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Gradient of dopamine responsiveness to dopamine receptor agonists in subregions of the rat nucleus accumbens

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Abstract

The present study sought to investigate the possibility that the degree of selectivity of dopamine D3/D2 receptor agonists such as quinelorane, 7-hydroxy-2-dipropylaminotetralin (7-OH-DPAT), quinpirole and apomorphine on dopamine D3 over D2 receptor subtypes can be assessed by measuring dopamine transmission in the shell vs. core compartments of the nucleus accumbens by using microdialysis in freely moving rats. Significant reductions in dialysate dopamine levels compared to vehicle-treated animals were observed in the shell of the nucleus accumbens with 3, 10 and 30 μg/kg quinelorane, 100 μg/kg 7-OH DPAT, 25 and 100 μg/kg quinpirole, and 100 μg/kg apomorphine. In the core subregion, significant reductions in dopamine were seen at 10 and 30 μg/kg quinelorane, 25 and 100 μg/kg 7-OH-DPAT, 100 μg/kg quinpirole and 100 μg/kg apomorphine. However, a significant shell/core dichotomy could only be observed in response to the lowest dose of quinelorane (3 μg/kg) with the shell being hyper-responsive compared with the core. The present findings suggest that quinelorane is one of the most selective dopamine D3 receptor agonists based on its ability to target the shell subregion of the nucleus accumbens.

Keywords: Microdialysis; Dopamine; Dopamine D3/D2 receptor agonist; Nucleus accumbens

1. Introduction

The nucleus accumbens occupies a prominent position in the ventral striatum and is the main target of the mesotelencephalic dopamine system. As such, the nucleus accumbens has been the framework of theories exploring the chemoarchitectural substrates of schizophrenia and other affective disorders as well as theories of reward and motivation, including aspects of drug addiction. The existence of subterritories within the nucleus accumbens has now been widely supported by histochemical, in vivo neurochemical, electrophysiological as well as morphological and ultrastructural studies (for a recent review, see Zahm, 2000). Specifically, the nucleus accumbens can be subdivided into a dorsolateral core, which primarily connects to somatic motor output systems, and a ventromedial shell, which is more intimately linked to viscero-endocrine effector systems (Zahm, 2000). Such a structural compartmentation is suggested by the various neurochemical and cellular features of the nucleus accumbens that designate specific afferent and efferent systems, probably involved in different behavioural functions. For example, the shell responds more sensitively than the core to a variety of stimuli, including drugs of abuse (Pontieri et al., 1995; Hedou et al., 1999; Heidbreder et al., 1999), restraint and pharmacological stress (Deutch and Cameron, 1992; Horger et al., 1995; Kalivas and Duffy, 1995; King et al., 1997), food (Bassareo and Di Chiara, 1999) and novelty (Rebec et al., 1997; Rebec, 1998). The shell subregion may also be an important target for mediating the therapeutic effects of antipsychotic drugs, since all antipsychotic agents increase Fos expression in the shell, but only those associated with extrapyramidal motor symptoms stimulate Fos expression in the core (Deutch, 1996). Furthermore, atypical neuroleptics such as clozapine or risperidone, which are characterised by a potent 5-HT_2 receptor antagonism, elicit greater changes in dopa-

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mine concentrations in the shell compared with the core of the nucleus accumbens. In contrast, typical neuroleptics such as haloperidol and raclopride, which are characterised by a potent dopamine D2 receptor antagonism, produce larger modifications in dopamine levels in the core than in the shell of the nucleus accumbens (Deutch and Cameron, 1992; Graybiel et al., 1990; Marcus et al., 1996; Merchant and Dorsa, 1993). Thus, the differential involvement of the shell and core in responding to a variety of stimuli and in particular to antipsychotic drug treatment suggests potential differences in the functional roles of these subregions in response to dopamine receptor agonists or antagonists.

A link between dopamine D3 receptors and schizophrenia has been originally suggested from the observation that dopamine D3 receptors are expressed preferentially in granule cells of the islands of Calleja and in medium-sized spiny neurones of the rostral and ventromedial shell of the nucleus accumbens, regions in which the dopamine D2 receptors are scarcely expressed (Diaz et al., 1994, 1995; Le Moine and Bloch, 1996). More recently, the distribution of the dopamine D3 receptor in the human brain has been shown to follow a rather similar pattern to that observed in the rat brain (Hall et al., 1996; Shafer and Levant, 1998; Suzuki et al., 1998; Gurevich and Joyce, 1999). Thus, dopamine D3-receptors may play a different role than dopamine D2 sites in the control of motor behaviour (Baik et al., 1995; Millan et al., 1995; Kelly et al., 1998; Shafer and Levant, 1998) and may be implicated in psychotic states and their treatment (Shafer and Levant, 1998).

