The Muscarinic Receptor Agonist Xanomeline Has an Antipsychotic-Like Profile in the Rat


Vernalis Research Limited, Winnersh, Wokingham, United Kingdom

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ABSTRACT

The muscarinic receptor agonist xanomeline was examined and compared with the antipsychotics clozapine and/or haloperidol in the following in vivo rat models: apomorphine-induced disruption of prepulse inhibition (PPI), amphetamine-induced hyperlocomotion, and the conditioned emotional response (CER) test. The effects of xanomeline were also assessed ex vivo on dopamine turnover in the rat medial prefrontal cortex. Under conditions of varying dose and prepulse intensity, xanomeline, like haloperidol, had no effect on PPI. In contrast, the muscarinic receptor antagonist scopolamine and the muscarinic receptor agonist pilocarpine both induced significant dose-dependent deficits in PPI. Haloperidol and xanomeline, but not pilocarpine, dose dependently reversed apomorphine-induced disruption of PPI. Thus, xanomeline induced a clear antipsychotic-like effect in PPI, whereas pilocarpine appeared to induce a psychotomimetic-like effect. Xanomeline attenuated amphetamine-induced hyperactivity at doses that had no effect on spontaneous activity, possibly indicating a separation between attenuation of limbic hyperdopaminergic function and the induction of hypolocomotion. Haloperidol and clozapine also reversed amphetamine-induced hyperlocomotion, but at similar doses to those that reduced spontaneous locomotion. Clozapine, but not haloperidol had an anxiolytic-like effect in the CER test. The effects of xanomeline in the CER test were similar to those of clozapine, although at the anxiolytic dose it tended to disrupt baseline levels of lever pressing. Finally, haloperidol, clozapine, pilocarpine, and xanomeline, all induced an increase in dopamine turnover in medial prefrontal cortex. The antipsychotic-like effects of xanomeline in the animal models used here suggest that it may be a useful treatment for psychosis.

Dysregulation of dopamine is generally considered to be central to the symptoms of schizophrenia. Over the past 40 years, however, accumulating data indicate that the cholinergic system may also play an important role. Key early findings include the discovery that compounds that nonspecifically block muscarinic cholinergic receptors induce a form of psychosis in normal humans and exacerbate the symptoms of schizophrenia (Neubauer et al., 1966a,b). Moreover, antimuscarinic agents were also reported to block the effects of typical antipsychotics on positive symptoms (Johnstone et al., 1983).

Although muscarinic receptor agonists have not been tested in controlled trials with schizophrenic patients, muscarinic receptor agonists and acetylcholinesterase (AChE) inhibitors both appear to be effective in the treatment of psychotic symptoms in neurodegenerative disorders. Bodick et al. (1997) reported that the functionally selective M3/M4 muscarinic receptor agonist xanomeline dose dependently reduced hallucinations, agitation, and delusions in a 6-month randomized double-blind, placebo-controlled parallel group trial with Alzheimer’s patients. Hutchinson and Fazzini (1996) found that the AChE inhibitor tacrine reduces or abolishes hallucinations in Parkinson’s disease, and Kaufer (1998) found that metrifonate and other AChE inhibitors alleviated neuropsychiatric symptoms in Alzheimer patients, including a reduction in hallucinations in a multicenter, 26-week, double-blind, placebo-controlled study. Although it seems likely that the neural basis for the psychotic symptoms in neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease differ from that occurring in schizophrenia, a recent report claiming that the antipsychotic agent olanzapine was effective in the treatment of psychotic symptoms in Alzheimer’s disease gives some credence to the suggestion that compounds that treat psychotic symptoms in Alzheimer’s disease may also be effective in schizophrenia (Street et al., 1999).

ABBREVIATIONS: AChE, acetylcholinesterase; 5-HT, 5-hydroxytryptamine; PPI, prepulse inhibition; CER, conditioned emotional response; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; dur, rate of lever pressing during each light presentation; pre, interval preceding each light presentation; CDP, chlordiazepoxide; ANOVA, analysis of variance; PAR, conditioned avoidance response; mPFC, medial prefrontal cortex.
Xanomeline, as well as other muscarinic receptor agonists, have been shown to have effects resembling atypical antipsychotics in a number of animal models of schizophrenia, notably dopamine hyperactivity models (see below). Because xanomeline does not have appreciable affinity for various other neurotransmitter receptors, including dopamine receptors, these findings implicate muscarinic receptors as potential targets for modulation of dopaminergic activity (Shannon et al., 1994; Bymaster et al., 1994). Hagan et al. (1987) showed that muscarinic receptor agonists and the anticholinesterase inhibitor phystostigmine blocked amphetamine-induced ipsilateral rotations in rats with unilateral 6-hydroxydopamine lesions to the nigrostriatal system. In contrast, muscarinic receptor antagonists increased amphetamine-induced rotations. Muscarinic receptor agonists have also been shown to decrease apomorphine-induced climbing (Shannon et al., 2000). In addition, various muscarinic receptor agonists resemble antipsychotics in inhibiting firing of A10 dopamine neurons, blocking amphetamine-induced c-fos expression in the nucleus accumbens, and inhibiting conditioned avoidance behavior in the rat (Bymaster et al., 1998a, 1999; Shannon et al., 1999, 2000). Interestingly, the inhibition of A10 firing by muscarinic receptor agonists is immediate, whereas chronic administration of the dopamine and 5-hydroxytryptamine (5-HT) receptor antagonist clozapine is necessary for this effect, coincident with its delayed therapeutic effect as an antipsychotic agent (Bymaster et al., 1998a; Shannon et al., 2000). Finally, antipsychotics and muscarinic receptor agonists have been shown to improve prepulse inhibition (PPI) disrupted by dopamine receptor antagonists or muscarinic receptor antagonists, respectively (Swerdlow et al., 1994; Jones and Shannon, 2000a,b).

