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Effects of anticholinergic drugs selective for muscarinic receptor subtypes on prepulse inhibition in mice

Makoto Ukai*, Ami Okuda, Takayoshi Mamiya

Department of Chemical Pharmacology, Faculty of Pharmacy, Meijo University, Nagoya 468-8503, Japan

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Abstract

The effects of anticholinergic drugs selective for muscarinic receptor subtypes on prepulse inhibition of acoustic startle response were determined in mice. The prepulse inhibition is associated with sensorimotor information processing in the brain. The anticholinergic agent scopolamine (0.3 mg/kg, s.c.) significantly attenuated prepulse inhibition, while the drug (1–10 mg/kg, s.c.) had no effects on startle amplitude as an indicator of startle response. The muscarinic M_1 receptor antagonist pirenzepine (0.1–10 μg/mouse, i.c.v.) and the muscarinic M_2 receptor antagonist AF-DX116 (11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one) (0.1–10 μg/mouse, i.c.v.) had no effects on prepulse inhibition or startle amplitude. The muscarinic M_3 receptor antagonist 4-DAMP (1,1-dimethyl-4-diphenylacetoxy-piperidinium iodide) (30 μg/mouse, i.c.v.) and the muscarinic M_4 receptor antagonist tropicamide (0.1 μg/mouse, i.c.v.) significantly attenuated prepulse inhibition, while tropicamide (0.01 μg/mouse, i.c.v.) but not 4-DAMP (10 and 30 μg/mouse, i.c.v.) produced a significant increase in startle amplitude. These results suggest that the blockade of muscarinic M_3 and M_4 receptors leads to the disruption of prepulse inhibition.

Keywords: Prepulse inhibition; Scopolamine; AF-DX116; 4-DAMP; Tropicamide; Pirenzepine; Muscarinic receptor; (Mouse)

1. Introduction

The startle response induced by acoustic stimulation has been demonstrated to be inhibited by prior acoustic stimulation with relatively small sound. This phenomenon is named prepulse inhibition, which is associated with sensorimotor information processing in the brain (Varty et al., 2001).

The prepulse inhibition has been reported to be impaired in schizophrenic patients (Braff et al., 1992). In particular, the nonselective dopamine receptor agonist apomorphine and the *N*-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine have been shown to impair prepulse inhibition in rodents (Mansbach et al., 1988; Mansbach and Geyer, 1989), while antipsychotic drugs improve the impairment of prepulse inhibition (Swerdlow and Geyer, 1993). The dysregulation of dopamine and glutamate is generally considered to be central to the symptom of schizophrenia and the disturbance of prepulse inhibition. In contrast, there are multiple lines of evidence suggesting the possible involvement of muscarinic cholinergic systems in the mechanisms of prepulse inhibition with acoustic startle response. Although the original findings with carbachol have been reported by Caine et al. (1992), carbachol dose-dependently enhances prepulse inhibition and attenuates startle amplitude. Scopolamine dose-dependently reduces prepulse inhibition and enhances startle amplitude (Jones and Shannon, 2000a; Geyer et al., 2001). Furthermore, trihexyphenidyl and benztropine have been demonstrated to significantly decrease prepulse inhibition, although muscarinic receptor agonists, such as pilocarpine, oxotremorine, and arecholine, as well as the cholinesterase inhibitors physostigmine and tacrine, have no effects on prepulse inhibition (Jones and Shannon, 2000b). Recently, the muscarinic receptor agonist xanomeline dose-dependently reverses the apomorphine-induced disruption of prepulse inhibition (Stanhope et al., 2001). At present, however, the contribution of muscarinic receptor subtypes to prepulse inhibition is inconclusive in rodents, particularly in rats.

In an attempt to further clarify the involvement of acetylcholine receptor subtypes in prepulse inhibition in mice, the

* Corresponding author. Tel.: +81-52-832-1781; fax: +81-52-834-8090.
E-mail address: ukai@ccmfs.meijo-u.ac.jp (M. Ukai).

