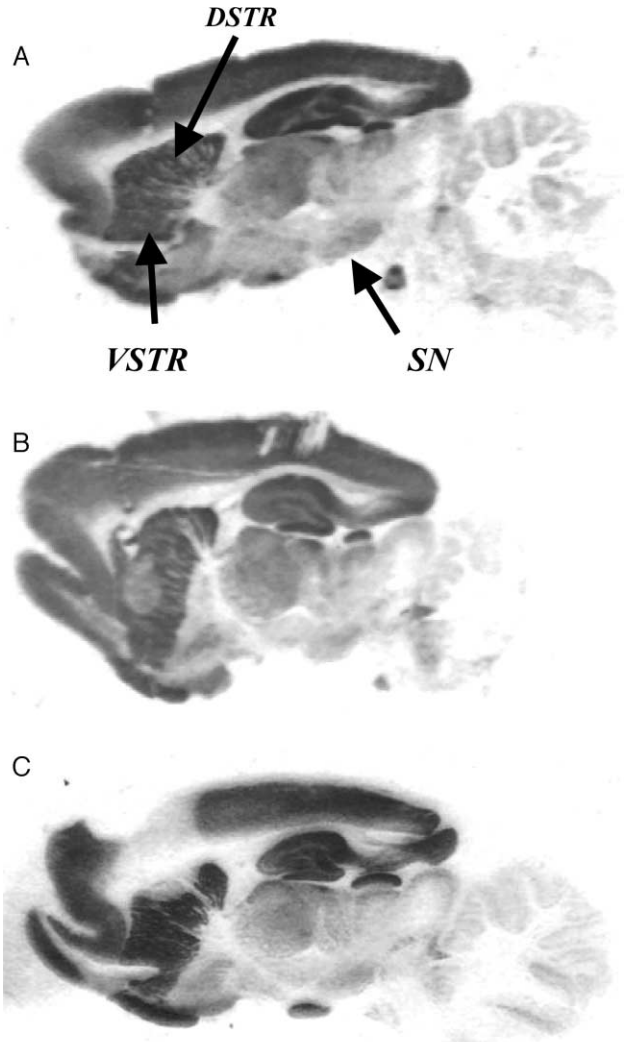

Fig. 4. Autoradiographs demonstrating the occupancy of muscarinic receptors in the striatum at 2 h following saline (A), or atropine (5 mg/ml: 50 nl) (B and C) injection into the ventral striatum (VSTR) and dorsal striatum (DSTR), respectively. The paler areas of receptor occupancy by atropine are indicated by the arrows.

3.5. Mapping the site of action of atropine in reducing catalepsy

The site of action of atropine was mapped using a modification of the technique described in Cameron and Crocker (1989) and Crocker and Cameron (1992). Initially, coronal sections from rats killed 2 h after 100 nl injections of atropine into the ventral striatum, which reduced raclopride-induced catalepsy, were visualised using a computerised image analysis system. This system comprised a video camera (attached to a computer) suspended over a light source on which the autoradiographic film is placed. The site and area of atropine's occupancy of muscarinic receptors was traced from the computer screen onto transparent sheets, along with appropriate anatomical landmarks, that could be overlaid on maps in the brain atlas of Paxinos and Watson (1986). When these areas of occupancy were overlapped, a common area of intersection was identified in the ventral striatum, which contained the muscarinic receptors whose blockade was associated with reducing catalepsy. Its location is shown diagrammatically in Fig. 5A. Next, areas of atropine occupancy from rats in which injections of 100 nl atropine into the dorsal striatum failed to reduce catalepsy were mapped. It was found that these areas did not overlap with the common area identified as reducing catalepsy. To determine the area more precisely, sagittal sections were prepared from rats that had received 50 nl injections of atropine into either the dorsal or ventral striatum. These sections were examined by image analysis and the common area of intersection associated with reductions in catalepsy was mapped using the same method described before. The level of muscarinic receptor occupancy in the functionally identified ventral 'atropine' site, shown in Fig. 5B, associated with reductions in raclopride-induced catalepsy, was $74 \pm 3.7\%$.