In the present study we have examined the effects of xanomeline in additional rat models of psychosis. These include the reversal of amphetamine-induced hyperlocomotion (Arnt, 1995) and apomorphine-induced disruption of prepulse inhibition (Swerdlow et al., 1994). We also examined the effects of xanomeline on dopamine turnover in the prefrontal cortex as measured ex vivo. Finally, the effects of xanomeline were examined and compared with the effects of antipsychotic agents in the conditioned emotional response (CER) test. Unlike the other tests, this latter test is used to detect anxiolytic-like profile in conflict models of anxiety in animals (Moore et al., 1994). Thus, this test was included to determine whether the effects of a muscarinic receptor agonist would resemble those of either typical or atypical antipsychotics.

Materials and Methods

Animals and Maintenance Conditions

Adult male rats were used. Lister hooded rats were obtained from Harlan Olac (Bicester, Oxon, UK) and Sprague-Dawley rats were obtained from Charles River (Margate, Kent, UK). All experiments were conducted between 9:00 AM and 5:00 PM. Rats were grouped housed under a 12-hour light/dark cycle (lights on at 8:00 AM), in a temperature- (20.5 ± 2.5°C) and humidity- (55 ± 15%) controlled environment. Unless otherwise mentioned food and water were freely available. All animal husbandry and experimental procedures were carried out in accordance with the UK Animals (Scientific Procedure) Act 1986 governing the health and welfare of laboratory animals.

Drugs

Haloperidol (Baker Norton, Harlow Essex, UK), xanomeline tartrate (Medicinal Chemistry Department, Vernalis Research Ltd., Wokingham, UK), d-amphetamine sulfate, apomorphine, scopolamine HBr, methylscopolamine, clozapine (Sigma-Aldrich, Poole, Dorset, UK), and pilocarpine (Tocris Cookson, St. Louis, MO) were used. All dose levels are expressed as free base. The pretreatment time, route of administration, vehicle, and dosing volume varied between experiments as outlined below.

PPI

Sprague-Dawley rats (300–350 g) were tested in four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA). Each chamber consisted of a Plexiglas cylinder (8.8 cm in diameter) mounted on a frame and secured to a base unit by four metal pin legs. Movement of the rat within the cylinder was detected by a piezoelectric accelerometer attached below the frame. A loudspeaker (Radio Shack Supertweeter) was mounted 24 cm above the cylinder and provided background white noise and acoustic stimuli (pulse and prepulses). The whole apparatus was housed in a ventilated and lit chamber (39 × 38 × 58 cm, length × width × height). Presentation of the acoustic stimuli was controlled by SR-LAB software and interface, which also digitized (0–4095), rectified, and recorded the responses from the accelerometer. Mean startle amplitude, the dependent measure, was determined by averaging 150, 1-ms readings taken at the beginning of the pulse stimulus onset. Sound levels of the auditory stimuli were calibrated using a noisemeter (model number 1422C; Dawe Instruments, Instrotech, St. Albans, UK).

On the day before PPI testing, rats were placed into the startle apparatus for a habituation session. After a period of 1 min during which rats were exposed to background noise (70 dB), the session began with rats being exposed to different auditory stimuli. The session began with 10 consecutive startle stimulus trials (120 dB), which lasted 40 ms. This was followed by 12 of each of two trial types: 1) startle stimulus (120 dB, 40 ms), and 2) startle stimulus preceded 100 ms by a prepulse 3 dB above background noise (70 dB) and lasting 20 ms. These two trial types were interspersed across six consecutive blocks, with two of each occurring in each block. The intertrial interval was variable between 20 and 40 s and sessions lasted approximately 18 min.

Test Session. After a habituation period of 1 min during which rats were exposed to background noise (70 dB), the session began with rats being exposed to different auditory stimuli. The session began with 10 consecutive startle stimulus trials (120 dB), which lasted 40 ms. This was followed by 12 of each of the following four trial types: startle stimulus (120 dB, 40 ms), and the startle stimulus preceded 100 ms by prepulses of 3, 6, or 12 dB above background noise (70 dB) and lasting 20 ms. These four trial types occurred across six consecutive blocks, with each trial type occurring twice within each block. Within each of these six blocks there was also one null stimulus that was the same as background noise (70 dB, 40 ms). The intertrial interval was variable between 20 and 40 s and sessions lasted approximately 35 min.

Drugs. All drugs were administered s.c. at a dose volume of 1 ml/kg. Scopolamine HBr, methylscopolamine, haloperidol, pilocarpine, and xanomeline were all dissolved in 0.9% NaCl. Apomorphine was dissolved in a solution of 0.1% ascorbic acid in 0.9% saline. In study 1, haloperidol (n = 8), pilocarpine (n = 9), xanomeline (n = 10), scopolamine HBr (n = 10–11), or methylscopolamine (n = 8) were tested alone to ascertain their effects on the startle response and PPI. In this study, all compounds were administered 30 min before rats were placed in the startle chambers, except pilocarpine, which was administered 20 min pretest. Study 2 assessed the ability of haloperidol (n = 8), pilocarpine (n = 8), and xanomeline (n = 9–10)
to reverse the effects of apomorphine on PPI. In this interaction study, haloperidol, pilocarpine, and xanomeline were administered 30 min pretest and apomorphine was administered 10 min pretest. Control rats were administered with the appropriate vehicle at the appropriate pretreatment times. Thus, all animals in study 2 received either apomorphine or its vehicle 10 min before the session and the target drug and its vehicle 30 min before the session.