However, recent studies point to the fact that caution is required in attributing the effects of dopamine D3 agonists to activation of dopamine D2- and/or D3-receptors (Levant, 1997). In the present study, we investigated the possibility that the degree of selectivity of a dopamine D3/D2 agonist such as quinelorane on dopamine D3 over D2 receptor subtypes can be assessed by measuring dopamine transmission in vivo in the shell vs. core compartments of the nucleus accumbens. Additional agonists with varying selectivity for dopamine D3 over D2 receptors such as 7-hydroxy-2-dipropylaminotetralin (7-OH-DPAT), quinpirole, and apomorphine, were also tested for comparative purposes.

2. Materials and methods

2.1. Subjects

Four groups of male Sprague–Dawley rats (Charles River, UK) weighing 250–300 g were housed in groups of four to five per cage in a temperature- and humidity-controlled environment with free access to food (restricted to 20 g/day after surgery) and water. Rats were kept on a 12-h light/dark cycle with lights on at 0700 h. All experimental procedures carried out in the present study were within the guidelines of the Animals (Scientific Procedures) Act 1986.

2.2. Surgical procedure

The animals were anaesthetised using a mixture of medetomidine (0.04 ml/100 g s.c.) and fentanyl (0.9 ml/kg i.p.). Once deep anaesthesia was obtained, rats were transferred to a stereotaxic frame (David Kopf, Topanga, CA) with the upper incisor bar set at −3.2 mm below the interaural line. Rats were put on a homeothermic blanket set at 37 °C throughout. An incision was made to reveal bregma, from which all co-ordinates were taken. Holes

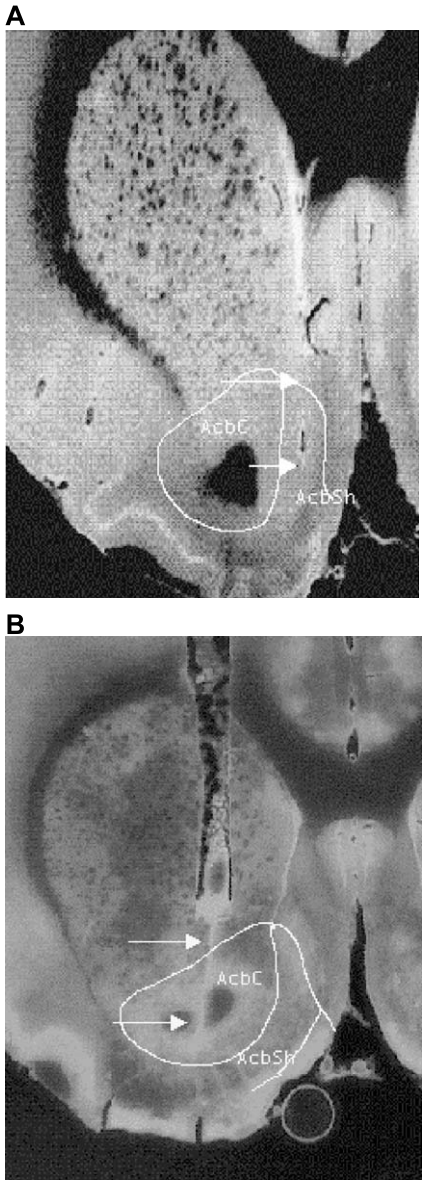

Fig. 1. Photomicrograph of a coronal section at the level of the shell (Panel A) and core (Panel B) subregions of the nucleus accumbens. Arrowheads indicate the segment of the microdialysis membrane in both subterritories of the nucleus accumbens.

Table 1
Basal levels (pmol) of dopamine in the shell vs. core subregions of the nucleus accumbens

Drug study	Shell (pM)	Core (pM)
Quinelorane	1821.3 ± 259.9	668.7 ± 163[a]
7-OH-DPAT	676.21 ± 78.9	437.36 ± 36.1
Quinpirole	997.3 ± 71.3	766.5 ± 46.8
Apomorphine	709.83 ± 67	1097.8 ± 92

[a] $P < 0.001$.

were then drilled for four anchor screws, and another for unilateral placement of an intracerebral cannula guide (CMA 11, Biotech, UK) into the shell or core subregions of the nucleus accumbens. The co-ordinates with respect to bregma were as follows (Paxinos and Watson, 1986): for the shell of the nucleus accumbens: +1.2 mm anterior (A) to bregma; 0.8 mm lateral (L) to the midsagittal sinus; 5.8 mm ventral (V) to the dura surface; for the core of the nucleus accumbens: A=+1.2 mm, L=2.0 mm, V=5.8 mm. The dura directly beneath the guide was broken, and the guide implanted. Using dental cement, the guide and a tether screw (Presearch, UK) placed posterior to the probe, were secured in place, and the wound sealed. Anaesthesia was reversed using a mixture of atipamezole (0.02 ml/100 g s.c.) and nalbuphine (0.02 ml/100 g s.c.). The rats were monitored until they regained their righting reflex. The animals were allowed to recover for 1 week before the dialysis experiment started. Eighteen hours prior to the start of experimentation, the animals were randomly assigned to one of six circular polycarbonate microdialysis cages (ø 285 mm; H: 355 mm) and left to acclimatise to their new environment.