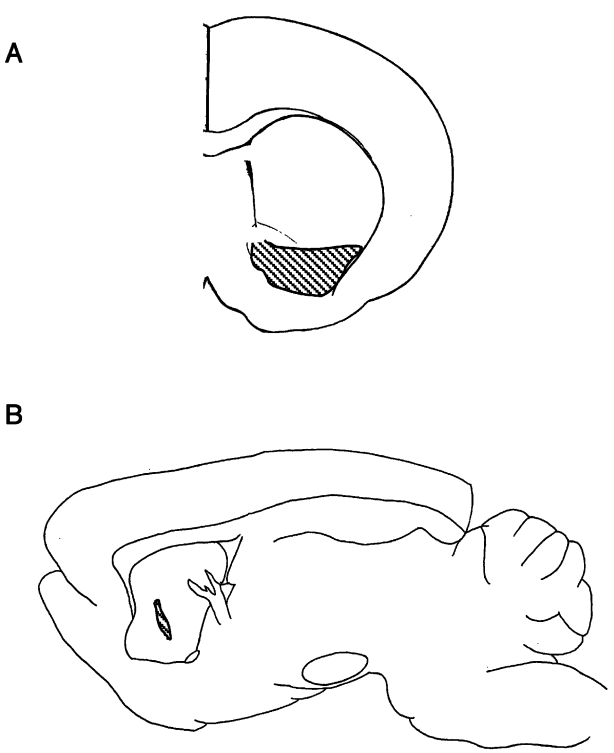

Fig. 5. Site of muscarinic receptors mediating reductions in raclopride-induced catalepsy. (A) The hatched area represents the common area of muscarinic receptor occupancy associated with reductions in raclopride-induced catalepsy. The area was mapped using 100-nl injections: $n = 7$ rats, $n = 35$ sections: approximate coordinates of atropine 'site' are A-P, $\sim +1.2$ mm; L, ~ 1 to 4 mm; and D-V, ~ -6.5 to -8 mm. (B) The hatched area represents the common area of muscarinic receptor occupancy associated with reductions in raclopride-induced increases in catalepsy. The area was mapped using 50-nl injections: $n = 6$ rats, $n = 13$ sections: approximate coordinates of atropine 'site' are A-P, -0.2 to $+0.45$ mm; L, ~ 2.4 mm; and D-V, -5.2 to -6.6 mm.

4. Discussion

The aim of the current study was to investigate further the interaction between cholinergic and dopaminergic drugs within the basal ganglia by determining the site of action of atropine in reducing the motor side effects resulting from dopamine D2 receptor blockade. It is well accepted that there is an association between the development of motor side effects and the actions of antipsychotic drugs at dopamine D2 dopamine receptors, which is supported by the relationship between the affinity of an antipsychotic drug for the dopamine D2 receptor and its propensity to produce motor side effects (Creese et al., 1976; Seeman et al., 1976). Further, both human studies using positron emission tomography (PET), and experimental studies have shown that motor side effects, such as muscle rigidity, occurred when threshold levels in the range of 74–82% of striatal dopamine D2 receptors are occupied by an antipsychotic drug (Farde et al., 1992; Hemsley and Crocker, 1998, 1999). However, it is unclear where muscarinic receptor antagonists act to reduce the effects of D2 blockade, thereby reducing the severity of motor side effects.

Atropine dose-dependently reduced catalepsy induced by raclopride, but had no effect on catalepsy when administered alone. This effect is consistent with reductions observed in two earlier studies using a selective dopamine D2 receptor antagonist to induce catalepsy (Ogren and Fuxe, 1988; Haraguchi et al., 1997). Other studies have investigated the effects of muscarinic receptor antagonists on catalepsy induced by antipsychotic drugs, such as haloperidol (Costall et al., 1972; Sayers et al., 1976; Haraguchi et al., 1997; Ushijima et al., 1997). Furthermore, these effects have been shown to be dose-dependent (Ushijima et al., 1997), thus, supporting the findings of the present study. However, the magnitude of these effects cannot be compared across studies due to differences in the muscarinic receptor antagonist used and the method of assessment of catalepsy.