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effects of the muscarinic M_1 receptor-preferring antagonist pirenzepine (Ukai et al., 1995, 1997; Eglen et al., 1996), the muscarinic M_2 receptor-preferring antagonist AF-DX116 (11-[[2-diethylamino-*O*-methyl]-1-piperidinyl]acetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepine-6-one) (Baratti et al., 1993; Billard et al., 1995), the muscarinic M_3 receptor-preferring antagonist 4-DAMP (1,1-dimethyl-4-diphenylacetoxy-piperidinium iodide) (Michel et al., 1989), and the muscarinic M_4 receptor-preferring antagonist tropicamide (Lazareno et al., 1990), in addition to the anticholinergic agent scopolamine, on prepulse inhibition and startle amplitude, which is an indicator of startle response, were determined in mice, although it is possible that the pharmacology of prepulse inhibition in mice is very different from rats in many ways.

2. Materials and methods

2.1. Animals

Male mice of ddY strain aged 6–8 weeks (Japan SLC, Hamamatsu, Shizuoka, Japan) were used in the study. Animals were at least kept for 4 days before starting the experiment. The temperature (24 ± 1%), humidity (55 ± 5%), and 12-h light/dark cycle (light period: 0730–1930 h) were set. Water and food were freely available. In addition, all efforts were made to minimize animal suffering, and to reduce the number of animals used according to the guiding principles for the care and use of laboratory animals approved by Faculty of Pharmacy, Meijo University and by the Japanese Pharmacological Society.

2.2. Drugs

Scopolamine hydrobromide (Sigma, St. Louis, USA), pirenzepine dihydrochloride, AF-DX116, 4-DAMP, and tropicamide (Tocris Cookson, Bristol, UK) were used. Drugs such as pirenzepine, AF-DX116, 4-DAMP, and tropicamide were dissolved in 0.9% isotonic saline solution (Otsuka Pharmaceutical, Tokyo, Japan) and injected 5 μl/mouse at a rate of 1 μl/10 s into the cerebral ventricle under ether (Wako, Osaka, Japan) anesthesia. The intracerebroventricular (i.c.v.) injection was made with a 4-mm-long needle (30 gauge) attached to a 50-μl Hamilton microsyringe according to the method of Haley and McCormick (1957). Scopolamine was subcutaneously administered 0.1 ml/10 g body weight. Scopolamine and pirenzepine were administered 30 min before putting mice into the holder, while AF-DX116, 4-DAMP, and tropicamide were administered 15 min before putting into the holder. The doses of cholinergic antagonists used in this study were lifted from previous reports (Ukai et al., 1995; Jones and Shannon, 2000b). In contrast, there is no context for the dose selection in the case of tropicamide because the reports on tropicamide result from in vitro assays (Rinken, 1995).

2.3. Apparatus

Startle chambers (SR-LAB; San Diego Instruments, San Diego, CA, USA) consisted of nonrestrictive Plexiglas cylinders, 3.8 cm in diameter and 13 cm long, resting on a Plexiglas platform in a ventilated and well-lit chamber connecting with a measurement cage containing a signal amplification sensor (Ukai and Okuda, 2003). A high-frequency speaker mounted 33 cm above the cylinder produced all acoustic stimuli. A piezoelectric accelerometer was mounted under each cylinder and detected transduced animal movements. A computer and interface assembly digitized and stored the data. A dynamic calibration system (SR-LAB; San Diego Instruments) was used to ensure comparable sensitivities across chambers. Sound levels were measured in decibels. The background white noise was 70 dB in the soundproof box of the loudspeaker from the upper part of the measurement cage.