2.3. Brain microdialysis procedure

Before implantation, microdialysis probes (CMA/11, 2 mm active cuprophane membrane length, Biotech) were placed in 70% ethanol, and perfused with artificial cerebrospinal fluid (aCSF) containing 125 mM nucleus accumbens, 2.5 mM KCl, 1.18 mM $MgCl_2 \cdot 6H_2O$, 1.26 mM $CaCl_2 \cdot 2H_2O$, and 2.0 mM Na_2HPO_4, adjusted to pH 7.4 with 85% H_3PO_4 (high-performance liquid chromatography grade) at 2–5 μl min^{-1}. Both inlet and outlet tubings of the probe were attached to a dual quartz lined two-channel liquid swivel (Instech 375/D/22QE) on a low mass spring counterbalanced arm, which in turn was connected to a gas tight syringe (CMA Exmire 1 ml, Biotech) on a microinfusion pump (Univentor 864, Biotech). The animals

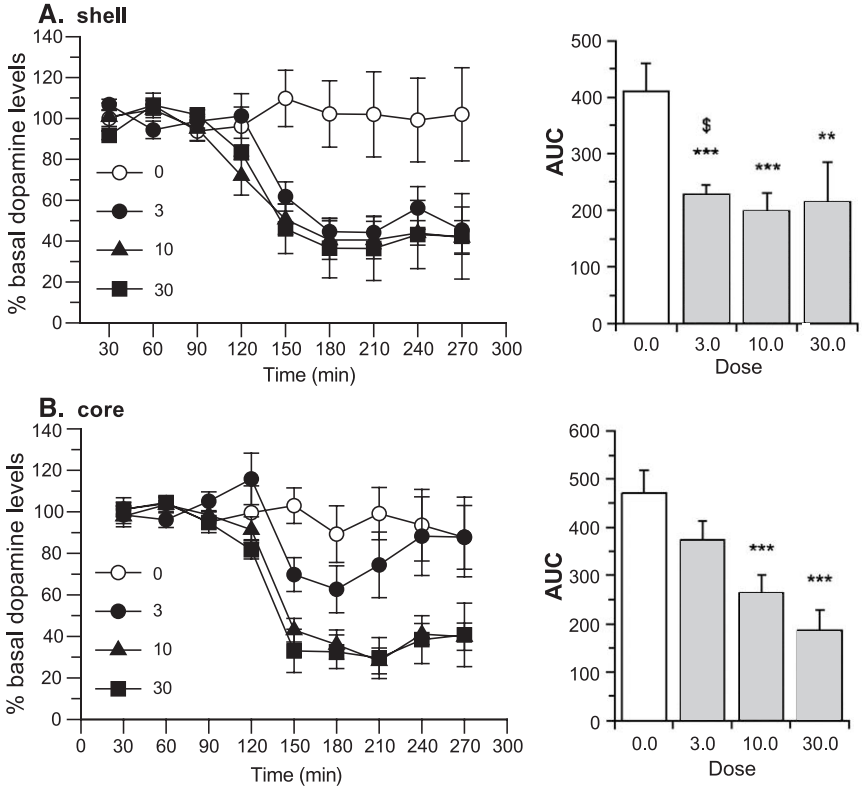

Fig. 2. Inhibition of dopamine release in (A) shell and (B) core of the rat nucleus accumbens by quinelorane (0, 3, 10, 30 μg/kg s.c.) as measured by in vivo microdialysis. Figures on the left show data expressed as mean (±S.E.M.) percent baseline dopamine (Shell: 1.8 ± 0.3 nM vs. Core: 0.7 ± 0.2 nM) and each data point represents the mean dialysate sampling over 30-min periods. Arrows indicate when drug was administered. Figures on the right show area under the curve (AUC) in response to drug. An overall ANOVA revealed significant main effects of dose [$F(3,42) = 13.2$; $P < 0.0001$] and time [$F(8,336) = 46.5$; $P < 0.0001$]; and a significant subregion × dose × time interaction [$F(24,336) = 0.8$; $P < 0.05$]. **$P < 0.01$; ***$P < 0.001$ in comparison to vehicle; $^{\$}P < 0.05$ in comparison to core.

were briefly anaesthetised with isoflurane to allow removal of the guide pin and insertion of the microdialysis probe into the guide cannula. Probes were perfused at 1 μl/min for 2 h before sample collection started. After this equilibration period, three basal samples were collected at 30-min intervals, before both shell and core animals were administered with test compound. Dialysate samples were collected into glass vials (Chromacol, UK) containing 5 μl 0.03% acetic acid for an additional 180-min period. Microdialysates were then assayed using high-performance liquid chromatography (HPLC). After the first microdialysis experiment, probes were removed and the guide pin replaced. Animals were returned to their home cages for 7 days before their second use, within the same study, according to a randomisation process.

