Activation of dopamine D₁ receptors enhances cholinergic transmission and social cognition: a parallel dialysis and behavioural study in rats

Benjamin Di Cara, Fany Panayi, Alain Gobert, Anne Dekeyne, Dorothée Sicard, Lotte De Groote and Mark J. Millan

Institut de Recherches Servier, Department of Psychopharmacology, 125 chemin de Ronde, 78290 Croissy-sur-Seine, France

Abstract

Although dopaminergic mechanisms are known to modulate cognitive function and cholinergic transmission, their pharmacological characterization remains incomplete. Herein, the role of D₁ sites was evaluated employing neurochemical and behavioural approaches. By analogy to the acetylcholinesterase inhibitor, galantamine (0.0025–0.63 mg/kg s.c.), the selective and high efficacy D₁ receptor agonist, SKF 82958, dose-dependently (0.0025–0.63), robustly and potently enhanced extracellular levels of acetylcholine (ACh) in the frontal cortex and hippocampus of freely moving rats. A further agonist, SKF 81297 (0.04–0.63), mimicked this action whereas the selective antagonist, SCH 23390 (0.00063–0.63), decreased levels of ACh. In the presence of SCH 23390 (0.08), the facilitatory influence of SKF 82958 (0.04) upon ACh levels was abolished. In a model of social memory (recognition of a juvenile by an adult rat), galantamine (0.04–0.63), SKF 82958 (0.01–0.16) and SKF 81297 (0.001–0.16) dose-dependently abrogated amnesic effects of the muscarinic receptor antagonist scopolamine (1.25). Further, under conditions of spontaneous loss of recognition, mimicking the effects of galantamine (0.04–2.5), SKF 82958 (0.01–0.16) and SKF 81297 (0.04–1.25) dose-dependently and specifically facilitated social recognition. Conversely, SCH 23390 (0.0025–0.04) exerted a modest negative influence upon social recognition and, in its presence, the pro-cognitive properties of SKF 82958 were blocked. In conclusion, D₁ receptors exert a tonic, facilitatory influence upon cholinergic transmission and social recognition. Although the relationship between these actions awaits further clarification, these data underpin the relevance of D₁ receptors to CNS disorders in which cholinergic transmission and social cognition are disrupted.

Accepted 19 December 2005; Reviewed 14 February 2006; Revised 11 May 2006; Accepted 19 June 2006; First published online 22 August 2006

Key words: Acetylcholine, cognition, dopamine, frontal cortex, microdialysis.

Introduction

Cholinergic pathways in many cerebral structures are implicated in the modulation of cognitive function (Everitt and Robbins, 1997; Sarter and Bruno, 1997). Of particular importance, the frontal cortex (FCX) and hippocampus are intensely innervated by cholinergic fibres originating in the nucleus basalis magnocellularis (NBM) and lateral septal area respectively (Ammassari-Teule et al., 1993; Bigl et al., 1982; Mesulam et al., 1983). A disruption of cerebral cholinergic networks may contribute to the cognitive deficits of Alzheimer’s disease, Parkinson’s disease, schizophrenia and other CNS disorders (Dubois et al., 1990; Lawrence and Sahakian, 1998). Accordingly, drugs which interact with cholinergic transmission, such as direct muscarinic and nicotinic agonists or inhibitors of acetylcholinesterase (AChE), are of interest as therapeutic agents (Bentley et al., 2004; Friedman, 2004). An alternative approach would be to target mechanisms which control the activity of cholinergic pathways.

Dopaminergic systems are of particular interest since dopamine fulfils a broad role in cognitive function, exerting its effects in several corticolimbic...
structures, at least partially in interaction with cholinergic neurons (Levin et al., 1990; Myhrer, 2003; Nieoullon, 2002). Interestingly, all five classes of dopaminergic receptor have been implicated in the modulation of mnemonic function and D_{1} receptor antagonists are attracting increasing interest as antipsychotic agents with a potentially improved influence upon cognitive function (Joyce and Millan, 2005; Laszy et al., 2005). Nonetheless, most attention has to date been devoted to dopamine D_{1} receptors (Desimone, 1995; Goldman-Rakic, 2005) which are expressed in many structures controlling mnemonic function, including the FCX (Dubois et al., 1986; Huang et al., 1992) and hippocampus (Huang et al., 1992; Kohler et al., 1991). In the FCX, D_{1} receptors participate in the control of working memory and attentional processes (Cai and Arnsten, 1997; Sawaguchi and Goldman-Rakic, 1991). Over a critical range of doses, stimulation of frontocortical D_{1} receptors has shown to improve (and blockade of D_{1} receptors to impair) working memory in rodents and primates (Arnsten et al., 1994; Cai and Arnsten, 1997; Seamans et al., 1998). Dopamine D_{1} receptors in the hippocampus have also been reported to enhance cognitive function though, as considered in the Discussion, closely related D_{2} receptors may be involved in these actions. Further, in both the FCX and the hippocampus, there is evidence that activation of D_{1} (D_{2}) receptors reinforces glutamatergic mechanisms of long-term potentiation (LTP), a molecular substrate of memory formation (Li et al., 2003; Yang, 2000).