2.4. Test session

Six different trial types were presented in the test session: 40-ms broadband 87-dB (prepulse-alone trial) and 118-dB (pulse-alone trial) bursts; three different prepulse (pp) + pulse (p) trials in which 40-ms-long 10-dB (pp10p118), 13-dB (pp13p118), or 17-dB (pp17p118) stimuli above a 70-dB background white noise preceded the 118-dB pulse by 100 ms (onset to onset); and a no-stimulus trial, in which only the background white noise was presented (Ukai and Okuda, 2003). Thus, the six trial types presented were: pulse alone; prepulse alone; pp10p118; pp13p118; pp17p118; and no stimulus. Each of the six different trial types was presented in a random order eight times. An intertrial interval was 30 s. The test session consisted of 48 trials. The session began with a 5-min acclimation period followed by test session. The vibration was examined 100 ms after auditory stimulation (pulse) as startle amplitude. The prepulse inhibition was obtained as follows: prepulse inhibition={[pulse-alone response − (prepulse + pulse response)]/(pulse-alone response)} × 100. In addition, the effects of drugs on startle amplitude were obtained after stimulation of pulse alone.

2.5. Statistical analysis

All of the results were expressed as mean ± S.E.M., and analyzed by Kruskal–Wallis analysis of variance by ranks. If there were significant H values, *post-hoc* comparisons were made using nonparametric Bonferroni/Dunn's multiple comparison test (two-tailed).

3. Results

3.1. Effects of scopolamine

Scopolamine (0.3 mg/kg, s.c.) significantly decreased prepulse inhibition [$H = 12.633$, $P < 0.05$], but the drug

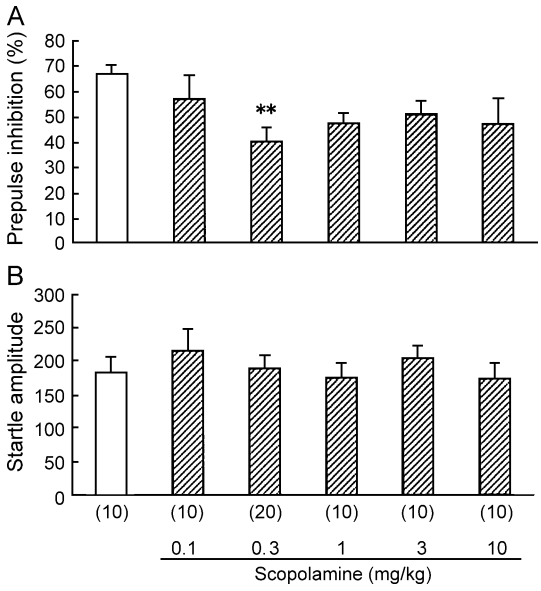

Fig. 1. Effects of scopolamine on prepulse inhibition (A) and startle amplitude (B) in mice. Each value represents the mean ± S.E. Scopolamine (s.c.) was given to mice 30 min before measurements. The number of mice used is shown in parentheses. $^{**}P<0.01$ vs. control.

(0.1–10 mg/kg, s.c.) failed to affect startle amplitude [$H=2.091$, $P>0.05$] (Fig. 1). Although all groups except the case of 0.3 mg/kg scopolamine consisted of 10 mice, a 0.3-mg/kg dose of scopolamine was administered to 20, but not 10, mice in an attempt to obtain conclusive results.

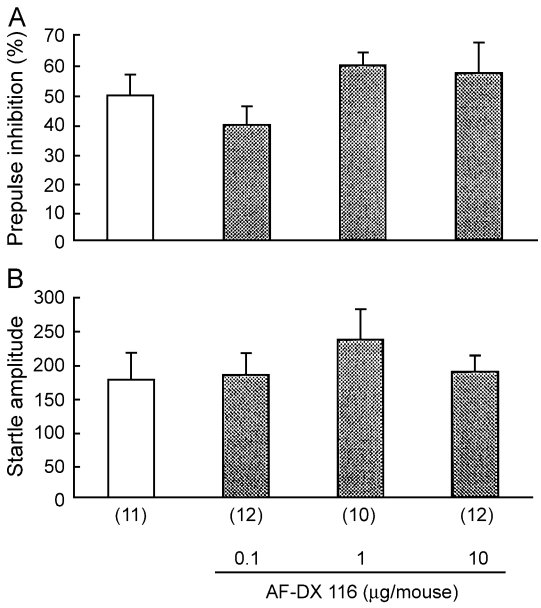

Fig. 3. Effects of AF-DX116 on prepulse inhibition (A) and startle amplitude (B) in mice. Each value represents the mean ± S.E. AF-DX116 (i.c.v.) was given to mice 15 min before measurements. The number of mice used is shown in parentheses.