2.4. Chromatographic analysis of brain microdialysates

Dialysate samples (10 μl) were analysed using HPLC coupled to electrochemical detection (HPLC-ECD) equipped with 50 μl loop, reverse-phase 2.1 × 150 mm Waters Symmetry C18 3.5 mm column and guard column, and an electrochemical amperometric detector (Antec-Decade, Leyden, the Netherlands), which was used in conjunction with a computerised chromatography workstation (Waters Millennium, USA). The column temperature was set at +35 °C, with the electrochemical detector voltage at +0.65 V. The mobile phase comprised of KH_2PO_4 0.07 M, OSA·Na 1 mM, EDTA.Na_2 0.1 mM in methanol (MeOH)/H_2O (v/v) at pH 2.75, which was filtered through a 0.22-μm filter (Millipore, Bedford, MA, USA), degassed under vacuum and delivered at a rate of 0.3 ml/min. The retention time and the area of the peaks of the endogenous components were compared to a standard solution containing 1000 and 200 pg/ml dopamine. The detection limits for dopamine was 2 fmol.

2.5. Histology

After completion of the final experiment, brains were removed and fixed in 4% paraformaldehyde in a phosphate buffer. Histological verification of probe placement was made via serial coronal sections (40 μm thick) using a cryostat. The sections were then processed for Fast Cresyl violet stain (Fig. 1).

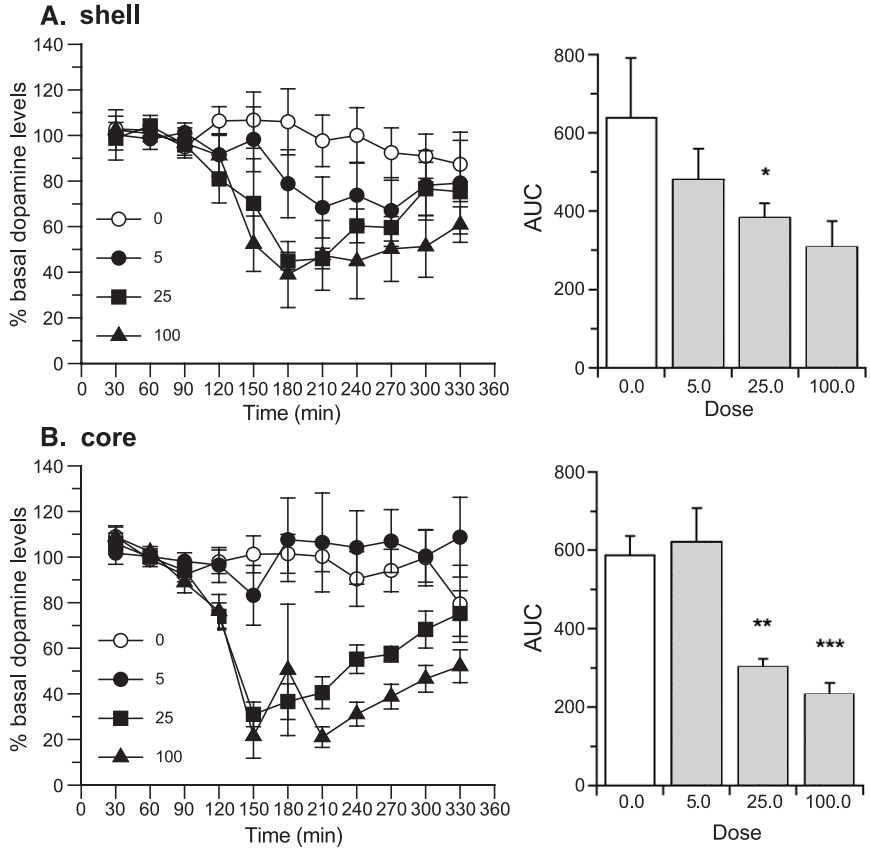

Fig. 3. Inhibition of dopamine release in (A) shell and (B) core of rat nucleus accumbens by 7-OH-DPAT (0, 5, 25, 100 μg/kg s.c.) as measured by in vivo microdialysis. The data are expressed as mean (± S.E.M.) percent baseline dopamine and each data point represents the mean dialysate sampling over 30-min periods. Arrows indicate when drug was administered. An overall ANOVA revealed significant main effects of dose [$F(3,50) = 14.0$; $P < 0.0001$] and time [$F(7,350) = 6.7$; $P < 0.0001$]; but no significant subregion × dose × time interaction. Post hoc on separate analysis of core and shell responses revealed that the dose effect was due to a significant reduction in dopamine levels in the shell at 100 μg/kg ($P = 0.04$) and in the core by 25 μg/kg ($P = 0.001$) and 100 μg/kg ($P = 0.0003$) in comparison to vehicle groups.