The FCX and hippocampus comprise possible sites of interaction between D_{1} receptors and cholinergic pathways in the modulation of mnemonic function. Supporting this notion, cholinergic nuclei projecting to the FCX and hippocampus are heavily innervated by dopaminergic pathways and contain a high density of D_{1} receptors (Huang et al., 1992; Rodrigo et al., 1998; Zaborsky and Cullinan, 1996). Further, several D_{1} receptor agonists elevated dialysis levels of ACh in the FCX (Acquas et al., 1994; Day and Fibiger, 1993; Steele et al., 1997) and hippocampus (Acquas et al., 1994; Day and Fibiger, 1994; Imperato et al., 1993). These observations suggest a facilitatory influence of D_{1} receptors upon ACh transmission. However, contradictory data (decreases in ACh levels or no effect) have been acquired for the selective antagonist, SCH 23390 (Acquas et al., 1994; Day and Fibiger, 1992; Steele et al., 1997), and available studies of the control of ACh release by D_{1} receptors present certain important limitations. Thus, only one study (Acquas et al., 1994) has directly compared the influence of D_{1} agonists upon cortical vs. hippocampal levels of ACh. Further, most investigations have employed only single drugs and/or a single dose; and few have demonstrated that the actions of agonists are specifically reversed by SCH 23390 (see Discussion). In addition, in all studies to date, AChE inhibitors were added to the perfusion medium to improve the detection of resting levels of ACh. This is problematic since AChE inhibitors modify the influence of pharmacological agents upon cholinergic transmission (Fuji et al., 1997; Gobert et al., 2003; Ichikawa et al., 2000; see Millan et al., 2004). Finally, apart from one paper (Steele et al., 1997), behavioural models of the influence of drugs upon cognitive function have not been performed in parallel.

In light of the above observations, the present study systematically evaluated the influence of activation and blockade of D_{1} receptors upon cholinergic transmission and cognitive function in rats. We employed a combined neurochemical and behavioural approach utilizing a model of social recognition (recognition of a juvenile by an adult rat) which has not, as yet, been employed to characterize the actions of D_{1} receptor ligands. Social recognition in rodents is subject to modulation by cholinergic mechanisms and dependent upon functionally intact muscarinic receptors for its full expression (Anglade et al., 1999; Perio et al., 1989; Soffié and Lamberty, 1988). Further, social recognition paradigms have previously been exploited to characterize the influence upon cognition of several classes of agent including vasopressin, serotonin (5-HT)_{1A} partial agonists, histamine H_{3} antagonists and cannabinoïd CB_{1} antagonists (Bielsky and Young, 2004; Dantzer et al., 1987; Insel and Fernald, 2004; Millan et al., 2004; Terranova et al., 1996). Herein, the following studies were undertaken. First, by use of a dialysis procedure not requiring the use of AChE inhibitors (Gobert et al., 2003; Ichikawa et al., 2000), the influence of the potent and selective D_{1} receptor agonists, SKF 82958 and SKF 81297 (Arnt et al., 1988; Cussac et al., 2004; O’Boyle et al., 1989), upon extracellular levels of ACh in the FCX and hippocampus was characterized in freely moving rats. Second, employing the social recognition test, the influence of D_{1} receptor agonists upon cognitive function both alone and in the presence of scopolamine was evaluated. Third, the antagonist, SCH 23390, was examined in parallel, together with its influence upon the actions of agonists. Finally, the effects of D_{1} receptor ligands were compared to those of the AChE inhibitor and clinically active pro-cognitive agent, galantamine (Bores et al., 1996; Wilkinson et al., 2004).
Materials and methods

Animals

Male Wistar rats were housed singly and had free access to food and water. Laboratory temperature was 21±1°C and humidity 60±5%. There was a 12 h light/dark cycle (lights on at 07:30 hours). All animal use procedures conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

Dialysis and chromatographic procedures

Surgery was performed on rats (Iffa Credo, l’Arbresle, France) weighing 225–250 g and anaesthetized with pentobarbital (60 mg/kg i.p.). As previously described (Gobert et al., 2003), rats were mounted in a Kopf stereotaxic frame and a single guide cannula (CMA/11) implanted in the FCX or dorsal hippocampus with coordinates as follows (in mm, according to Paxinos and Watson, 1994): AP+2.2, L±0.6, DV–0.2, and AP–3.8, L±2.0, DV–2.0 respectively. Rats were allowed to recover for 5 d before dialysis. On the dialysis day, a cuprophan CMA/11 probe (FCX 4 mm, hippocampus 2 mm length; 0.24 mm o.d.) was lowered into position. The probe was perfused at 1 µl/min with a phosphate-buffered solution containing NaCl (147.2 mM), KCl (4 mM) and CaCl₂ (2.3 mM), adjusted to pH 7.2. After implantation, a 150-min period was respected in order to achieve stable baseline. Thereafter, 20-min dialysate samples were collected for 240 min. Three basal samples were collected prior to drug administration (SKF 82958, SKF 81297, SCH 23390, galantamine or scopolamine). Concerning interaction studies, SCH 23390 or scopolamine were injected 20 min prior to SKF 82958. Regarding chromatographic procedure, ACh was quantified in the absence of AChE inhibitors. Dialysate samples (20 µl) were collected on 10 µl acetic acid 0.1%, and 20-µl aliquots were then analysed. The mobile phase was composed of Na₂HPO₄ (50 mM) and Proclin (0.5%; BAS, Congleton, Cheshire, UK), adjusted to pH 8.2 with H₃PO₄. The stationary phase was composed of a cation exchange (530 x 1.0 mm, BAS), a first immobilized enzyme reactor containing choline oxidase/catalase (55 x 1 mm, BAS) and a second containing choline oxidase/AChE (55 x 1 mm, BAS) maintained at 35°C. An electrochemical detector (Decade, Antec-Leyden, The Netherlands) was used for quantification. A radial glassy carbon electrode coated with a peroxidase-redox polymer (BAS) was set at +100 mV vs. Ag/AgCl. The mobile phase was delivered at a constant flow rate of 0.14 ml/min. The sensitivity of the assay for ACh was 0.1 pg injected in a volume of 20 µl.