Amphetamine-Induced Hyperlocomotion

Sprague-Dawley rats weighing 350 to 550 g were used. The rats were placed in the experimental room on the day of the procedure. Animals were administered the test compound or saline s.c. 40 min pretest (1 ml/kg) and were returned to their home cages (n = 7–8). Immediately before testing, the animals were administered s.c. with d-amphetamine (0.5 mg/kg) or saline (1 ml/kg). The animals were then placed individually in an activity monitor cage (48 × 27 × 20 cm, length × width × height) and locomotor activity was measured for 1 h (Activity Monitor AM1052; Benwick Electronics, Linton Instrumentation, Norfolk, UK). The activity monitor cage was positioned in a frame equipped with infrared light sources and photocells in a 7 by 4 matrix. The light beams crossed the cage 3 cm above its base. Locomotor activity was assessed by counting the total number of beam breaks that occurred during a 1-h test period.

Dopamine Turnover in Rat Prefrontal Cortex

Sprague-Dawley rats (300–350 g) were weighed before s.c. administration of drug or vehicle control (1 ml/kg, n = 7–8 depending upon study). In study 1, rats were administered either vehicle control (8% Tween in saline), haloperidol (0.1 or 1 mg/kg), or clozapine (1 or 10 mg/kg). In studies 2 and 3, rats were administered either vehicle control (8% Tween in saline, study 2; or saline, study 3) or pilocarpine (1 or 3 mg/kg, study 2; or 3 or 10 mg/kg, study 3). In the final study, rats were administered either saline control or xanomeline (1, 3, 10, or 30 mg/kg). Drug treatments were coded and administration to each animal determined by a Latin square, with tissue dissection conducted blind to treatment. In all experiments, animals were sacrificed 60 min postinjection and the prefrontal cortex dissected on ice. Tissues were then snap frozen on dry ice and stored at −80°C before analysis.

Prefrontal cortex tissue content of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by high-performance liquid chromatography as described below. Dopamine turnover (T/O) was then defined by the equation T/O = ([DOPAC] + [HVA])/[dopamine].

Tissue samples were homogenized in Eppendorf tubes in ice-cold 0.1 M perchloric acid (10 µl/mg of tissue) and centrifuged at 10,000 rpm for 10 min. The resultant supernatant was then spun through Acrodisc 0.45-µm filters and finally diluted either 1:5 or 1:10 in ice. Tissues were then snap frozen on dry ice and stored at −80°C before analysis.

Hooded Lister rats (initial weights 250–300 g) that were food-deprived to approximately 90% of their free feeding weight were trained in 24 operant chambers (Coulbourn Instruments, Allentown, PA) with associated Coulbourn pellet dispensers and operant levers. These chambers were housed in outer sound- and light-attenuating shells equipped with a ventilation fan that also helped to mask external noise. A single operant lever was positioned on the left side of the front panel of the operant chamber, 2 cm above the grid floor. Coulbourn animal test cage grid floor shockers were used to deliver shock (0.4 mA, 0.5 s) to the grid floors (model E-10-10SF). Formula P Noyes food pellets (45 mg) could be delivered to the food magazine centered on the front wall of the chamber approximately 2 cm above the floor. A houselight (4.0 mA, 28 V) was positioned on the front wall of the chamber, above the food magazine, approximately 1.0 cm below the ceiling. The operant chambers were controlled and the number of lever presses recorded by an Acorn computer programmed in Paul Fray Arachnid software (Cambridge, UK).

By using the methods described by Stanhope and Dourish (1996), hungry rats were trained in darkened chambers to lever press for food pellets on a random interval 90-s schedule. Rats then received training during which presentation of a houselight was sometimes paired with mild electric footshock. These sessions were approximately 60 min in length and usually conducted 5 days/week. During these sessions, food pellets continued to be available on a random interval 90-s schedule as before. However, the rats were now presented with two, 2-min-long periods of houselight illumination. The first light presentation occurred approximately 20 min after the start of the session (range 15–25 min) and the second light presentation occurred approximately 20 min after the first light presentation (range 15–25 min). Light presentations were occasionally followed by a 0.5-s, 0.4 mA footshock. In a given session, footshock could follow neither, one, or both of the light presentations. The rate of lever pressing during each of the light presentations (the “dur” period) and the rate of lever pressing in the 2-min interval preceding each light presentation (the “pre” period) was recorded. These response rates were used to calculate suppression ratios using the following formula: response rate during light/(response rate dur light) + (response rate pre light)]. A suppression ratio of 0 indicates that the light has evoked conditioned fear and has completely suppressed lever pressing, whereas a suppression ratio of 0.5 indicates the response rate is unchanged by the light presentation, i.e., the complete absence of fear.

Once a reliable suppression ratio was observed, occasional test sessions occurred. Test sessions were identical to training sessions except that footshock was never delivered after the first light presentation and treatment with either vehicle or a test compound occurred before the session began. Drug tests occurred no more frequently than once per week and a baseline training session was always carried out before each test session.

Drugs. Before drug test sessions, the rats were semirandomly assigned to groups such that the groups were matched for the rate of lever pressing and the level of conditioned suppression displayed during baseline training. Drug treatment in each study was orthogonal to drug treatment administered to the animals in the previous test. Rats with suppression ratios of greater than 0.15 or that did not lever press in both pre periods in the previous baseline session were excluded a priori from experiments.