2.6. Drugs

Four separate groups of rats were used in this experiment, each receiving doses of a different drug. The drugs chosen for this study differ in potency and selectivity for the dopamine D2 and D3 receptors but display similar behavioural profiles, typically hypolocomotion at low doses (Storey et al., 1995; Daly and Waddington, 1993). Treatment was randomly assigned within each group so that each animal received no more than three different doses of either quinelorane (3, 10, 30 μg/kg), 7-OH-DPAT (5, 25, 100 μg/kg), quinpirole (5, 25, 100 μg/kg) or apomorphine (5, 25, 100 μg/kg). All compounds (Sigma) were dissolved in saline and administered subcutaneously.

2.7. Data analysis

Basal dialysate levels of dopamine were analysed by a $2 \times 4 \times 3$ analysis of variance (ANOVA) with main factors of subregion (shell vs. core) and drug (quinelorane, 7-OH-DPAT, quinpirole, apomorphine) and a repeated measurements factor of time (three blocks of 30 min each). Time course changes in dialysate dopamine levels were expressed as percentage of baseline. The raw data were analysed by a $2 \times 4 \times 6$ ANOVA with main factors of subregion (shell vs. core) and drug dose and a repeated measurements factor of time (six blocks of 30 min each). Total dopamine release was calculated using the trapezoid rule for determination of area under the curve (AUC). The differences between individual means were assessed with the post hoc Fischer's test. Statistical significance was set at a probability level of $P < 0.05$ for all tests.

3. Results

3.1. Basal dialysate levels of dopamine in the shell vs. core subregions of the nucleus accumbens

Basal levels of dopamine were shown to be significantly higher in the shell region (1.8 ± 0.26 nM) compared with its core counterpart (0.7 ± 0.16 nM) in the quinelorane study ($F(1,52) = 5.544$; $P < 0.001$). However, significant differences between basal levels in core and shell subre-

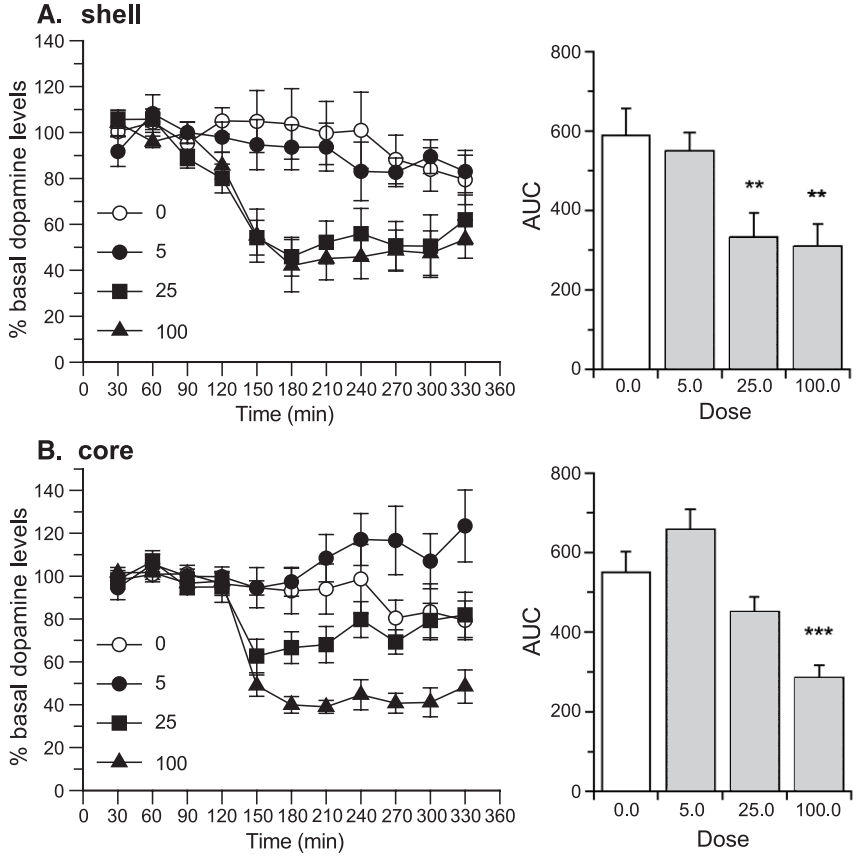

Fig. 4. Inhibition of dopamine release in (A) shell and (B) core of rat nucleus accumbens by quinpirole (0, 5, 25, 100 μg/kg s.c.) as measured by in vivo microdialysis. The data are expressed as mean (± S.E.M.) percent baseline dopamine and each data point represents the mean dialysate sampling over 30-min periods. Arrows indicate when drug was administered. An overall ANOVA revealed significant main effects of dose [$F(3,48) = 14.0$; $P < 0.0001$;] and time [$F(7,336) = 10.7$; $P < 0.0001$]; but no significant subregion × dose × time interaction. Post hoc on separate analysis of core and shell responses revealed that the dose effect was due to a significant reduction in dopamine levels in the shell at 25 μg/kg ($P = 0.006$) and 100 μg/kg ($P = 0.004$) and in the core by 100 μg/kg ($P = 0.001$) in comparison to vehicle groups.

gions were not observed in any of the other studies (see Table 1).