Atropine significantly and dose-dependently reduced catalepsy at doses of 10 and 5 mg/kg, which occupied

83 ± 16.7% and 69 ± 4.9% of striatal muscarinic receptors, respectively. Atropine alone (5 mg/kg) did not occupy striatal or nigral dopamine D2 receptors, nor affect the levels of dopamine D2 occupancy by raclopride, indicating that its effects were not mediated by indirect changes in dopamine D2 blockade. As expected, raclopride did not occupy muscarinic receptors, nor did the addition of raclopride affect the binding of atropine to muscarinic receptors.

There is little published information about the levels of muscarinic receptor occupancy by muscarinic receptor antagonists in the context of behavioural or motor endpoints. Haraguchi et al. (1997) reported that scopolamine reduced catalepsy induced by a range of dopamine D1 and D2 receptor antagonists, and found that higher levels of muscarinic occupancy were associated with greater reductions in catalepsy. These findings are consistent with those from in vivo and in vitro studies, which demonstrated an inverse relationship between the incidence of motor side effects and the affinity of a variety of antipsychotic drugs for muscarinic receptors in the striatum (Miller and Hiley, 1974; Snyder et al., 1974) and those of the current study.

The location of the muscarinic receptors involved in the modulation of motor side effects is unclear. The striatum has a high concentration of muscarinic (Boyson et al., 1988) and dopamine receptors (Bouthenet et al., 1987), and has been implicated in the production of catalepsy (Fog, 1972; Sanberg, 1980). The substantia nigra pars compacta also contains high concentrations of dopamine D2 (Bouthenet et al., 1987) and muscarinic receptors (Gronier and Rasmussen, 1998). Both regions play key roles in the motor regulation and behaviour (Double and Crocker, 1995) and were investigated as possible sites of muscarinic modulation of dopamine D2-related motor changes.

Injections of 50–500 nl atropine into the ventral striatum produced significant reductions in raclopride-induced catalepsy, similar to those obtained following subcutaneous injection of atropine. However, no significant effects on catalepsy were observed following injection of atropine into the dorsal striatum or substantia nigra (Fig. 3). Atropine injections of 50 nl into the ventral striatum reduced catalepsy significantly, whereas injections of the same volume into the dorsal striatum and substantia nigra produced non-significant changes in catalepsy scores. These findings provide strong support for the involvement of muscarinic receptors located in the ventral striatum mediating the effects of atropine in reducing raclopride-induced catalepsy.

This conclusion was strengthened by the functional localization of the muscarinic receptors involved in reducing raclopride-induced catalepsy using a novel technique developed by us to localise striatal dopamine and muscarinic receptors mediating changes in behaviour (Cameron and Crocker, 1989; Crocker and Cameron, 1992) and muscle tone (Hemsley and Crocker, 2001). In the current study, we found a common area of muscarinic receptor occupancy in the ventral striatum in all rats exhibiting reductions in raclopride-induced catalepsy. Further, no overlap was found between this 'atropine' site and areas of muscarinic receptor occupancy in rats receiving dorsal injections of atropine, which caused no decrease in catalepsy. The 'atropine' site (Fig. 5) was also shown to mediate changes in raclopride-induced muscle rigidity, assessed as increases in tonic electromyographic (EMG) activity (Hemsley and Crocker, unpublished observations). The level of muscarinic occupancy in the ventral 'atropine' site found to be associated with reductions in catalepsy and increased EMG activity was 74%. Despite levels up to 94% occupancy being determined in the dorsal striatum, no effects on catalepsy were observed, again supporting the concept of a site-specific effect of atropine. These findings provide further support for the role of the ventral site in the expression of behaviour.

The results in the current study suggest that a threshold of muscarinic receptor occupancy may need to be reached before effects on catalepsy are observed. Thus, following dorsal striatal injections of 500 nl atropine, there was 58% occupancy of ventral striatal receptors which was not associated with a significant decrease in catalepsy (Fig. 3). However, ventral striatal occupancies in excess of 74% were associated with decreases in catalepsy (Fig. 3), suggesting a threshold between 58% and 74%. This range is also consistent with values obtained from the study in which atropine was administered subcutaneously, where striatal occupancies of 69% or greater were associated with significant reductions in catalepsy (Fig. 1).

