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Stimulation of the $\alpha 4\beta 2$ nicotinic receptor by 5-I A-85380 improves auditory gating in DBA/2 mice

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Abstract

Abnormal auditory gating is a symptom of schizophrenia which has been proposed to be mediated through the $\alpha 7$ nicotinic acetylcholine receptor (nAChR). It has been shown that the non-selective nicotinic agonist nicotine has an influence on auditory gating in part by acting on the $\alpha 4\beta 2$ nAChR. The goal of this study was to determine the effect of 5-I A-85380, an agonist for the $\alpha 4\beta 2$ nAChR, in an inbred mouse model with a deficiency for auditory gating. Anesthetized DBA/2 mice were administered 5-I A-85380 alone and in combination with the $\alpha 4\beta 2$ nAChR antagonist, dihydro- β -erythroidine, or the $\alpha 7$ nAChR antagonist, α -bungarotoxin. A recording electrode in the CA3 region of the hippocampus recorded P20-N40 waveforms in response to two auditory stimuli. The amplitudes of the response to the first and second clicks were used to determine TC ratios, the measure of auditory gating. 5-I A-85380 significantly decreased the TC ratios by selectively increasing the response amplitudes to the first click with no significant influence on the response amplitudes to the second click. The effect was blocked by dihydro- β -erythroidine whereas α -bungarotoxin had no effect on response amplitude to either click. Although the $\alpha 7$ nAChR may mediate the hippocampal response of DBA/2 mice to the second click, the $\alpha 4\beta 2$ nAChR appears to modulate the response to the first click. Thus, the present study implicates the involvement of more than one subtype of nAChR in the auditory gating of DBA/2 mice, specifically the $\alpha 4\beta 2$ nAChR, and its role in the response amplitude to the first stimulus.

Keywords

nicotinic acetylcholine receptor; schizophrenia; hippocampus; 5-I A-85380; auditory gating; DBA/2

1. Introduction

The inability to correctly process sensory information is a cardinal symptom of schizophrenia. In particular, the inability to filter incoming auditory stimuli results in schizophrenia patients becoming overwhelmed or “flooded” by extraneous stimuli (Venables, 1964). The phenomenon of filtering repetitive auditory stimuli in order to properly focus attention is known as auditory gating. Auditory gating is deficient in schizophrenia patients (Freedman et al.,

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2003; Leonard et al., 2002). It has been proposed that the inability of these patients to properly gate auditory stimuli is due to a dysfunction of inhibitory circuits involving the hippocampus (Adler et al., 1998). One method for measuring the response to auditory stimuli in humans is by electroencephalographic recordings of the P50 auditory evoked potential. This potential is a measure of the brain's electrical response in the form of evoked waves to two identical auditory stimuli separated by 500 milliseconds (ms). In control subjects the amplitude of the P50 waveform response to the second stimulus (test stimulus) is decreased compared to the response to the first stimulus (conditioning stimulus). In schizophrenia patients however, there is no decrease in the P50 waveform amplitude to the second auditory stimulus indicating a failure to activate an inhibitory circuit which results in sensory stimuli overload (Venables, 1992).

The P20-N40 waveform complex in rodents is analogous to the P50 auditory evoked potential recordings in humans (Bickford-Wimer et al., 1990; Bickford and Wear, 1995; Simpson and Knight, 1993; Stevens et al., 1998). This waveform complex is defined as a positive inflection occurring 20–40 ms following stimulus onset and continuing through to the following negativity. This complex has been shown to have less variability than either individual component (Hashimoto et al., 2005). Data from both human and rodent studies have implicated nicotinic acetylcholine receptors (nAChRs) in the auditory gating deficit experienced by schizophrenia patients. Specifically, the $\alpha 7$ nAChR subtype has a decreased density in the CA3 region of the hippocampus and the dentate gyrus of post-mortem brain tissue in schizophrenia patients as compared to controls (Breese et al., 1997; Freedman et al., 1995). Genetic linkage studies indicate that the P50 auditory evoked potential deficit of schizophrenia patients corresponds to chromosome 15q14 which is the locus of the $\alpha 7$ nAChR gene (Chini et al., 1994; Freedman et al., 1997; Orr-Urtreger et al., 1995).