3.2. Effects of selective antagonists for cholinergic receptor subtypes

Pirenzepine (0.1–10 μg/mouse, i.c.v.) failed to affect prepulse inhibition [$H=1.238$, $P>0.05$] or startle amplitude [$H=0.053$, $P>0.05$] (Fig. 2). AF-DX116 (0.1–10 μg/mouse, i.c.v.) hardly influenced prepulse inhibition [$H=6.225$,

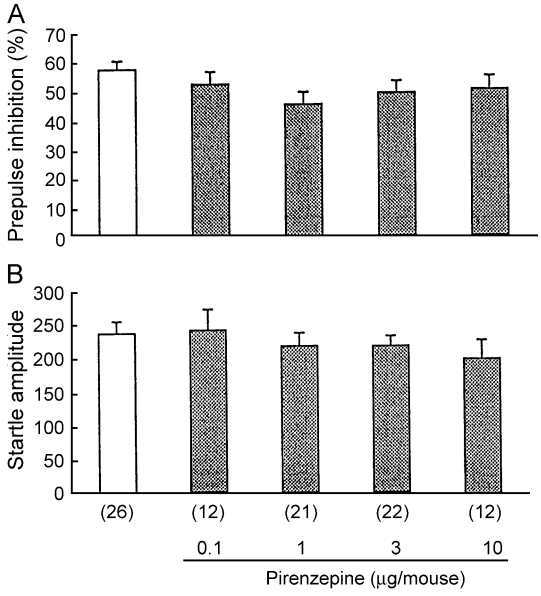

Fig. 2. Effects of pirenzepine on prepulse inhibition (A) and startle amplitude (B) in mice. Each value represents the mean ± S.E. Pirenzepine (i.c.v.) was given to mice 30 min before measurements. The number of mice used is shown in parentheses.

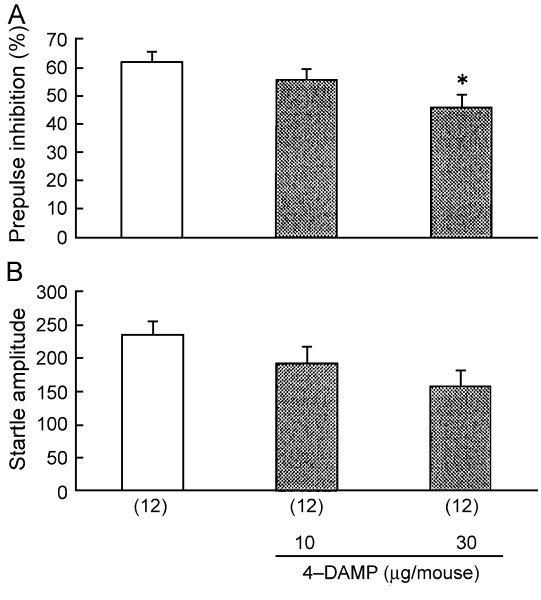

Fig. 4. Effects of 4-DAMP on prepulse inhibition (A) and startle amplitude (B) in mice. Each value represents the mean ± S.E. 4-DAMP (i.c.v.) was given to mice 15 min before measurements. The number of mice used is shown in parentheses. $^{*}P<0.05$ vs. control.