Social recognition test

Experiments were carried out on Wistar rats (Elevage Janvier, le Genest-Saint-Ise, France) weighing 240–260 g on arrival. Juvenile Wistar rats (25–30 d old) were used in recognition tests. The adult rats were individually housed for 2 d prior to testing. The procedure used was essentially that described previously (Millan et al., 2004; Perio et al., 1989). On the test day, each adult was placed in its home cage on the observation table. A 5-min period of habituation to the environment was allowed before beginning the experiment. Thereafter, an unfamiliar juvenile rat was introduced into the home cage of the adult rat for a first 5-min session (T1). An observer unaware of drug treatment recorded the total duration (in seconds) of social investigation (time spend by the adult rat in sniffing, following, biting, jumping or crawling over or under the juvenile). Thereafter, a second 5-min session (T2) was initiated with the same (or a different) juvenile rat, and the total duration of social investigation was scored by the same observer. This procedure was conducted with the same juvenile, in order to assess the influence of the drug upon social recognition, or with a different juvenile, as an index of specificity of its action. In a first set of experiments, a 120-min delay was allowed between T1 and T2. SKF 82958, SKF 81297 or galantamine were administered immediately after T1. In interaction studies, SKF 82958 was administered immediately after T1 and SCH 23390 1 min latter. In a second set of experiments, T1 immediately preceded T2, and the same juvenile was presented. To evaluate their potential amnesic effects, SCH 23390 or scopolamine were injected 30 min before T1. In the interaction study with SCH 23390, SKF 82958 was administered 15 min before SCH 23390. In interaction studies with scopolamine (inhibition of its amnesic properties), SKF 82958 or SKF 81297 were administered 15 min before scopolamine.

Data representation and statistical analysis

In dialysis experiments, values are means±S.E.M. of observations from groups of n=5–9, and are expressed relative to baseline values (defined as 100%). Statistical comparisons of data were undertaken using ANOVA with time as a repeated measure. Two-way (drug x time) and three-way (drug x drug x time) ANOVAs were used for dose–response and interaction studies respectively. If significant, the ANOVA
was followed by post-hoc comparisons to vehicle groups by Dunnett’s test. In the social recognition test, adult rats displaying durations of social investigation of <60 s during T1 were not examined further. Values are means ± S.E.M. (n = 5–9 rats per group) of total duration of social investigation (in seconds). Statistical evaluations were carried out using one-way or two-way ANOVAs followed, where appropriate, by Dunnett’s test or Newman–Keuls tests. In all experiments, p values of <0.05 were considered significant.

Chemicals and drugs

Drugs were dissolved in sterile water plus a few drops of lactic acid if necessary. Solutions were adjusted to pH >5.0. All drugs were injected by the subcutaneous (s.c.) route in a volume of 1.0 ml/kg. SKF 82958 [(±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine hydrobromide], SKF 81297 [(±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine hydrobromide], SCH 23390 [(R)-(+)8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol hydrochloride] and scopolamine (hydrochloride) were purchased from Sigma (Chesnes, France), and galantamine hydrobromide from Tocris (Bristol, UK).

Results

Influence of the AChE inhibitor, galantamine, upon dialysis levels of ACh in the FCX and hippocampus of freely moving rats (Figure 1)

Resting levels of ACh were 3.73 ± 0.13 pg/20 μl in the FCX and 1.13 ± 0.07 pg/20 μl in the hippocampus. In both structures, single subcutaneous injection of vehicle induced a modest (150% of basal values) and transient (40 min) increase in extracellular levels of ACh. Subcutaneous administration of the AChE inhibitor, galantamine, robustly and dose-dependently (0.0025–0.63 mg/kg) elevated ACh levels reaching, at the dose of 0.63 mg/kg, 664 ± 111% of basal values (100%) in FCX. ANOVA with time as the repeated measure revealed a significant main effect of group (F<sub>3,27</sub> = 5.9, p < 0.01) and time (F<sub>11,44</sub> = 49.6, p < 0.01), and a group × time interaction (F<sub>33,297</sub> = 9.2, p < 0.01). Post-hoc analysis (Dunnett’s test, p < 0.05) indicated that the influence of galantamine was different from vehicle at doses ≥0.01 mg/kg. Administration of galantamine (0.04–0.63 mg/kg) also elevated hippocampal ACh levels, reaching 361 ± 42% of basal values (0.63 mg/kg). ANOVA indicated a significant main effect of group (F<sub>3,27</sub> = 4.7, p < 0.05) and time (F<sub>11,44</sub> = 20.1, p < 0.01) and a group × time interaction (F<sub>33,297</sub> = 3.2, p < 0.01). Galantamine, at doses of 0.16 or 0.63 mg/kg, induced a more pronounced effect than vehicle (Dunnett’s test, p < 0.05) upon ACh levels.

Influence of SKF 82958 and SKF 81297 upon dialysis levels of ACh in the FCX and hippocampus (Figure 2)

Administration of the D<sub>1</sub> receptor agonist, SKF 82958, robustly elevated ACh levels in both the FCX (group: F<sub>4,44</sub> = 6.6, p < 0.01; time: F<sub>11,44</sub> = 27.0, p < 0.01; group × time: F<sub>44,244</sub> = 4.8, p < 0.01) and hippocampus (group: F<sub>4,44</sub> = 24.6, p < 0.01; time: F<sub>11,44</sub> = 34.6, p < 0.01; group × time: F<sub>44,277</sub> = 7.6, p < 0.01). The increases in
ACh levels were dose-dependent (0.0025–0.63 mg/kg) and sustained over the total duration (180 min) of evaluation. The maximal increase in ACh levels was reached at a dose of 0.63 mg/kg and was comparable in the FCX and hippocampus (285 ± 46% and 343 ± 41% of baseline values respectively). SKF 81297 also elicited a pronounced, dose-dependent (0.04–0.63 mg/kg) and sustained increase in ACh levels in both the FCX (group: $F_{4,24} = 12.0$, $p < 0.01$; time: $F_{11,44} = 53.4$, $p < 0.01$; group × time: $F_{44,264} = 6.0$, $p < 0.01$) and hippocampus (group: $F_{5,22} = 2.0$, $p < 0.01$; time: $F_{11,44} = 19.9$, $p < 0.01$; group × time: $F_{44,224} = 1.9$, $p < 0.01$). Its maximal effect, observed at the dose of 0.63 mg/kg, was somewhat more pronounced in the FCX (300 ± 29% of baseline values) than in the hippocampus (232 ± 2% of baseline).