Pharmacologically, nicotine, a non-selective nAChR agonist, has been shown to improve deficient auditory gating in schizophrenia patients (Adler et al., 1993). The improvement in this deficit is proposed to be mediated through the $\alpha 7$ nAChR subtype. Rodent studies have demonstrated that nicotinic agonists, which correct the auditory gating deficit, act by selectively decreasing the amplitude of the test response and/or increasing the amplitude of the conditioning response (Radek et al., 2006; Stevens et al., 1998; Stevens and Wear, 1997). An alteration in the size of response amplitudes is reflected in the TC ratio. The TC ratio is the amplitude of the evoked response to the test stimulus (TAMP) divided by the amplitude of the evoked response to the conditioning stimulus (CAMP). A low TC ratio (≤ 0.4) indicates normal gating while a greater TC ratio (> 0.4) reflects an auditory gating deficit (Stevens et al., 1998). The decrease in amplitude of the test response indicates an inhibition of firing of a subpopulation of pyramidal cells in the hippocampus in response to the test stimulus (Adler et al., 1998). A strain of inbred mice, the DBA/2, models the auditory gating deficit experienced by schizophrenia patients. Similar to the reduction in hippocampal $\alpha 7$ nAChRs observed in human post-mortem brain tissue from schizophrenia patients, the DBA/2 strain of mice has reduced numbers of $\alpha 7$ receptors in the hippocampus, as well as a lack of inhibition of the P20-N40 response to the test stimulus (Adams et al., 2001; Stevens et al., 1996). Further support for the role of the $\alpha 7$ receptor in the gating deficit in DBA/2 mice is the improvement observed after administration of DMXB-A, also known as GTS-21, which is a selective $\alpha 7$ partial agonist (de Fiebre et al., 1995; Kem, 1997; Simosky et al., 2001; Stevens et al., 1999). Recently, a Phase I clinical study was conducted to assess the efficacy of DMXB-A in non-smoking schizophrenia patients on cognitive functioning and P50 sensory inhibition. The results showed improvements in both P50 sensory inhibition and neurocognitive measures following administration of this compound (Olinic et al., 2006).

Correction of the gating deficit in schizophrenia patients after administration of nicotine may be due in part to its activation of high-affinity neuronal nicotinic receptors. These high-affinity

nAChRs consist of $\beta 2$ subunits in conjunction with $\alpha 4$ subunits with or without other α -subunits (Flores et al., 1992; Gerzanich et al., 1998; Lindstrom et al., 1987). Assessment of the role of $\alpha 4\beta 2$ receptors in the gating deficit has not been as well explored as that of the $\alpha 7$ receptor. It was first noted by Stevens and Wear (1997) that ABT-418, an $\alpha 4\beta 2$ agonist, produced an increase in the conditioning amplitude of P20-N40 auditory evoked potentials in DBA/2 mice. However, ABT-418 is not selective for the $\alpha 4\beta 2$ receptor (Briggs et al., 1995; Papke et al., 1997) and was found to also decrease test amplitude (TAMP) which was blocked by administration of the $\alpha 7$ antagonist α -bungarotoxin (α -BTX) (Stevens and Wear, 1997). Radioligand binding studies of hippocampal homogenates from schizophrenic smokers suggested a decrease in the binding of both ^3H -nicotine and ^3H -cytisine, a radioligand selective for $\alpha 4\beta 2$, as compared to that of non-schizophrenic smokers, indicating a decrease in the high-affinity receptor levels in schizophrenic hippocampus (Breese et al., 2000; Freedman et al., 1995). In postmortem hippocampus and thalamus from non-schizophrenic smokers there is an increase in ^3H -nicotine binding indicating an up-regulation of high-affinity nicotinic receptors (Breese et al., 1997), while schizophrenic smokers fail to show a similar up-regulation of these receptors with nicotine exposure. This suggests an abnormal response of these receptors to cigarette smoking in the schizophrenic population (Adams and Stevens, 2007; Breese et al., 2000). Data regarding the genes encoding the $\alpha 4$ and $\beta 2$ subunits indicate that molecular variants in only one of the genes does not alone confer susceptibility to schizophrenia, but an interaction between variants in the $\alpha 4$ gene along with the $\beta 2$ gene do produce a significant risk for schizophrenia (De Luca et al., 2006).