3.2. Quinelorane-induced changes in dialysate dopamine levels in the shell vs. core subregions of the nucleus accumbens

Quinelorane produced a significant decrease in dialysate dopamine levels in both the shell and core subregions of the nucleus accumbens (Fig. 2) However, the lowest dose of quinelorane (3 μg/kg) produced only a slight transient decrease in dopamine levels in the core of the nucleus accumbens, whereas the same dose of quinelorane induced a marked decrease in dialysate dopamine levels in the shell of the nucleus accumbens. The overall ANOVA yielded significant main effects of quinelorane dose [$F(3,42) = 13.2$; $P < 0.0001$] and time [$F(8,336) = 46.5$; $P < 0.0001$] and a significant subregion × dose × time interaction [$F(24,336) = 0.8$; $P < 0.05$]. Post hoc analysis of core vs. shell at 3 μg/kg revealed a significant difference ($P = 0.0015$).

3.3. 7-OH-DPAT-induced changes in dialysate dopamine levels in the shell vs. core subregions of the nucleus accumbens

7-OH-DPAT produced a significant decrease in dialysate dopamine levels in both the shell and core subregions of the nucleus accumbens (Fig. 3) The overall ANOVA yielded significant main effects of 7-OH-DPAT dose [$F(3,50) = 14.0$; $P < 0.0001$] and time [$F(7,350) = 6.7$; $P < 0.0001$] but no significant subregion × dose × time interaction. Post hoc on separate analysis of core and shell responses revealed that the dose effect was due to a significant reduction in dopamine levels in the shell at 100 μg/kg dose ($P = 0.04$) and in the core by 25 μg/kg ($P = 0.001$) and 100 μg/kg ($P < 0.001$) doses in comparison to vehicle groups. No significant differences between subregions were observed at any dose level.

3.4. Quinpirole-induced changes in dialysate dopamine levels in the shell vs. core subregions of the nucleus accumbens

Quinpirole produced a significant decrease in dialysate dopamine levels in both the shell and core subregions of the