In each drug test, a group of rats was treated with a benzodiazepine receptor agonist, either chlordiazepoxide (CDP) or diazepam, which served as a positive control within each experiment. Due to different pretest times, routes of administration and vehicle between the positive control compound and the target compounds, in the clozapine and the haloperidol experiments each drug treatment group received two injections. In the clozapine experiment, there were five groups of rats (n = 7–9) given the following pair of treatments 1) vehicle/vehicle, 2) vehicle/2.5 mg/kg clozapine, 3) vehicle/5.0 mg/kg clozapine, 4) vehicle/10 mg/kg clozapine, and 5) 10 mg/kg CDP/vehicle. The first injection (CDP or its vehicle of 1% methylcel- lulose) was administered p.o. in a dosing volume of 2 ml/kg 40 min before the rat was placed into the operant chamber. The second injection [clozapine or its vehicle of 20% polyethylene glycol 400 in acified ph-cycloextrin (25 M citric acid solution in 10% ph-cycloextrin, w/v)] was administered s.c. in a dosing volume of 2 ml/kg 10 min before the rat entered the chamber.
In the haloperidol experiment, five groups of rats (*n* = 10–11) were given pairs of injections with the first injection being either diazepam or its vehicle and the second injection of either haloperidol or its vehicle as follows: 1) vehicle/vehicle, 2) vehicle/0.01 mg/kg haloperidol, 3) vehicle/0.03 mg/kg haloperidol, 4) vehicle/0.1 mg/kg haloperidol, and 5) 4.0 mg/kg diazepam/vehicle. Haloperidol was purchased in an aqueous solution of 5 mg/ml in Serenac ampoules. This solution of haloperidol was diluted to the appropriate concentrations by using 0.9% saline vehicle. In this experiment and in the following xanomeline study, diazepam was dissolved in 8% Tween 80 and administered at a dosing volume of 1 ml/kg. Both injections were s.c. and occurred approximately 30 min before the rats being placed into the operant chambers.

In the xanomeline study (*n* = 7–9), both xanomeline and the positive control compound diazepam were administered using the same vehicle, dosing volume, route, and pretreatment time as used for diazepam in the preceding haloperidol study. Therefore, the animals in the xanomeline study received only one injection of vehicle; 2.5, 5.0, 10, or 20 mg/kg xanomeline; or 4.0 mg/kg diazepam.

**Data Analysis**

**PPI.** The dependent measures in the PPI studies were startle amplitude after the 120-dB startle stimulus (measured in arbitrary units) and percentage of PPI. The latter was calculated according to the following formula: [100 − (startle amplitude on prepulse-pulse trials/startle amplitude on pulse alone trials) × 100]. For study 1, the main effect of treatment on startle reactivity was analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test. Percentage of PPI was analyzed using two-way ANOVA with the factors drug treatment and prepulse intensity (three levels: 3, 6, and 9 dB). A main effect of treatment was followed by Newman-Keuls test. If, however, there was an interaction between treatment and prepulse intensity then a one-way ANOVA was performed on each prepulse-pulse trial separately followed by Newman-Keuls test, where appropriate.

Apomorphine (0.5 mg/kg) routinely increases startle amplitude and impairs PPI in our hands. Therefore, this was the dose chosen to disrupt PPI in the drug interaction experiments in study 2. Before conducting statistical analysis on the target drug effects in study 2, the predicted effects of apomorphine on both the startle response and percentage of PPI were confirmed. A *t* test was used to determine whether apomorphine affected the magnitude of the startle response to the 120-dB stimulus in the interaction studies (i.e., the apomorphine/vehicle group were compared with the vehicle/vehicle-treated group). For the PPI measure in the drug interaction experiments, a mixed two-way analysis of variance, including the vehicle/vehicle-treated group and the vehicle/apomorphine-treated group as a between-subjects factor and prepulse intensity as a within-subjects factor, was conducted to confirm that apomorphine significantly disrupted PPI. Once the expected disruption in PPI was confirmed, a two-way analysis of variance (with drug treatment as the between-subjects factor and prepulse intensity as the within-subjects factor) was conducted to determine whether any dose of the test compound significantly reversed the disruptive effects of apomorphine on PPI. Only the groups treated with apomorphine were included in this analysis. One-way ANOVA with only the apomorphine-treated groups was also used to determine whether the target drug in combination with apomorphine had any effects on the startle response to the 120-dB stimulus. Significant effects of drug treatment were followed by post hoc multiple group comparisons with a Newman-Keuls test.

**Amphetamine-Induced Hyperlocomotion.** Two-way ANOVA was used to assess whether there was a significant effect of amphetamine and a possible interaction between the test compound and amphetamine. If a significant interaction was detected, separate one-way ANOVAs were conducted to determine whether significant drug effects were induced in the animals pretreated with 1) vehicle or 2) d-amphetamine. Significant main effects in the separate one-way ANOVAs were followed by post hoc Dunnett’s *t* tests.

**Dopamine Turnover in Rat Prefrontal Cortex.** Drug-induced changes in dopamine turnover were assessed for statistical significance by one-way ANOVA followed post hoc by Dunnett’s *t* tests, where appropriate.

**CER.** A mixed within-subjects (trials) and between-subjects (drug treatment) ANOVA was conducted separately on the rate of lever pressing during the pre and dur periods and on the suppression ratios. Significant interactions were followed by one-way analysis of variance on the data collected for each trial. Significant main effects of drug treatment were analyzed using post hoc Dunnett's *t* test. In experiments in which significant drug treatment × trial interactions were identified, the data for each trial are presented separately. Otherwise, the data for each experiment are presented collapsed across the two test trials.

Suppression ratios could not be calculated if no lever presses occurred during the pre light periods. Thus, animals that failed to respond during either the first or the second light presentation were excluded from the statistical analysis.

**Results**

**Prepulse Inhibition**

**Study 1: Effect of Haloperidol, Scopolamine, Pilocarpine, and Xanomeline.** Haloperidol. Haloperidol induced a significant dose-dependent decrease in startle amplitude (*F*[4,35] = 2.6, *P* < 0.05) with a minimum effective dose of 0.3 mg/kg, compared with vehicle (Fig. 1A). In contrast, haloperidol had no significant effect on percentage of PPI (*F*[4,35] = 1.4; Fig. 1B). The treatment by prepulse intensity interaction on percentage of PPI also failed to reach statistical significance (*P* < 1; data not shown).