Recently, a study by Radek et al. (2006) specifically addressed the involvement of $\alpha 4\beta 2$ nAChRs in the auditory gating deficit in unanesthetized DBA/2 mice. It was determined that nicotine improved auditory gating in DBA/2 mice, in part, by significantly increasing the conditioning amplitude. Co-administration of the $\alpha 4\beta 2$ antagonist dihydro- β -erythroidine (DH β E) blocked the increase in CAMP observed with nicotine. Nicotine is a non-selective agonist (Papke et al., 2007) but the administration of DH β E and the resultant blockade of the increase in CAMP suggests a role for the high-affinity nicotine receptor in the auditory gating deficit of DBA/2 mice (Papke et al., 2007; Radek et al., 2006). Therefore, to further our understanding of $\alpha 4\beta 2$ nAChR participation in the auditory gating deficit we studied the response of anesthetized DBA/2 mice to a relatively selective $\alpha 4\beta 2$ agonist, 5-I A-85380 (Koren et al., 1998; Mukhin et al., 2000). The effects of 5-I A-85380 upon auditory gating were assessed with and without prior central administration of either DH β E or α -BTX.

2. Results

In concert with previous studies, anesthetized DBA/2 mice showed a deficit in auditory gating as evidenced by high TC ratios (test amplitude/conditioning amplitude ≥ 0.4) during baseline recordings, prior to drug administration (Fig. 1 and Fig. 5). The $\alpha 4\beta 2$ agonist 5-I A-85380 produced significant decreases in TC ratios following intraperitoneal (IP) administration of 0.1, 0.3 and 1 mg/kg doses as compared to averaged baseline recordings (Fig. 1 and Fig. 5). Repeated measures analysis (MANOVA) revealed a significant decrease in TC ratio over time at the highest three doses [$F_{(13, 143)} = 4.74$, $P < 0.001$; $F_{(13, 130)} = 6.33$, $P < 0.001$; $F_{(12, 108)} = 4.36$, $P < 0.001$; for 0.1, 0.3 and 1 mg/kg respectively] as compared to baseline. A Fisher's protected least-significant difference (PLSD) a posteriori analysis showed a significant decrease in TC ratio at the 5 through 35 minute time points after 5-I A-85380 injection for the 0.3 and 1 mg/kg doses, while the 0.1 mg/kg dose produced a significant decrease at the 5 and 15 through 40 minute time points after 5-I A-85380 injection (Fig. 1). The lowest dose of 5-I A-85380 tested (0.03 mg/kg) did not produce a statistically significant change in TC ratio at any particular time point but did result in an overall significant difference as compared to baseline [$F_{(13, 143)} = 1.86$, $P = 0.040$].