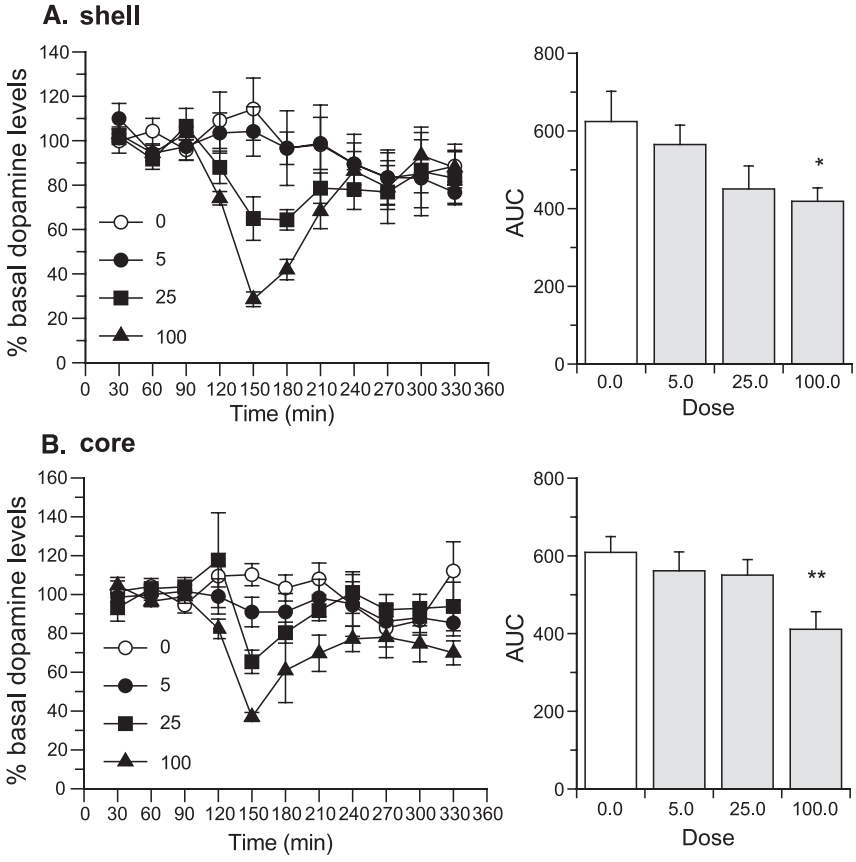

Fig. 5. Inhibition of dopamine release in (A) shell and (B) core of rat nucleus accumbens by apomorphine (0, 5, 25, 100 μg/kg s.c.) as measured by in vivo microdialysis. The data are expressed as mean (± S.E.M.) percent baseline DA and each data point represents the mean dialysate sampling over 30-min periods. Arrows indicate when drug was administered. An overall ANOVA revealed significant main effects of dose (0, 5, 25, 100 μg/kg s.c.) [$F(3,34) = 6.0$; $P < 0.01$] and time [$F(7,238) = 6.3$; $P < 0.0001$]; but no significant subregion × dose × time interaction. Post hoc on separate analysis of core and shell responses revealed that the dose effect was due to a significant reduction in dopamine levels in both regions by the 100 μg/kg in comparison to vehicle groups (shell $P = 0.003$; core $P = 0.004$).

nucleus accumbens (Fig. 4) The overall ANOVA yielded significant main effects of quinpirole dose [$F(3,48)=12.3$; $P<0.0001$] and time [$F(7,336)=10.7$; $P<0.0001$] but no significant subregion × dose × time interaction. Post hoc on separate analysis of core and shell responses revealed that the dose effect was due to a significant reduction in dopamine levels in the shell at 25 μg/kg ($P=0.006$) and 100 μg/kg ($P=0.004$) doses and in the core by 100 μg/kg dose ($P=0.001$) in comparison to vehicle groups. No significant differences between subregions were observed at any dose level.

3.5. Apomorphine-induced changes in dialysate dopamine levels in the shell vs. core subregions of the nucleus accumbens

Apomorphine produced a significant decrease in dialysate dopamine levels in both the shell and core subregions of the nucleus accumbens (Fig. 5) The overall ANOVA yielded significant main effects of apomorphine dose [$F(3,34)=6.0$; $P<0.01$] and time [$F(7,238)=6.3$; $P<0.0001$] but no significant subregion × dose × time interaction. Post hoc on separate analysis of core and shell responses revealed that the dose effect was due to a significant reduction in dopamine levels in both regions by the 100 μg/kg in comparison to vehicle groups (shell $P=0.003$; core $P=0.004$). No significant differences between subregions were observed at any dose level.

4. Discussion

The goal of the present study was to compare the effect of a range of dopamine receptor agonists with varying dopamine D2/D3 receptor affinities on extracellular dopamine levels in the shell vs. core subregions of the rat nucleus accumbens. Within the quinelorane treatment groups, a significant difference in dopamine levels was observed between the shell and core subregion of the nucleus accumbens, with levels in the shell being significantly higher than those in the core. However, across the four groups, basal microdialysate levels of dopamine were not shown to be significantly different in the shell region of the nucleus accumbens compared with the core. All 4 drugs tested (quinelorane, 7-OH-DPAT, quinpirole and apomorphine) produced a significant decrease in dopamine in both the shell and core. However, a shell/core dichotomy was observed in response to the lowest dose of quinelorane (i.e. 3 μg/kg) with the shell being hyper-responsive compared with its core counterpart.

In the present study, no significant overall difference in basal dialysate dopamine levels was observed. Within the literature, there is no general agreement as to whether extracellular dopamine levels are higher or lower in the shell vs. core of the nucleus accumbens. Studies in Sprague–Dawley rats have reported either higher basal levels of dopamine in the shell compared with the core (Barrot et al., 2000), lower levels of dopamine in the shell than in the core (King et al., 1997; Pierce and Kalivas, 1995), or no significant differences between the two subterritories of the nucleus accumbens (Cadoni and Di Chiara, 1999, 2000; Cadoni et al., 2000; Kalivas and Duffy, 1995; Pontieri et al., 1995; Sokolowski et al., 1998). Similarly, studies performed on Wistar rats have shown either higher levels in the core compared with shell (Heidbreder and Feldon, 1998; Hedou et al., 1999) or no significant differences in basal dopamine levels between the two subregions (Murphy et al., 2000; Wu et al., 1999). Finally, recent studies investigating conditioned dopamine release in the nucleus accumbens of Lister hooded rats failed to detect significant differences in basal levels between the shell and core subterritories (Ito et al., 2000). Thus, the evidence for differences in basal extracellular dopamine levels between subterritories of the nucleus accumbens is inconclusive and may be a consequence of differences in experimental conditions and environmental factors such as housing conditions or stress related events.