![Fig. 1](https://example.com/fig1.png)
Pilocarpine. A strong nonlinear tendency for pilocarpine to decrease startle amplitude at the 3.0-mg/kg dose (Fig. 2A) was observed at the doses tested. However, this effect did not achieve statistical significance ($F_{[3,32]} = 1.4$). In contrast, pilocarpine induced a significant and dose-dependent impairment in percentage of PPI ($F_{[3,32]} = 8.7; P < 0.01$), with 10 mg/kg pilocarpine significantly impairing PPI relative to vehicle (Fig. 2B). This effect of pilocarpine on percentage of PPI was not influenced by prepulse intensity ($F < 1$ for the interaction; data not shown).

Xanomeline. Xanomeline significantly and dose dependently decreased startle amplitude with a minimum effective dose of 30 mg/kg ($F_{[3,36]} = 3.2; P < 0.04$; Fig. 2C). Xanomeline had no significant effects on percentage of PPI at the doses used ($F_{[3,36]} = 1.9$; Fig. 2D) nor was there an interaction between xanomeline treatment and prepulse intensity ($F_{[6,72]} = 1.9$; data not shown).

Scopolamine. Scopolamine was tested as both the methyl and HBr salts within the same experiment. Neither salt form of this drug significantly affected startle amplitude at the doses tested ($F_{[4,46]} = 2.1; P < 0.1$; Fig. 3A). However, there was a strong tendency toward a decrease, most notably after 0.1 mg/kg of the methyl salt form of scopolamine. In contrast, there was a pronounced and significant effect of drug treatment on percentage of PPI ($F_{[4,46]} = 2.9; P < 0.03$; Fig. 3B). Scopolamine HBr dose dependently reduced percentage of PPI, and this was significant compared with vehicle after the 0.1-mg/kg dose. The group administered 0.1 mg/kg methylscopolamine did not differ from vehicle on the percentage of PPI measure. There was no significant interaction between treatment and prepulse intensity on percentage of PPI measure ($F < 1$; data not shown).

Study 2: Effect of Haloperidol, Pilocarpine, and Xanomeline on Apomorphine-Induced Deficits in PPI. Haloperidol. In this study, apomorphine when administered alone, had no significant effects on mean startle amplitude to the 120-dB stimulus ($t_{(14)} = 0.025; P = 0.81$; Fig. 4A). As predicted, however, apomorphine significantly impaired percentage of PPI ($F_{[1,14]} = 20; P < 0.001$; Fig. 4B); this effect did not interact with prepulse intensity ($F < 1$). In rats
pretreated with haloperidol, ANOVA revealed no main effect of drug treatment on the startle amplitude to the 120-dB stimulus ($F_{1,100} = 2.7; P = 0.01$; Fig. 5A). Xanomeline pretreatment did not affect this apomorphine-induced increase in startle amplitude ($F_{[4,41]} = 1.7$), although as can be seen in Fig. 5A, the 3-mg/kg dose produced a nonsignificant trend toward a reversal of the apomorphine-induced increase in startle amplitude. As in the haloperidol experiment, apomorphine significantly reduced percentage of PPI compared with vehicle treatment ($F_{[1,18]} = 6.2; P < 0.02$; Fig. 5B). This effect of apomorphine did not significantly interact with prepulse intensity ($F_{[2,36]} = 3.1; P = 0.06$; data not shown). Xanomeline pretreatment reversed the apomorphine-induced deficit in PPI ($F_{[4,41]} = 5.3; P = 0.001$; Fig. 5B), and this effect did not interact with prepulse intensity ($F_{[8,82]} = 1.2$; data not shown). Post hoc analysis demonstrated that rats pretreated with the highest dose of xanomeline (30 mg/kg) differed significantly from the group given vehicle before apomorphine.

Pilocarpine. As with the xanomeline experiment, there was a significant increase in startle amplitude after apomorphine ($t_{[14]} = 2.53; P = 0.02$; Fig. 5C). Pilocarpine pretreatment significantly reversed this apomorphine-induced increase in startle amplitude ($F_{[4,35]} = 4.7; P < 0.01$). Post hoc analysis showed that rats pretreated with 10 mg/kg pilocarpine differed significantly from rats administered vehicle before apomorphine treatment. As in the previous two studies, apomorphine induced a robust deficit in percentage of PPI ($F_{[1,14]} = 6.6; P < 0.02$; Fig. 5D). This effect did not interact with prepulse intensity ($F < 1$; data not shown). However, in contrast to haloperidol and xanomeline, pilocarpine did not affect the apomorphine-induced deficit in PPI. There was no significant effect of pilocarpine pretreatment ($F < 1$) nor an interaction between pilocarpine treatment and prepulse intensity ($F < 1$; data not shown).
Amphetamine-induced hyperlocomotion. When administered in the absence of a test compound, 0.5 mg/kg d-amphetamine consistently increased locomotor activity from a baseline of approximately 1200 to approximately 3700 beam breaks (Figs. 6–8). This induction of hyperlocomotion was confirmed by the finding of a significant main effect of amphetamine compared with vehicle treatment in all three studies ($F_{[1,17]} = 90.33, F_{[1,69]} = 77.48$ and $F_{[1,56]} = 209.31$; all $P$ values < 0.0001).

At a dose of 0.1 mg/kg, haloperidol significantly reduced baseline levels of locomotor activity ($F_{[3,28]} = 12.7; P < 0.01$). Both 0.03 and 0.1 mg/kg haloperidol significantly reduced the hyperlocomotion induced by d-amphetamine ($F_{[3,28]} = 36.7; P < 0.01$; Fig. 6).