Analysis of conditioning and test amplitudes showed there was no significant change in test amplitudes as compared to baseline after 5-I A-85380 injection (IP) at any of the four doses tested (Fig. 2). In contrast, the conditioning amplitude showed significant increases at the 0.1, 0.3, and 1 mg/kg doses [$F_{(13, 143)} = 4.70$, $P < 0.001$; $F_{(13, 130)} = 3.94$, $P < 0.001$; $F_{(12, 108)}$, $P = 0.004$, respectively], but not at the 0.03 mg/kg dose [$F_{(13, 143)} = 1.19$, $P = 0.29$]. The time course of significant increases in CAMP, as determined with a Fisher's PLSD a posteriori analysis, varied with the dose. At the 0.1 mg/kg dose there was a significant increase which began 15 minutes following 5-I A-85380 injection and lasted for the remainder of the recording time (40 minutes). At the 0.3 mg/kg dose, significance was observed 5 minutes post-injection and lasted for 25 minutes with a final significant difference at the 35 minute time point. Although the 1 mg/kg dose of 5-I A-85380 resulted in an increase in CAMP as compared to baseline, significance was reached only at the 5 and 25 minute time points (Fig. 2).

To verify that 5-I A-85380 was increasing CAMP through the $\alpha 4\beta 2$ receptor, DH β E (1 μ l of 30 nM) was administered via intracerebroventricular (ICV) injection and followed 20 minutes later by an injection of 5-I A-85380 (1mg/kg, IP) (Fig. 3). Because this was a competition study, the 1mg/kg dose of 5-I A-85380 was chosen as it produced a significant decrease in TC ratios (Fig. 1). Twenty minutes of records were obtained following ICV injection to confirm that DH β E alone did not produce effects on either CAMP or TAMP (Fig. 3). Repeated measures analysis showed a significant blockade in 5-I A-85380-induced reduction of TC ratios with DH β E administration [$F_{(16, 160)} = 0.83$, $P = 0.65$] by blockade of the increase in CAMP observed with this dose of 5-I A-85380 alone [$F_{(16, 160)} = 1.50$, $P = 0.11$] (Fig. 3).

To determine if the $\alpha 7$ nAChR played any role in the observed effect of 5-I A-85380, the $\alpha 7$ nicotinic receptor antagonist, α -BTX (1 μ l of 1.25 nM), was administered ICV 20 minutes prior to injection of 5-I A-85380 (1 mg/kg, IP) (Fig. 4). Repeated measures analysis showed that α -BTX failed to prevent significant increases in CAMP following 5-I A-85380 injection [$F_{(16, 112)} = 2.43$, $P = 0.0040$]. A Fisher's LSD a posteriori analysis showed significant increases in CAMP at the 35, 45 and 60 minute time points following 5-I A-85380 injection as compared to baseline (Fig. 4). The administration of α -BTX failed to prevent the 5-I A-85380 decrease in TC ratio [$F_{(16, 112)} = 6.45$, $P < 0.0010$]. A Fisher's PLSD a posteriori analysis indicated significant decreases in TC ratio 10 minutes following 5-I A-85380 injection and persisting through the remainder of the recording (60 minutes). α -BTX alone, prior to 5-I A-85380 administration, produced no significant effects on CAMP, TAMP or TC ratios.

3. Discussion

The goal of the present study was to test an agonist with relative selectivity for the $\alpha 4\beta 2$ receptor to confirm the findings of Radek et al. (2006) on the role of the $\alpha 4\beta 2$ nAChRs in auditory gating. 5-I A-85380 is a halogenated analog of A-85380. This compound has been shown to have a very high affinity for nAChRs in rat brain ($K_i = 11$ pM for the $\alpha 4\beta 2$ receptor) with binding that is saturable and reversible (Koren et al., 1998; Mukhin et al., 2000). The 125 I- or 123 I-radiolabeled forms of this compound have been shown to readily cross the blood-brain barrier in vivo in mice, rhesus monkey and baboon with high specificity and low toxicity (Chefer et al., 1998; Musachio et al., 1998; Musachio et al., 1999; Vaupel et al., 1998). 125 I-5-I A-85380 binding density in rat brain was comparable to the binding densities of 3 H-cytisine and 3 H-nicotine indicating that 5-I A-85380 is labeling a population of $\alpha 4\beta 2$ nAChRs (Mukhin et al., 2000). It was determined that the binding of the radiolabeled 5-I A-85380 in rat brain is not inhibited by mecamylamine (a noncompetitive nAChR antagonist), atropine (a muscarinic0 antagonist), naloxone (an antagonist at opiate receptors), or haloperidol (an antagonist at D₂-like dopamine receptors) (Mukhin et al., 2000). Competition studies in rat brain between unlabeled 5-I A-85380 and the radiolabeled $\alpha 7$ antagonist α -BTX resulted in a K_i value of 250 nM. This value of affinity for the $\alpha 7$ receptor is 25,000 times less than the measured affinity