A link between dopamine D3 receptors and schizophrenia was originally suggested from the observation that dopamine D3 receptors are expressed preferentially in granule cells of the islands of Calleja and in medium-sized spiny neurones of the rostral and ventromedial shell of the nucleus accumbens, regions in which the dopamine D2 receptor expression is much lower (Diaz et al., 1994, 1995; Le Moine and Bloch, 1996). More recently, the distribution of the dopamine D3 receptor in the human brain has been shown to follow a similar pattern to that observed in the rat brain (Hall et al., 1996; Shafer and Levant, 1998; Suzuki et al., 1998; Gurevich and Joyce, 1999). Thus, dopamine D3 receptors may play a different role than dopamine D2 sites in the control of motor behaviour (Baik et al., 1995; Millan et al., 1995; Kelly et al., 1998; Shafer and Levant, 1998) and may be implicated in psychotic states and their treatment (Shafer and Levant, 1998; Harrison, 1999; Kerwin and Owen, 1999). The sensitivity of the shell region of the nucleus accumbens to drugs of abuse, stress and antipsychotic agents highlights the importance of this region, and subsequently the dopamine D3 receptor, as a target for mediating the therapeutic effects in a number of psychiatric disorders. Chronic antipsychotic treatment alters dopamine D3 receptor expression in rodents (Buckland et al., 1992, 1993). Changes in the density of D3 receptors in psychotic patients can be normalised by antipsychotic treatment (Gurevich et al., 1997). Furthermore, dopamine D3 receptor gene polymorphisms may be associated with the occurrence of schizophrenia (Shaikh et al., 1996). Finally, dopamine D3 receptors seem to be involved in the modulation of reward (Pilla et al., 1999; Vorel et al., 2002). In the present study, quinelorane was the only agonist to exhibit a core/shell differentiation in inhibition of dopamine release. Quinelorane's potential to

preferentially inhibit dopamine release in the shell of nucleus accumbens at low doses could be due to the degree of selectivity it displays for dopamine D3 over D2 receptors. Kreiss et al. (1995) showed that the potency of 11 dopamine receptor agonists to inhibit neuronal firing in vivo was significantly correlated with affinity for the rat dopamine D3 receptor. Specifically, quinelorane, which displayed 200-fold selectivity for the dopamine D3 receptor has been shown to be the most potent inhibitor of dopamine neuronal activity when compared to 7-OH-DPAT, quinpirole, and apomorphine which have selectivities of 60-, 138- and 2-fold, respectively. However, although the same rank order of potency is suggested by the neurochemistry data in the present study, specific targeting of the shell subregion of the nucleus accumbens does not suggest this degree of functional selectivity. Quinelorane was the only agonist to show a shell/core dichotomy, and only at the lowest dose tested therefore exhibiting a maximum of 3-fold selectivity in this model. The fact that a difference in basal levels of dopamine between the two subregions was also only observed in the animals treated with quinelorane raises the question as to whether the elevated dopamine levels in the shell during the study contributed to the shell/core dichotomy. However, previous unpublished in house studies with quinelorane where shell and core dopamine levels have not been significantly different from each other (approximately 1000 pM) have also shown a shell/core dichotomy suggesting that basal levels of dopamine within the shell are not significantly influencing the magnitude of the quinelorane response in this case. Thus, the absence of a significant shell/core dichotomy in response to treatment with the other dopamine D3/D2 receptor agonists used in this study suggests that these molecules do not show the selectivity required in this assay.

In accordance with these observations, reported selectivities of dopamine D3/D2 receptor agonists in in vitro assays are also extremely variable and dependent on the assay used, the radioligand, and whether it is the rat or human receptor that is under investigation. For example, radioligand binding studies show that the selectivity of quinelorane for the dopamine D3 receptor ranges between 95-fold in displacement of [^{125}I]iodosulpiride from Chinese hamster ovary cells transfected with human dopamine D3 or D2L receptor subtypes (Sokoloff et al., 1992) and 1.7-fold in autoradiographic studies using displacement of [^{3}H]quinpirole in rat striatum (D2 rich) compared to rat vestibulocerebellum (dopamine D3 receptor rich, Flietstra and Levant, 1998). In vitro functional assays also show variability. For example, Sautel et al. (1995) report that the functional selectivity of quinelorane is 21-fold in a mitogenesis assay measuring [^{3}H]thymidine incorporation, whereas Coldwell et al. (1999) found that the binding selectivity of quinelorane at human dopamine D3 receptors was 25-fold, whilst functional selectivity, as measured by cytocellular microphysiometry was reduced to 8-fold compared to quinpirole, with a functional selectivity of 25-fold, and proved to be the most selective agonist in this assay.

The ability of dopamine D3/D2 agonists to produce differential effects on dopamine release in subregions of rat nucleus accumbens depends, as one might expect, on the degree of selectivity of the agonist for the dopamine D3 receptor. However, caution must be exercised when attributing selectivity based on in vitro studies as variability in assay conditions can have marked effects on the observed result. In agreement with in vivo electrophysiological studies (Kreiss et al., 1995), the data from the present study suggest that quinelorane is the most selective of the agonists investigated for the rat dopamine D3 receptor based on its ability to target the shell subregion of the nucleus accumbens. Therefore, we conclude that quinelorane at the correct doses may provide a tool to specifically target the activity of the dopamine D3 receptor in the shell region of the nucleus accumbens.

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