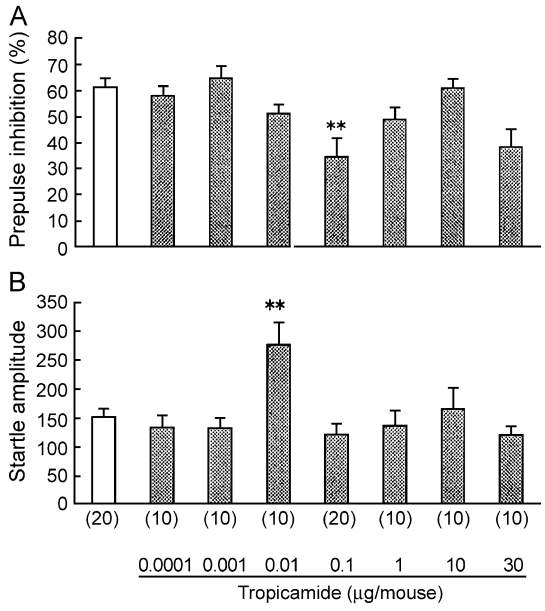

Fig. 5. Effects of tropicamide on prepulse inhibition (A) and startle amplitude (B) in mice. Each value represents the mean ± S.E. Tropicamide (i.c.v.) was given to mice 15 min before measurements. The number of mice used is shown in parentheses. $^{**}P<0.01$ vs. control.

$P>0.05$] or startle amplitude [$H=1.376$, $P>0.05$] (Fig. 3). 4-DAMP (30 μg/mouse, i.c.v.) significantly decreased prepulse inhibition [$H=6.343$, $P<0.05$] but not startle amplitude [$H=5.416$, $P>0.05$] (Fig. 4). Tropicamide (0.1 μg/mouse, i.c.v.) significantly decreased prepulse inhibition [$H=22.032$, $P<0.01$], while it (0.01 μg/mouse, i.c.v.) significantly increased startle amplitude [$H=14.304$, $P<0.01$] (Fig. 5).

4. Discussion

Prepulse inhibition is one of the models of sensorimotor information processing (Swerdlow et al., 1992). Although prepulse inhibition is relevant to various nervous systems in the brain (Furuya et al., 1999; Klamer et al., 2001), there are multiple lines of evidence suggesting the possible involvement of muscarinic cholinergic systems in the mechanisms of prepulse inhibition of acoustic startle response (Fendt and Koch, 1999; Jones and Shannon, 2000a,b; Stanhope et al., 2001). In this study, the result that the anticholinergic agent scopolamine (0.3 mg/kg, s.c.) impaired prepulse inhibition is in accord with previous reports in rats (Jones and Shannon, 2000a, b). Although the effects of scopolamine on prepulse inhibition were not dose-dependent, the finding is consistent with a previous report (Fendt and Koch, 1999). This nonlinear dose–response curve for scopolamine demands cautious interpretation of our data. We have no exact explanation for the observation that scopolamine at higher doses does not influence prepulse inhibition. Although scopolamine has been shown to have a slightly higher affinity for the M_3 receptor subtype with a twofold to threefold lower affinity for other muscarinic receptor subtypes (Bolden et al., 1992), higher doses of scopolamine might affect different types of muscarinic receptors, resulting in nonsignificant effects of scopolamine.

Since the muscarinic M_1 receptor subtype has previously been postulated to be important in cognition (Ukai et al., 1995, 1997), it might be expected that muscarinic M_1 receptor-preferring antagonists would be most effective in disrupting prepulse inhibition. However, the muscarinic M_1 receptor-preferring antagonist pirenzepine (0.1–10 μg/mouse) and the muscarinic M_2 receptor-preferring antagonist AF-DX116 (0.1–10 μg/mouse) did not influence prepulse inhibition or startle amplitude, suggesting that muscarinic M_1 and M_2 receptors are not closely involved in prepulse inhibition. The doses of pirenzepine used were identical with those used in a previous study (Ukai et al., 1995, 1997). The higher doses of pirenzepine and AF-DX116 were not examined because it is likely that the specific effects reduce. In particular, the doses of anticholinergic agents used in the present study were almost equivalent to, or far beyond, those used in previous studies (Taira, 1998; Ukai et al., 1995, 1997).