Figure 2. Influence of the $D_1$ receptor agonists, SKF 82958 and SKF 81297, upon dialysis levels of acetylcholine (ACh) in the frontal cortex (FCX) and hippocampus (Hipp) of freely moving rats. Data represent the mean ± S.E.M. of observations from groups of $n = 5–8$. They are expressed as percent of baseline (average of the three samples preceding drug injection). (a, b) Arrow indicates injection (s.c.) of vehicle (Veh) or SKF 82958. (c, d) Arrow indicates injection (s.c.) of Veh or SKF 81297. * $p < 0.05$, SKF 82958 or SKF 81297 vs. respective Veh.

Influence of SCH 23390 upon dialysis levels of ACh in the FCX and hippocampus, alone and in combination with SKF 82958 (Figure 3)

The $D_1$ receptor antagonist, SCH 23390, induced a long-lasting suppression of ACh levels both in the FCX (group: $F_{4,22} = 4.0$, $p < 0.05$; time: $F_{11,44} = 16.2$, $p < 0.01$; group × time: $F_{44,262} = 1.7$, $p < 0.01$) and in the hippocampus (group: $F_{4,28} = 5.8$, $p < 0.01$; time: $F_{11,44} = 12.9$, $p < 0.01$; group × time: $F_{44,318} = 2.1$, $p < 0.01$). The reductions in ACh levels were dose-dependent (0.00063–0.63 mg/kg) and, at the dose of 0.63 mg/kg, sustained over the 180 min of evaluation. At this dose, the magnitude of the effect of SCH 23390 was comparable in the FCX and hippocampus: 43 ± 6% and 53 ± 6% of basal values.
Regarding the interaction study in the FCX (Figure 3c), a three-way ANOVA with time as the repeated measure indicates a significant group \times time interaction ($F_{11,286} = 2.1, p < 0.01$). Additionally, the main effect of SCH 23390 ($F_{1,26} = 10.3, p < 0.01$) and SKF 82958 ($F_{1,26} = 16.1, p < 0.01$) was observed when administered in combination with vehicle. However, in the presence of SCH 23390 (0.08 mg/kg), SKF 82958 (0.04 mg/kg) did not increase ACh levels ($F_{1,26} = 0.4, p > 0.05$). In the hippocampus (Figure 3d), analysis indicated a group \times time interaction ($F_{11,264} = 5.3, p < 0.01$), and a significant effect of SCH 23390 ($F_{1,24} = 44.9, p < 0.01$) and SKF 82958 ($F_{1,24} = 30.6, p < 0.01$). In the presence of SCH 23390 (0.08 mg/kg), the effect of SKF 82958 was abolished ($F_{1,24} = 2.9, p > 0.05$).

**Influence of SKF 82958 upon the scopolamine-induced increase in ACh levels in the FCX and hippocampus (Figure 4)**

Administration of the muscarinic antagonist, scopolamine, induced a robust, long-lasting and dose-dependent (0.04–2.5 mg/kg) increase in ACh levels, in the FCX (group: $F_{5,28} = 14.4, p < 0.01$; time: $F_{11,55} = 30.4, p < 0.01$; group \times time: $F_{55,360} = 3.9, p < 0.01$) and the hippocampus (group: $F_{5,28} = 10.2, p < 0.01$; time: $F_{11,55} = 31.9, p < 0.01$; group \times time: $F_{55,360} = 5.3,$

![Figure 3. Influence of the D1 receptor antagonist, SCH 23390, alone or in combination with SKF 82958 upon dialysis levels of acetylcholine (ACh) in the frontal cortex (FCX) and hippocampus (Hipp). Data represent the mean ± S.E.M. of observations from groups of $n = 5–8$. They are expressed as percent of baseline (average of the three samples preceding drug injection). (a, b) Arrow indicates injection (s.c.) of vehicle (Veh) or SCH 23390. (c, d) Left arrow indicates injection (s.c.) of SCH 23390 or Veh. Right arrow indicates injection (s.c.) of Veh or SKF 82958. * $p < 0.05$, SCH 23390 vs. respective Veh. · $p < 0.05$, SCH 23390–Veh or Veh–SKF 82958 vs. respective Veh–Veh. § $p < 0.05$, SCH 23390–SKF 82958 vs. respective Veh–SKF 82958.](http://ijnp.oxfordjournals.org/)

---

B. Di Cara et al.
Scopolamine, at a dose of 2.5 mg/kg, elicited a less pronounced maximal increase in ACh levels in the FCX compared to the hippocampus (246 ± 19% and 368 ± 58% of baseline respectively). Regarding the interaction study (Figure 4c), cortical levels of ACh were increased by either scopolamine (1.25 mg/kg), SKF 82958 (0.04 mg/kg) and their combination. A three-way ANOVA with time as the repeated measure, indicated a group x time interaction ($F_{11,264} = 2.0$, $p < 0.01$). Further analysis revealed that SKF 82958 did not affect the influence of scopolamine upon ACh levels ($F_{11,99} = 0.4$, $p > 0.05$).