In rats that were pretreated with vehicle, clozapine significantly reduced photobeam breaks with a minimum effective dose of 1 mg/kg, although the effects of 3 mg/kg just failed to reach statistical significance ($F_{[4,35]} = 2.8; P < 0.05$). Clozapine significantly reversed the effects of amphetamine pretreatment with an minimum effective dose of 1 mg/kg ($F_{[4,34]} = 7.4; P < 0.01$; Fig. 7).

Xanomeline had no significant effects on the baseline levels of locomotor activity ($F_{[4,35]} = 1.9$) but significantly reduced d-amphetamine-induced hyperactivity at a minimum effective dose of 1 mg/kg ($F_{[4,35]} = 31.9; P < 0.01$; Fig. 8).

Dopamine turnover in rat prefrontal cortex. Basal dopamine, DOPAC, and HVA content in vehicle-treated animals, from which dopamine turnover was calculated, are shown in Table 1A.

The antipsychotic agents haloperidol and clozapine both dose dependently and significantly increased dopamine turnover in rat prefrontal cortex (Table 1B, study 1). Thus, at 0.1 and 1.0 mg/kg haloperidol increased turnover by 34% ($P < 0.05$; Dunnett's test) and 55% ($P < 0.01$), respectively, above the control ratio. Clozapine was less potent, its administration leading to an increase in dopamine turnover of 19% (nonsignificant) and 47% ($P < 0.01$) above the control ratio after 1 and 10 mg/kg, respectively.

Pilocarpine had no effect on prefrontal cortex dopamine turnover at 1 mg/kg but did significantly increase turnover at 3 mg/kg, by 67% compared with the control ratio ($P < 0.01$; Table 1B, study 2). The effect at 3 mg/kg observed in study 2 was replicated in study 3 (55% increase above control ratio; $P < 0.05$) in which the effect of higher doses of pilocarpine were also investigated. Higher doses of the muscarinic agonist (10 and 30 mg/kg) had no significant effect on the same neurochemical measure in this study, thereby producing a "bell-shaped" dose-response curve with a narrow window of efficacy.

Xanomeline dose dependently increased dopamine turnover with a minimum significantly effective dose of 30 mg/kg (51% above control ratio; $P < 0.01$; Table 1B, study 4). Administration of 10 mg/kg xanomeline resulted in a raised dopamine turnover ratio compared with control (24% increase), which fell just short of statistical significance.

Conditioned Emotional Response. Clozapine. Clozapine administered at 5 but not 10 mg/kg significantly decreased rates of lever pressing during the pre light period ($F_{[4,35]} = 2.7; P < 0.05$; Fig. 9A). Although this effect tended to be observed on the second trial but not the first trial, the treatment × trial interaction did not achieve statistical significance ($F_{[4,35]} = 1.5; P > 0.05$). In contrast, there was a significant interaction between drug treatment and trial on the rates of lever pressing during the light stimulus ($F_{[4,35]} = 3.9; P < 0.01$), but the source of this interaction could not be determined. Separate one-way ANOVAs on the response rates during the light for each trial showed a significant effect of drug treatment on both trial 1 and trial 2 ($F$ values$_{[4,35]} = 6.0$ and 2.8, respectively; $P$ values < 0.05). In both cases post hoc Dunnett's t test showed that CDP but not clozapine significantly increased lever pressing during the
light period compared with vehicle (Fig. 9B). Although the effects of clozapine on light response rates were not statistically significant, both 10.0 mg/kg clozapine and CDP significantly increased suppression ratios compared with vehicle ($F_{(4,35)} = 5.1; P < 0.01$; Fig. 9C). The trial by drug treatment interaction for suppression ratios was not statistically significant ($F_{(4,35)} = 1$).

Haloperidol. Only two of the 11 rats in the group treated with the highest dose of 0.1 mg/kg haloperidol were able to respond during the pre period of both trials. For the purpose of presentation, the results of these two animals are included in the figure, although their results are excluded from the statistical analyses. Up to a dose of 0.03 mg/kg, haloperidol did not significantly affect pre light rates of lever pressing ($F_{(3,37)} = 1$; Fig. 10A). However, there was a significant effect of drug treatment on lever pressing during the light ($F_{(3,37)} = 15; P < 0.01$). Post hoc Dunnett’s $t$ test demonstrated a significant increase in lever pressing during the light after diazepam relative to vehicle (Fig. 10A). A significant effect of drug treatment on suppression ratios ($F_{(3,37)} = 31; P < 0.01$) was again due to a significant difference between the diazepam and vehicle group (Fig. 10B). There were no interactions between drug treatment and trial on any of the measures in this experiment ($F$ values < 1).

Xanomeline. After 10 and 20 mg/kg xanomeline the number of rats reaching the criteria of at least one response in

### Table 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug (mg/kg)</th>
<th>Drug Dose (s.c.) 60 min before Sacrifice</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Haloperidol</td>
<td>100 ± 7 134 ± 7a 155 ± 6b</td>
<td>$F_{(4,35)} = 7.83$</td>
</tr>
<tr>
<td>1</td>
<td>Clozapine</td>
<td>100 ± 7 119 ± 7</td>
<td>$F_{(4,35)} = 7.83$</td>
</tr>
<tr>
<td>2</td>
<td>Pilocarpine</td>
<td>100 ± 14 122 ± 4 167 ± 12a 108 ± 13 82 ± 12</td>
<td>$F_{(3,28)} = 7.36$</td>
</tr>
<tr>
<td>3</td>
<td>Pilocarpine</td>
<td>100 ± 13 155 ± 9a 124 ± 7</td>
<td>$F_{(3,28)} = 7.36$</td>
</tr>
<tr>
<td>4</td>
<td>Xanomeline</td>
<td>100 ± 10 100 ± 10 93 ± 8</td>
<td>$F_{(4,34)} = 7.86$</td>
</tr>
</tbody>
</table>

*Significantly different to baseline, $P < 0.05$.  
bSignificantly different from baseline, $P < 0.01$.  