In contrast, the muscarinic M_3 receptor-preferring antagonist 4-DAMP (30 μg/mouse) and the muscarinic M_4 receptor-preferring antagonist tropicamide (0.1 μg/mouse) significantly impaired prepulse inhibition. The stimulation of muscarinic M_3 receptors has been reported to inhibit the release of glutamate in the striatum (Niittykoski et al., 1999), implying the possibility that the blockade of muscarinic M_3 receptors with 4-DAMP facilitates the release of glutamate, leading to the enhancement of glutamatergic neurotransmission in the brain. However, there seems to be a discrepancy because the nonselective NMDA receptor antagonist dizocilpine inhibits prepulse inhibition, suggesting that NMDA does not play a major role in the effects of 4-DAMP. It is, moreover, likely that 4-DAMP affects muscarinic M_1 besides M_3 receptors (Michel et al., 1989). Since the present result demonstrates that pirenzepine failed to affect prepulse inhibition, it is unlikely that muscarinic M_1 receptors contribute to the effects of 4-DAMP.

Tropicamide is considered to be a muscarinic M_4 receptor-specific ligand with relatively high affinity for M_1 (Rinken, 1995). Since the results show that pirenzepine (0.1–10 μg), a muscarinic M_1 receptor-preferring antagonist, had no significant effects on prepulse inhibition, the effects of tropicamide are associated with muscarinic M_4 receptor. It is very likely that the nonlinear dose effects reflect not only different types of receptors, but different locations within the regulatory circuitry of prepulse inhibition. For example, muscarinic receptors in the hippocampus, striatum, septum, and pedunculopontine nucleus probably all impact prepulse inhibition in different ways. When the drugs are given systemically, all sites are affected.

The startle amplitude is an indicator of startle response, which is elicited by sudden intense acoustic stimulation and

is composed of a twitch of facial, neck, and limb muscles (Fendt and Koch, 1999). Although scopolamine, pirenzepine, AF-DX116, and 4-DAMP were without any effects on startle amplitude, tropicamide exclusively at a dose of 0.01 μg/mouse increased startle amplitude. Scopolamine has been reported to produce nonsignificant effects on startle amplitude (Jones and Shannon, 2000b), whereas, to date, there are no reports regarding the effects of tropicamide on it. The lack of dose-dependent effects of tropicamide on startle amplitude remains to be determined.

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References

Baratti, C.M., Opezzo, J.W., Kopf, S.R., 1993. Facilitation of memory storage by the acetylcholine M2 muscarinic receptor antagonist AF-DX 116. Behav. Neural Biol. 60, 69–74.

Billard, W., Binch III, H., Crosby, G., McQuade, R.D., 1995. Identification of the primary muscarinic autoreceptor subtype in rat striatum as M2 through a correlation of in vivo microdialysis and in vitro receptor binding data. J. Pharmacol. Exp. Ther. 273, 273–279.

Bolden, C., Cusack, B., Richelson, E., 1992. Antagonism by antimuscarinic and neuroleptic compounds at the five cloned human muscarinic cholinergic receptors expressed in Chinese hamster ovary cells. J. Pharmacol. Exp. Ther. 260, 576–580.

Braff, D.L., Grillion, C., Geyer, M.A., 1992. Gating and habituation of the startle reflex in schizophrenic patients. Arch. Gen. Psychiatry 49, 206–215.

Caine, S.B., Geyer, M.A., Swerdlow, N.R., 1992. Hippocampal modulation of acoustic startle and prepulse inhibition in the rat. Pharmacol. Biochem. Behav. 43, 1201–1208.

Eglen, R.M., Hegde, S.S., Watson, N., 1996. Muscarinic receptor subtypes and smooth muscle function. Pharmacol. Rev. 48, 531–565.

Fendt, M., Koch, M., 1999. Cholinergic modulation of the acoustic startle response in the caudal pontine reticular nucleus of the rat. Eur. J. Pharmacol. 370, 101–107.

Furuya, Y., Kagaya, T., Ogura, H., Nishikawa, Y., 1999. Competitive NMDA receptor antagonists disrupt prepulse inhibition without reduction of startle amplitude in a dopamine receptor-independent manner in mice. Eur. J. Pharmacol. 364, 133–140.

Geyer, M.A., Krebs-Thomson, K., Braff, D.L., Swerdlow, N.R., 2001. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berlin) 156, 117–154.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects of produced by intracerebral injection of drugs in the conscious mouse. Br. J. Pharmacol. 12, 12–15.