Influence of galantamine upon social recognition in a procedure with a 120-min inter-session delay (Figure 5)

The total duration of social investigation during the first session (T1) was 98 ± 5 s, and this value was reproducible throughout the studies reported herein. Injection of vehicle immediately after T1 did not
modify the total duration of social investigation in the
second session (T2), indicating a comparable level of
social investigation between the two sessions (no
recognition of a juvenile). By contrast, galantamine
robustly decreased the duration in social investigation
during T2, as indicated by the increased difference
in duration of social investigation between the two
sessions (T2 – T1). One-way ANOVA revealed a main
effect of drug ($F_{4, 35} = 31.3$, $p < 0.01$) and post-hoc
analysis (Dunnett’s test) indicated a dose-dependent
(0.04–2.5 mg/kg) effect of galantamine ($p < 0.05$).
Reflecting its specificity of action upon the recognition
of a juvenile, the maximally active dose of galantamine
(2.5 mg/kg) was ineffective when the familiar juvenile
rat was replaced by a different juvenile during T2.
Two-way ANOVA indicated a significant main effect
of juvenile ($F_{1, 24} = 19.8$, $p < 0.01$) and drug ($F_{1, 24} = 28.9$,$p < 0.01$), and a juvenile × drug interaction ($F_{1, 24} = 21.2$,$p < 0.01$).

**Figure 5.** Influence of galantamine upon social recognition
with a 120-min inter-session delay. Dose–response curve for
the improvement of retention by the acetylcholinesterase
inhibitor, galantamine, and control for the specificity of its
action with a different juvenile. Data are mean ± S.E.M. of
observations from groups of $n = 5–9$ and represent the
difference in social investigation duration between the two
sessions (T2 – T1). * $p < 0.05$, galantamine vs. vehicle (Veh).
# $p < 0.05$, different juvenile–galantamine vs. same
juvenile–galantamine.

**Influence of SKF 82958 and SKF 81297 upon
social recognition in a procedure with a 120-min
inter-session delay (Figure 6)**

SKF 82958 dose-dependently (0.01–0.16 mg/kg) re-
duced the total duration of social investigation
during T2 ($F_{3, 23} = 34.3$, $p < 0.01$), attaining a difference
(T2 – T1) of $-49 ± 9$ s at a dose of 0.16 mg/kg. At this
dose, SKF 82958 was ineffective in the presence of a
different juvenile rat during the second session,
demonstrating its specificity of action upon recogni-
tion of a juvenile (juvenile: $F_{1, 10} = 33.3$, $p < 0.01$; group: $F_{1, 10} = 47.5$, $p < 0.01$; juvenile × group: $F_{1, 10} = 27.3$, $p < 0.01$). A further agonist, SKF 81297, also
dose-dependently reduced the total duration of social
investigation during T2 ($F_{4, 31} = 6.9$, $p < 0.01$), and a
maximal difference (T2 – T1) of $-34 ± 5$ s was obtained

**Figure 6.** Influence of SKF 82958 and SKF 81297 upon
social recognition with a 120-min inter-session delay.
Dose–response curve for the improvement of retention by D$_1$
receptor agonists, SKF 82958 and SKF 81297, and control for
the specificity of their actions with different juveniles. Data
are mean ± S.E.M. of observations from groups of $n = 6–9$ and
represent the difference in social investigation duration
between the two sessions (T2 – T1). * $p < 0.05$, SKF 82958 or
SKF 81297 vs. respective vehicle (Veh). # $p < 0.05$, different
juvenile–SKF 82958 vs. same juvenile–SKF 82958. § $p < 0.05$, different
juvenile–SKF 81297 vs. same juvenile–SKF 81297.
at a dose of 1.25 mg/kg. The specificity of action of SKF 81297 was also confirmed using a different juvenile during T2 (juvenile: $F_{1,11} = 26.4, p < 0.01$; group: $F_{1,11} = 35.6, p < 0.01$; juvenile × group: $F_{1,11} = 5.8, p < 0.05$).

**Influence of SCH 23390 upon actions of SKF 82958 in a procedure with a 120-min inter-session delay (Figure 7)**

By itself, SCH 23390 (0.01 mg/kg) did not modify the difference in total duration of social investigation between the two sessions. However, in its presence, the reduction in duration of social investigation during T2 induced by SKF 82958 (0.04 mg/kg) was abolished, providing a further demonstration of the specificity of the influence of SKF 82958 upon recognition of a juvenile. Two-way ANOVA indicated a significant main effect of SKF 82958 ($F_{1,26} = 28.0, p < 0.01$) and SCH 23390 ($F_{1,26} = 14.6, p < 0.01$), and a SKF 82958 × SCH 23390 interaction ($F_{1,26} = 15.4, p < 0.01$).

**Influence of scopolamine upon social recognition in a procedure without an inter-session delay (Figure 8)**

In the absence of an inter-session delay, the total duration in social investigation during T2 was markedly reduced compared to T1. The decrease in duration reflects the recognition of a juvenile between the two sessions. In the presence of vehicle, total duration was $108 ± 4$ s and $43 ± 4$ s for T1 and T2 respectively. The muscarinic antagonist, scopolamine, robustly reduced (0.16–2.5 mg/kg) the difference (T2 – T1) in duration of social investigation (one-way ANOVA: $F_{3,33} = 13.5, p < 0.01$), reflecting its deleterious influence upon recognition of a juvenile. The most robust effect of scopolamine was exerted at a dose of 1.25 mg/kg, which was selected for subsequent interaction studies.

**Influence of SKF 82958 and SKF 81297 upon the actions of scopolamine in a social recognition procedure without an inter-session delay (Figure 9)**

The reduction in recognition of a juvenile by scopolamine (1.25 mg/kg) was dose-dependently abrogated by SKF 82958 (0.0025–0.04 mg/kg; $F_{3,26} = 32.8, p < 0.01$) which, under these conditions of spontaneous recognition, did not itself modify the total duration of social investigation ($F_{3,31} = 2.6, p > 0.05$). Likewise, SKF 81297 dose-dependently (0.0016–0.04 mg/kg) blocked the deleterious effect of scopolamine (1.25 mg/kg) upon recognition of a juvenile ($F_{3,31} = 9.6, p < 0.01$), but did not itself modify the total duration of social investigation ($F_{3,26} = 2.5, p > 0.05$).