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**Fig. 9.** Effects of clozapine (s.c.) and CDP (p.o.) on mean lever press rates on trial 1 (□) and trial 2 (■): before (A) and during (B) the light presentation, and on the calculated (C) suppression ratios. Mean ± S.E.M. * indicates significantly different relative to the vehicle-treated control group ($n = 7–9/group$).

**Fig. 10.** Effects of haloperidol (s.c.) and diazepam (DZM, s.c.) on mean lever press rates (A) before (□) and during (■) the light presentations, and on the calculated (B) suppression ratios (bottom). Mean ± S.E.M. * indicates significantly different relative to the vehicle-treated control group ($n = 10–11/group$).
Xanomeline had similar effects to haloperidol and/or clozapine in all of the models used in the present study. Like haloperidol and clozapine, xanomeline enhanced dopamine turnover in the prefrontal cortex and reversed amphetamine-induced hyperactivity at doses not disrupting locomotor activity alone. Xanomeline also reversed the disruptive effects of apomorphine in the prepulse inhibition model, which is claimed to have both face and construct validity vis a vis schizophrenia symptomology (Swerdlow et al., 1994). Like the atypical antipsychotic clozapine, xanomeline reduced conditioned suppression in the CER test. These findings and published data suggest that xanomeline may be an effective antipsychotic agent in humans.

Haloperidol alone had no effect on PPI but reversed the PPI deficit induced by apomorphine. Others have also demonstrated this effect of haloperidol, although the minimum effective dose of haloperidol is higher in some studies (Swerdlow et al., 1994; Varty and Higgins, 1995). Haloperidol may also enhance PPI in normal rats, but procedural changes are used to generate low baseline PPI values (Depoortere et al., 1997). An enhancement of PPI in normal rats by an antipsychotic drug would not be expected using the parameters used here.

Whereas xanomeline (30 mg/kg) reversed the apomorphine-induced deficit in PPI, pilocarpine up to a dose of 10 mg/kg had no effect. It could be argued that a higher dose of pilocarpine could reverse the disruption in PPI induced by apomorphine. This seems unlikely, however, given that at 10 mg/kg pilocarpine alone completely abolished PPI in untreated rats (Fig. 2B), an effect that is typical of a psychotomimetic as opposed to an antipsychotic action of drugs (see below).

Shannon et al. (1999, 2000) have shown that at 30 mg/kg xanomeline also impairs conditioned avoidance response (CAR) in rats and that this effect can be blocked by scopolamine. However, these authors showed that at 10 mg/kg pilocarpine also decreases CARs. The difference in the profile of pilocarpine in the PPI and CAR models is unclear. However, in this study we specifically manipulated the dopamine system, whereas the pharmacological basis for the CAR procedure may be more complex (Arnt, 1982; Ellenbroek, 1993).

We have also demonstrated that scopolamine HBr impairs PPI (0.1 mg/kg) with no effect on startle. In contrast, at the dose tested, methylscopolamine, which acts mainly in the periphery, tended to reduce startle without affecting PPI. Thus, scopolamine may decrease startle and impair PPI via peripheral and central mechanisms, respectively. The decrease in startle with scopolamine concurs with some previous studies (Davis, 1980). However, Fendt and Koch (1999) showed that scopolamine injections into the caudal pontine reticular nucleus increased startle. Thus, scopolamine acting at peripheral and central muscarinic receptors may have opposite effects on startle. Fendt and Koch (1999) also showed that scopolamine injected into the pontine reticular nucleus significantly impaired PPI, although the effect was not dose-related. In contrast, we saw a dose-related reduction in PPI with scopolamine HBr. These findings with scopolamine concur with clinical data indicating that anticholinergics are psychotomimetic (Neubauer et al., 1966a,b).

Xanomeline, like classical and atypical antipsychotics, attenuated amphetamine-induced hyperactivity (Arnt, 1995). This result is in keeping with previous findings for pilocarpine (Proctor et al., 1967). Whether reversal of amphetamine-induced hyperactivity is a predictor of antipsychotic activity is unknown. However, the effect of drugs on locomotor hyperactivity induced by dopaminergic agonists has been validated and used routinely as an animal model to assess limbic dopaminergic function (Costall et al., 1980; Arnt et al., 1994). Thus, attenuation of limbic dopaminergic function via
muscarnic receptor activation may reduce the reported hyperdopaminergic state observed in schizophrenic patients and represent an alternative class of drug to existing therapies.

Interestingly, clozapine attenuated amphetamine-induced hyperactivity only at doses that also reduced spontaneous locomotor activity. In contrast, haloperidol attenuated amphetamine-induced hyperactivity at a dose 3-fold lower than that reducing spontaneous activity. This is in contrast with a previous study, which although finding a similar dose separation for haloperidol, demonstrated that clozapine attenuated amphetamine-induced hyperactivity at a dose 6.5-fold lower than that reducing spontaneous locomotor activity (Arnt, 1995). Procedural differences between our study and that of Arnt (1995) may explain this discrepancy. In particular, in the study of Arnt (1995) the effects of clozapine on spontaneous locomotor activity and amphetamine-induced hyperactivity were assessed in different experiments published 4 years apart (Sanchez et al., 1991; Arnt, 1995), whereas currently the comparison was made in a single experiment.