The maximum level of muscarinic receptor occupancy assessed in the substantia nigra was 22% at 2-h post-injection, which may well be insufficient to modify raclopride-induced catalepsy, particularly in view of a report that nigral muscarinic receptors do mediate changes in catalepsy (De Montis et al., 1979). Experiments are in progress to determine whether higher levels of nigral muscarinic receptor occupancy are associated with reductions in raclopride-induced catalepsy. In addition, it is possible that atropine acts at sites other than the striatum to reduce catalepsy and additional sites within the basal ganglia are also being investigated. Possible sites include the subthalamic nucleus and entopeduncular nucleus, both sites we have shown to be involved in dopamine-mediated control of motor function (Hemsley and Crocker, unpublished observations), and contain muscarinic receptors (Kayadjanian et al., 1997; Gronier and Rasmussen, 1998).

The current findings suggest a specific role for muscarinic receptors in the ventral striatum in reducing dopamine D2 receptor antagonist-mediated catalepsy. This role can be considered in the context of the anatomical and functional heterogeneity of the striatum. For example, there are differences in the afferent neurones terminating in the dorsal and ventral regions. The dorsal striatum receives projections from the sensorimotor cortex and sub-

stantia nigra, while the ventral striatum receives input from both the ventral tegmental area and substantia nigra (McGeorge and Faull, 1989; Carelli and West, 1991), together with some input from the sensorimotor cortex. Thus, the ventral striatum is an interface between limbic and motor brain regions and could therefore be postulated to integrate motivational and motor commands. In contrast, the motor area for the hind limb of the rat is located within the dorsal striatum, and therefore, it is also reasonable to suggest that a blockade of muscarinic receptors at this site could result in modifications of catalepsy.

The failure of dorsal striatal injections of atropine to modulate raclopride-induced catalepsy may relate to the nature of the cataleptic state. Although movement of the limbs is primarily assessed as a measure of catalepsy, catalepsy is a behavioural syndrome that affects the entire body. More pertinent, however, is the fact that cataleptic rats have tonic muscular activation and maintain the use of their limbs (Fog, 1972). Raclopride-treated animals in the current study could move normally, albeit less frequently and at a slower pace than an untreated animal. Therefore, catalepsy appears to involve impairments in the programming or initiation of motor responses, possibly involving motivation, which utilises the integrating and processing capacity of the ventral striatum.

This concept is supported by studies showing catalepsy (Ossowska et al., 1990; Wardas et al., 1995) and muscle rigidity (Hemsley and Crocker, 2001), observed following discrete intracerebral injections of dopamine receptor antagonists to be mediated by receptors in the ventral, but not dorsal striatum. The current findings, by suggesting that dopamine and muscarinic receptor mechanisms in the ventral striatum play a key role in the regulation of behaviour and motor function, add to an increasing body of evidence supporting the importance of functional heterogeneity within the striatum (Eagle et al., 1999).

The importance of the ventral striatum in the expression of behaviour is further strengthened by a consideration of studies investigating dopamine agonist-induced behaviours. We reported the functional localization of dopamine D2 receptors mediating dopamine agonist-elicited stereotyped behaviour to a ventral striatal site (Cameron and Crocker, 1989). These findings were consistent with those of other workers who reported that dopamine receptors located in the ventral striatum regulate the induction of dopamine agonist-induced stereotypy and locomotor activity (Jicha and Salamone, 1991). It is also well established that muscarinic receptor antagonists (Scheel-Kruger, 1970) and agonists (Arnfred and Randrup, 1968) affect dopamine agonist-elicited behaviours. In a study to localise the striatal muscarinic receptors involved in modulating apomorphine-induced stereotypy using an irreversible muscarinic ligand (Crocker and Cameron, 1992), muscarinic receptors were localised in the same discrete ventral striatal area as the D2 receptors mediating stereotypy. Again, these findings emphasise the importance of the ventral striatum in mediating interactions between dopamine and acetylcholine.

In summary, the present study demonstrates an interaction between the cholinergic and dopaminergic systems within the ventral region of the striatum, whereby atropine dose-dependently modulates the effects of raclopride in producing cataleptic behaviours. These findings have important implications for the understanding of the way in which the striatum regulates the expression of behaviour.

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