**Influence of SCH 23390 upon social recognition in a procedure without an inter-session delay: blockade by SKF 82958 (Figure 10)**

At a dose of 0.01 mg/kg, SCH 23390 slightly but significantly increased the duration of social investigation during T2 ($F_{3,27} = 3.1, p < 0.05$). This action of
SCH 23390, revealed by a reduction in T2 – T1 values, reflects its deleterious influence upon recognition of a juvenile. Although this action was seen neither at higher (0.0025 mg/kg) nor at lower doses (0.04 mg/kg), it was specific inasmuch as it was abolished by pre-treatment with SKF 82958 (0.04 mg/kg), which was itself inactive under these conditions of spontaneous recognition. Two-way ANOVA revealed a significant main effect of SCH 23390 ($F_{1,19}=22.7, p<0.01$) and SKF 82958 ($F_{1,19}=4.0, p>0.01$), and a SCH 23390 × SKF 82958 interaction ($F_{1,19}=13.0, p<0.01$).

**Figure 9.** Influence of SKF 82958 or SKF 81297 upon the amnesic actions of scopolamine in a social recognition procedure without inter-session delay. Data are mean ± S.E.M. of observations from groups of $n=7–9$ and represent the difference in social investigation duration between the two sessions (T2 – T1). * $p<0.05$, vehicle (Veh)–Veh vs. Veh–scopolamine. # $p<0.05$, SKF 82958–scopolamine or SKF 81297–scopolamine vs. respective Veh–scopolamine. —○—, Vehicle; —●—, scopolamine (1.25 mg/kg).

**Discussion**

**Technical considerations**

Without exception, all previous studies of the modulation of ACh release by $D_1$ receptor ligands have employed AChE inhibitors in the perfusion medium to boost resting levels of ACh which are difficult to detect by conventional analytical procedures. By contrast, the present study was able to forego the use of AChE inhibitors by employing a choline-oxidase-loaded ‘pre-column’ to degrade choline, the high levels of which swamp those of ACh (Gobert et al., 2003; Ichikawa et al., 2002). The absence of AChE inhibitors in the perfusion medium is an important point since an artificial elevation in ACh levels may ‘over-activate’ cholinergic autoreceptors leading to marked alterations in the reactivity of cholinergic neurons to diverse classes of pharmacological agents (Fujii et al., 1997; Liu and Kato, 1994; Millan et al., 2004). Accordingly, basal levels of ACh in the FCX and hippocampus herein were some 10-fold lower than those in previous studies of $D_1$ agonists, yet still reproducibly detectable. Upon administration of vehicle, basal levels of ACh displayed an abrupt and short-lived increase in both FCX and hippocampus, an observation mimicking other studies of ACh release – although the use of AChE inhibitors blunts this effect of vehicle (Shirazi-Southall et al., 2002). This induction of ACh release upon handling reflects the well-documented responsiveness of cholinergic
pathways to manipulations which modify attentional status (Gobert et al., 2003; Sarter and Bruno, 1997).

**Facilitation by D₁ agonists of cholinergic transmission in the FCX and hippocampus**

Previous studies of the D₁ receptor ligands, dihydrexidine, CY 208,243 and A-77646, reported increases in dialysis levels of ACh in the FCX (Acquas et al., 1994; Day and Fibiger, 1993; Steele et al., 1997), observations extended herein to the highly selective agonists, SKF 81297 and SKF 82958, which potently provoked dose-dependent and sustained elevations in extracellular levels of ACh. Their effects attained increases in ACh levels comparable to the AChE inhibitor, galantamine (Figure 1; Bores et al., 1996; Wilkinson et al., 2004). Underpinning the specificity of their actions, active doses of SKF 81297 and SKF 82958 were close to those effective in other functional models (Chausmer and Katz, 2002; Ralph-Williams et al., 2002) and, in the presence of the selective antagonist, SCH 23390, no increase in ACh levels was observed with SKF82598. These data are in line with the abrogation by SCH 23390 of increases in ACh release elicited by dihydrexidine, A-77636 and the non-selective D₁/D₂ agonist, apomorphine (Acquas et al., 1994; Day and Fibiger, 1993, 1994; Steele et al., 1997). In the latter studies, only variable affects of SCH 23390 alone (administered at single doses) were seen. In contrast, a modest, dose-dependent and significant reduction in ACh levels was observed herein, consistent with a tonic inhibitory influence of D₁ receptors upon cortical cholinergic neurons. This difference to previous work may, as outlined above, reflect the absence of AChE inhibitors in the perfusion medium thereby increasing sensitivity for detection of mild decreases in ACh levels. SCH 23390 possesses significant agonist properties at 5-HT₂C sites (Millan et al., 2001), but they are unlikely to be involved since selective 5-HT₂C agonists, such as Ro-60,0175, do not modify cholinergic transmission (B. Di Cara et al., unpublished observations).

In the only comparative study to date, Acquas et al. (1994) reported that A-77636 was of similar efficacy in enhancing ACh levels in the hippocampus vs. FCX. Although that study did not examine full dose-response relationships, this finding is underpinned herein with the observation that SKF 81297 and SKF 82958 elicited robust elevations in hippocampal levels of ACh over similar doses ranges to the FCX, and with no marked difference in maximal effect. Further, by analogy to the FCX, the actions of SKF 82958 were abolished by SCH 23390 underlying the selectivity of its actions. SCH 23390 reduced basal ACh levels in hippocampus, corresponding to the previous findings of Imperato et al. (1993). Thus, D₁ receptor control of cholinergic transmission in the hippocampus and FCX appears, in principle, to be similar. Interestingly, certain other drug classes, such as partial agonists at 5-HT₁A receptors – which likewise possess broad-based cognitive properties – evoke similar effects in the FCX and hippocampus (Millan et al., 2004). In contrast, others have regionally specific actions: for

![Figure 10. Influence of (a) SCH 23390 alone, and (b) blockade of its actions by SKF 82958, in a social recognition procedure without inter-session delay. Data are mean ± S.E.M. of observations from groups of n = 5–9 and represent the difference in social investigation duration between the two sessions (T2−T1). * p < 0.05, SCH 23390 vs. vehicle (Veh). # p < 0.05, Veh–SCH 23390 vs. Veh–Veh. § p < 0.05, SKF 82958–SCH 23390 vs. Veh–SCH 23390.](http://ijnp.oxfordjournals.org/)

example, dopamine D₃ receptors antagonists increase ACh levels in FCX but not in the hippocampus (Gobert et al., In Press; Joyce and Millan, 2005).