Although xanomeline attenuated amphetamine-induced hyperactivity at doses of 1 to 10 mg/kg, the drug had no effect on spontaneous activity over the same dose range, indicating the possibility of a wide separation between attenuation of limbic hyperdopaminergic function and the onset of sedation.

The antipsychotics haloperidol and clozapine, and the muscarinic receptor agonists pilocarpine and xanomeline, all induced an increase in medial prefrontal cortex (mPFC) dopamine turnover, assessed ex vivo. The increase in dopamine turnover observed is likely to result from an increase in mPFC dopamine release. For clozapine, an increase in dopamine turnover is consistent with previous microdialysis studies, in which the atypical neuroleptics clozapine and olanzapine potentiated dopamine release in rat mPFC (Kuroki et al., 1999). Interestingly, haloperidol had either no effect or a mild effect on mPFC dopamine release in these studies, in agreement with intracortical infusion studies (Pehek and Yamamoto, 1994). Thus, clozapine and olanzapine have a more pronounced effect on dopamine function in cortical than subcortical areas, whereas haloperidol has the opposite profile, leading to the suggestion that this is representative of an atypical antipsychotic action. However, other studies, including those described here, have reported an enhancement by haloperidol of mPFC dopamine metabolism or release (Karoum and Egan, 1992). Thus, conclusions regarding the “typical” or “atypical” nature of the muscarinic receptor agonists cannot be drawn from the present study. However, the recent finding that xanomeline preferentially increases extracellular dopamine and expression of certain immediate early genes in the PFC/nucleus accumbens relative to the striatum is consistent with an atypical antipsychotic action (Perry et al., 2001).

The efficacious dose of xanomeline that increased dopamine turnover in rat mPFC (30 mg/kg) correlates well with the effects of the drug in PPI. This is the same dose required to reduce startle in normal rats and is also the minimum efficacious dose for the drug to reverse apomorphine-induced deficit in PPI. This correlation supports the contention that an increase in mPFC dopamine function may contribute to an antipsychotic-like effect of the drug.

In contrast, pilocarpine had neither a clear dose-dependent effect on dopamine turnover (increasing this measure at only 3 mg/kg, but not at higher doses) nor does it reverse apomorphine-induced deficits in PPI. It is interesting that pilocarpine actually produces a PPI deficit in its own right at 10 mg/kg, the dose at which the drug no longer increases dopamine turnover. It seems likely that other nonspecific effects of the drug are engaged at this dose, resulting in the behavioral and neurochemical profiles observed. The less specific pharmacology of pilocarpine may also underlie the absence of an effect on PPI at doses around 3 mg/kg, which increases mPFC dopamine turnover. Interestingly, however, this is the same dose that resulted in a clear tendency to reduce startle.

Other aspects of xanomeline’s pharmacology could account for its antipsychotic profile in PPI and set it apart from pilocarpine. Xanomeline is an agonist at 5-HT\(_{1A}\) and 5-HT\(_{1B}\) receptors and an antagonist at 5-HT\(_{2}\) receptors (Watson et al., 1998). However, these effects are unlikely to account for xanomeline’s effects in PPI because agonist actions at 5-HT\(_{1A}\) or 5-HT\(_{1B}\) receptors are reported to disrupt PPI and the 5-HT\(_{2}\) receptor antagonist MDL 100907 does not reverse apomorphine-induced disruption of PPI (Sipes and Geyer, 1994; Geyer et al., 1999). It seems more likely that differences in selectivity and/or efficacy for muscarinic receptor subtypes (e.g., M\(_1\), M\(_2\), or M\(_4\)) may account for the differences between pilocarpine and xanomeline (Bymaster et al., 1998b; Felder et al., 2000; Shannon et al., 2000).

In the CER model of anxiety clozapine (10 mg/kg) and xanomeline (5.0 mg/kg), but not haloperidol, had anxiolytic-like profiles as demonstrated by an increase in the suppression ratio. These effects were demonstrable under conditions where the clinically effective anxiolytics clordiazepoxide or diazepam also induced anxiolysis. However, the effects of xanomeline appear to be less robust than those of clordiazepoxide, diazepam, and clozapine, which increased suppressed responding without reducing baseline response rates. In contrast, xanomeline nonsignificantly reduced baseline responding at the same dose that tended to increase suppressed responding, and it is this combination of effects that have resulted in a significant change in the suppression ratio. Although this would suggest a cautious interpretation, the tendency to increase responding in the conditioned stimulus period at the same doses that reduced baseline responding is not a profile commensurate with a general nonspecific reduction in lever pressing such as that seen with haloperidol. Xanomeline’s profile is similar to that obtained with high doses of ipsapirone (10 mg/kg) or diazepam (10–30 mg/kg; Stanhope and Dourish, 1996). The lack of an anxiolytic-like effect with haloperidol concurs with its lack of effect in conflict tasks (Moore et al., 1994).

In summary, xanomeline dose dependently reverses apomorphine-induced deficits in PPI, and over the same doses and time course increases dopamine turnover in rat PFC. In addition, it reverses amphetamine-induced hyperlocomotion. These data taken together provide further evidence that xanomeline may have antipsychotic potential. The profile observed in the CER model, moreover, suggests that its neuroleptic action may be more similar to that of clozapine than haloperidol. The ability of xanomeline to induce these effects in rats is consistent with recent clinical data from patients with Alzheimer’s disease (Bodick et al., 1997). The predictive validity of these data for the antipsychotic effects of muscarinic receptor agonists in schizophrenic patients awaits the results of clinical trials.
References


Address correspondence to: Dr. N. R. Mirza, Head of Pharmacology, Neuro-Search A/S, 93 Pederstrupvej, DK-2750, Ballerup, Denmark. E-mail: max@neurosearch.dk