Jones, C.K., Shannon, H.E., 2000a. Effects of scopolamine in comparison with apomorphine and phencyclidine on prepulse inhibition in rats. Eur. J. Pharmacol. 391, 105–112.

Jones, C.K., Shannon, H.E., 2000b. Muscarinic cholinergic modulation of prepulse inhibition of the acoustic startle reflex. J. Pharmacol. Exp. Ther. 294, 1017–1023.

Klamer, D., Engel, J.A., Svenssow, L., 2001. The nitric oxide synthase inhibitor, L-NAME, block phencyclidine-induced disruption of prepulse inhibition in mice. Psychopharmacology (Berlin) 156, 182–186.

Lazareno, S., Buckley, N.J., Roberts, F.F., 1990. Characterization of muscarinic M4 binding sites in rabbit lung, chicken heart, and NG108-15 cells. Mol. Pharmacol. 38, 805–815.

Mansbach, R.S., Geyer, M.A., 1989. Effects of phencyclidine and phencyclidine biology on sensorimotor gating in the rat. Neuropsychopharmacology 2, 299–308.

Mansbach, R.S., Geyer, M.A., Braff, D.L., 1988. Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology (Berlin) 94, 507–514.

Michel, A.D., Stefanich, E., Whiting, R.L., 1989. Direct labeling of rat M3-muscarinic receptors by [^{3}H]4-DAMP. Eur. J. Pharmacol. 166, 459–466.

Niittykoski, M., Ruotsalainen, S., Haapalinna, A., Larson, J., Sirvio, J., 1999. Activation of muscarinic M3-like receptors and β-adrenoceptors, but not M2-like muscarinic receptors or α-adrenoceptors, directly modulates corticostriatal neurotransmission in vitro. Neuroscience 90, 95–105.

Rinken, A., 1995. Subtype-specific changes in ligand binding properties after solubilization of muscarinic receptors from baculovirus-infected Sf9 insect cell membranes. J. Pharmacol. Exp. Ther. 272, 8–14.

Stanhope, K.J., Mirza, N.R., Bickerdike, M.J., Bright, J.L., Harrington, N.R., Hesselink, M.B., Kennett, G.A., Lightowler, S., Sherdown, M.J., Syed, R., Upton, R.L., Wadsworth, G., Weiss, S.M., Wyatt, A., 2001. The muscarinic receptor agonist xanomeline has an antipsychotic-like profile in the rat. J. Pharmacol. Exp. Ther. 299, 782–792.

Swerdlow, N.R., Geyer, M.A., 1993. Clozapine and haloperidol in an animal model of sensorimotor gating deficits in schizophrenia. Pharmacol. Biochem. Behav. 44, 741–744.

Swerdlow, N.R., Caine, S.B., Braff, D.L., Geyer, M.A., 1992. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. J. Psychopharmacol. 6, 176–190.

Taira, C.A., 1998. Muscarinic receptor subtype involvement in brain cholinergic stimulation by intracerebroventricular neostigmine in sinoaortic denervated rats. Gen. Pharmacol. 31, 583–588.

Ukai, M., Okuda, A., 2003. Endomorphin-1, an endogenous μ-opioid receptor agonist, improves apomorphine-induced impairment of prepulse inhibition in mice. Peptides 24, 741–744.

Ukai, M., Shinkai, N., Kameyama, T., 1995. κ-Opioid receptor agonists improve pirenzepine-induced disturbance of spontaneous alternation performance in the mouse. Eur. J. Pharmacol. 281, 173–178.

Ukai, M., Itoh, J., Kobayashi, T., Shinkai, N., Kameyama, T., 1997. Effects of the κ-opioid dynorphin A(1–13) on learning and memory in mice. Behav. Brain Res. 83, 169–172.

Varty, G.B., Walters, N., Cohen-Williams, M., Carey, G.J., 2001. Comparison of apomorphine, amphetamine and dizocilpine disruptions of prepulse inhibition in inbred and outbred mice strains. Eur. J. Pharmacol. 424, 27–36.