Possible mechanisms of action of D₁ receptor ligands. A role for D₅ receptors?

An intriguing question, not directly addressed herein, concerns the sites of action of D₁ receptor ligands. Hersi et al. (1995b) demonstrated that local administration of the partial agonist, SKF 38393, into the hippocampus elevated ACh release whereas its perfusion into the septum, the origin of septo-hippocampal cholinergic neurons, was ineffective. Correspondingly, there is anatomical evidence that hippocampal D₁ sites facilitatory to ACh release may be localized on cholinergic terminals themselves (Hersi et al., 1995b). However, a further important issue concerns a possible role of D₅ receptors since ‘selective’ D₁ receptor ligands fail to discriminate D₁ from D₅ receptors. Supporting a role for the latter, the elevation in hippocampal ACh release elicited by SKF 38393 in rats was eliminated by antisense probes directed against D₅ but not D₁ receptors (Laplante et al., 2004) and it was absent in mice genetically deprived of D₅ receptors (Hersi et al., 2000). Thus, recruitment of D₅ receptors in the hippocampus, a structure in which they predominate over D₁ receptors (Bergson et al., 1995; Ciliax et al., 2000), may underlie the elevation in ACh levels by ‘D₁’ receptor agonists. Moreover, basal levels of ACh were diminished in mice with genetically invalidated D₅ receptors, consistent with SCH 23390-induced decreases in dialysis levels of ACh and suggestive of a tonic, facilitatory influence of D₅ receptors upon ACh release in the hippocampus. Inasmuch as the density of D₁ receptors is greater than that of D₅ sites in the FCX and NBM, the origin of frontocortical cholinergic neurons (Ciliax et al., 2000), a role of the former in the modulation of ACh release in the FCX appears likely, although this awaits direct demonstration. Their location awaits evaluation and, in addition to the FCX or NBM, populations in other structures such as the nucleus accumbens (Zmarowski et al., 2005) may indirectly modulate ACh release in the FCX.

Facilitative influence of D₁ receptors upon social memory

Systemic administration of D₁ receptor agonists facilitates cognitive function in rodents in a variety of experimental paradigms (Hersi et al., 1995a; Hotte et al., 2005; Steele et al., 1997). Nonetheless, they are not active under all conditions, single (high) doses have generally been employed, most studies have examined only one drug – usually SKF 38393 – and, surprisingly, it has not been demonstrated that the actions of agonists are blocked by SCH 23390 (Hersi et al., 1995a; Hotte et al., 2005). The present study is, then, of significance in demonstrating that SKF 81297 and SKF 82958 both exert potent, dose-dependent, robust and SCH 23390-reversible actions...
in a social recognition paradigm. Their actions were seen at low doses similar to those active in other models of activity at D₁ receptors (Haile and Kosten, 2001). In addition, by analogy to galantamine and other AChE inhibitors (Winslow and Camacho, 1995), their actions were expressed specifically in that time spent in social investigation during the second session was not modified upon presentation of a novel juvenile.

This is the first report that stimulation of D₁ receptors enhances social memory, although in line with the present observations, SCH 23390 abrogated the improvement in social recognition elicited by ethanol (Prediger et al., 2004). The muscarinic antagonist, scopolamine, perturbs social recognition (Millan et al., 2004; Perio et al., 1989; Sofié and Lambert, 1988; Terranova et al., 1996) and SKF 81297 and SKF 82958 dose-dependently blocked its ‘amnesic’ actions. These observations accord with work in other paradigms showing that D₁ receptor agonists alleviate the cognitive deficits provoked by scopolamine, as well as old age, stress, adrenalectomy and lesions of cholinergic neurons (Hersi et al., 1995a; Mizoguchi et al., 2000, 2004; Steele et al., 1997).

By analogy to ACh release, both alone and in interaction with scopolamine, monotonic dose–response curves were observed with D₁ agonists, with no evidence for inflection at higher doses. This is interesting since Steele et al. (1997) observed a biphasic, cognitive profile for dihydrexidine in a passive avoidance test in rats, consistent with studies of frontocortical working memory in primates (Cai and Arnsten, 1997; Murphy et al., 1996; Zahrt et al., 1997) that excessive stimulation of D₁ receptors may be deleterious to cognitive function. Nonetheless, a low level of activity at D₁ receptors is associated with poor social recognition inasmuch as SCH 23390 elicited a specific reduction when tested alone. This observation corresponds to previous findings with certain other models that SCH 23390 decreases cognitive function (Barros et al., 2001; Runyan and Dash, 2004; Seamans et al., 1998; Zahrt et al., 1997). As discussed above, this effect probably reflects interruption of tonic activity at D₁ receptors, although inverse agonist actions at constitutively active D₁ receptors cannot be excluded (Tiberi and Caron, 1994).

**Possible modes of action of D₁ receptors in modulating social cognition**

Social memory involves a complex network of olfactory, subcortical and cortical structures (Bielsky and Young, 2004; Davis, 2004). Accordingly, there are essentially three mechanisms via which D₁ receptor agonists might affect performance. First, in modulating the detection of chemosensory cues (rat odour) by actions at ‘olfactory receptor neurons’ innervating mitral cells in the olfactory bulb. This is, however, unlikely since D₁ receptors are not localized on olfactory afferents and D₁ receptor ligands do not to modify their activity (Coronas et al., 1999; Hisia et al., 1999). Further, although there is one isolated study indicating that SKF 38393 modulates odour discrimination, its effects were facilitatory not inhibitory (Yue et al., 2004). In addition, the control experiment with a novel juvenile excludes a role of sensory input in the effects of D₁ agonists, and scopolamine has been demonstrated not to modify odour discrimination in the rat (Doyle et al., 1998, 2003). Second, D₁ receptor ligands may act in the olfactory bulb itself or in interconnected downstream structures involved in the formation of social memory: the medial amygdala and lateral septum, the pyriform, perirhinal and entorhinal cortices, and the final site of integration, the hippocampus (Bielsky and Young, 2004; Davis, 2004). Although the density of D₁ receptors in the olfactory bulb is low, they are functionally active (Brunig et al., 1999; Coronas et al., 1999). Further, the other corticolimbic structures mentioned above contain significant populations of D₁ receptors, and based on microinjection studies, the amygdala, entorhinal cortex and hippocampus are all possible sites for an influence of D₁ agonists upon social memory (Bach et al., 1999; Barros et al., 2001; Li et al., 2003). A third possibility would be that drugs act on D₁ receptors in the FCX, a structure fulfilling a more general, facilitative role in the formation of working memory and attention (Granon et al., 2000; Mizoguchi et al., 2000, 2004).

Thus, the precise site(s) of action of D₁ agonists in modulating social memory remain(s) to be ascertained, as well as the role(s) of D₁ compared to D₅ receptors. As mentioned above, D₅ sites modulate hippocampal ACh release but behavioural evidence for a role in cognition is not, as yet, available. Indeed, mice with genetically deleted D₅ receptors showed no specific alterations in cognitive function in comparison to D₅ receptor knockout mice which revealed marked deficits in spatial memory, a mode of cognition integrated in the hippocampus (El-Ghundi et al., 1999; Holmes et al., 2001; Smith et al., 1998).

**Relationship between alterations in ACh release and cognitive properties of D₁ agonists**

Doses of SKF 81297 and SKF 82958 active in the social recognition procedure correspond closely to those which facilitated cholinergic transmission in
the hippocampus and FCX. Further, social memory is dependent upon functionally intact cholinergic networks and enhanced both by AChE inhibitors and by agonists at muscarinic and nicotinic receptors (Perio et al., 1989; Winters et al., 2000). It might, then, be conjectured that the D₁ receptor-mediated elevations in extracellular levels of ACh are directly related to the actions of SKF 81297 and SKF 82958 in the social memory paradigm. However, as for other classes of cognitive agent (see Millan et al., 2004 for discussion), mechanisms underlying blockade of scopolamine-induced amnesia remain poorly understood, and SKF 82958 did not amplify its facilitation of ACh release. In fact, SKF 81297 and SKF 82958 may recruit D₁ receptors post-synaptic to cholinergic terminals and co-localized with muscarinic and nicotinic receptors on pyramidal neurons. Furthermore, in the hippocampus and FCX, the influence of D₁ receptors may be exerted in interaction with glutamatergic receptors mediating LTP (Li et al., 2003; Yang, 2000).

Irrespective of the precise interrelationship between D₁ receptors and cholinergic pathways in the control of cognitive function, an intriguing finding was that SKF 82958 and galantamine exerted an additive influence both upon ACh levels and upon social memory. This observation raises the interesting possibility that the co-administration of D₁ agonists and AChE inhibitors may exert a particularly pronounced improvement of cognitive performance. Moreover, relative to the use of high doses of an AChE inhibitor alone, the therapeutic window to side-effects may be improved. Finally, these findings also suggest that drugs that combine D₁ agonist and AChE inhibitor properties might be of considerable therapeutic interest.

Summary and conclusions

In conclusion, selective stimulation of dopamine D₁ receptors markedly and specifically reinforced cholinergic transmission in the FCX and hippocampus, and enhanced social memory in rats. By contrast, selective blockade of D₁ receptors exerted a negative influence upon ACh release in both structures and suppressed social recognition. The relationship between these neurochemical and behavioural effects of D₁ ligands remains to be defined, as well as their precise mechanisms of action. Nonetheless, these data provide compelling evidence for the physiological role of D₁ receptors in the control of mnemonic function, and offer a novel behavioural paradigm for the characterization of their roles. From a physiological perspective, D₁ receptors may be involved in social interactions in animals, such as offspring recognition and pair bonding (Bielsky and Young, 2004). Therapeutically, D₁ receptors are targets for restoring cholinergic transmission and improving the cognitive deficits of neurological and psychiatric disorders such as Alzheimer’s disease, Parkinson’s disease and schizophrenia. Although the relationship between social memory in man and in rodent remains unclear, schizophrenia is characterized by a disruption of ‘social’ cognition (Insel and Fernald, 2004; Lee et al., 2004). Further studies of ACh release and social recognition may provide important insights into novel therapeutic strategies for enhancing mnemonic function in schizophrenia and other CNS disorders.

Acknowledgements

None.

Statement of Interest

None.

References


Bach ME, Barad M, Son H, Zhuo M, Lu YF, Shih R, Mansuy I, Hawkins RD, Kandel ER (1999). Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. Proceedings of the National Academy of Sciences USA 96, 5280–5285.


Bielsky IF, Young LJ (1999). Dopamine D_{1} receptor agonists A77636 or SKF81297 on cortical acetylcholine release. Synapse 37, 125–145.


Goldman-Rakic PS (2005). The relevance of the dopamine-D_{1} receptor in the cognitive symptoms of schizophrenia. Neuropsychopharmacology 21, S170–S181.

agents into rat prefrontal cortex. *Journal of Neuroscience* 20, 1208–1215.


Myhrer T (2003). Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based...