for the $\alpha 4\beta 2$ nAChR (as determined by competition studies with 0.5 nM ^3H -epibatidine in rat brain) (Mukhin et al., 2000). Mukhin et al. (2000) also determined that ^{125}I -5-I A-85380 did not bind to any brain region in $\beta 2$ -knockout mice as measured by in vitro autoradiography, indicating its lack of affinity for the $\alpha 7$ nAChR. The efficacy for 5-I A-85380 determined via two-electrode voltage clamp in *Xenopus* oocytes expressing human $\alpha 4\beta 2$ resulted in EC_{50} values (fit by a two-component concentration response curve) of 10 nM and 18 μM when oocytes were injected in a 1:1 subunit cDNA α : β ratio and an EC_{50} of 7.6 nM when injected in a 1:5 subunit cDNA α : β ratio (Zwart et al., 2006). Overall, these previously published reports indicate a high-selectivity of the nAChR agonist, 5-I A-85380, for a population of neuronal $\alpha 4\beta 2$ receptors. Thus, our present study utilized this compound to determine the effect of $\alpha 4\beta 2$ nAChRs on mice with a deficiency for auditory gating, to further elucidate the role of this receptor subtype in schizophrenia.

Our findings are in concert with those of Radek et al. (2006) in that activation of a population of $\alpha 4\beta 2$ nAChRs produces an increase in CAMP with a resultant decrease in TC ratios in DBA/2 auditory gating deficient mice. A decrease in TC ratio is indicative of an improvement in auditory gating (Stevens et al., 1998). Although our TC ratios did not reach a value indicative of normal auditory gating (≤ 0.4), after 5-I A-85380 administration, there were significant decreases at three of the doses tested as compared to baseline, indicating an improvement (Stevens et al., 1998). It had been previously determined that stimulation of $\alpha 7$ nAChRs produces a decrease in TAMP in DBA/2 mice auditory evoked potentials with a resultant lowering of TC ratios (de Fiebre et al., 1995; Kem, 1997; Simosky et al., 2001; Stevens et al., 1999). Radek et al. (2006) found that the increase in CAMP produced by the nonselective agonist nicotine could be prevented by DH β E (11.0 $\mu\text{mol/kg}$, IP) pretreatment 5 minutes prior to the nicotine administration (6.2 $\mu\text{mol/kg}$, IP). However, the decrease in TC ratio was still significant with DH β E pretreatment indicating that nicotine was acting on $\alpha 4\beta 2$ and $\alpha 7$ receptors since both the significant increase in CAMP and decrease in TC ratio was prevented by pre-administration with a high dose of mecamylamine (5.0 $\mu\text{mol/kg}$). Previous studies in our lab have confirmed that vehicle control injections to DBA/2 mice had no impact upon TC ratios (Hashimoto et al., 2005).

In the present study, a significant decrease in TC ratios was not found after administration of DH β E (1 μl of 30 nM, ICV) alone, suggesting that CAMP is not under tonic control by $\alpha 4\beta 2$ nAChRs. In contrast to the study by Radek et al. (2006) our administration of DH β E was directly into the cerebral ventricle. This route of administration (ICV) was chosen because DH β E may also interact with other $\beta 2$ -containing nAChRs in the peripheral nervous system (Harvey and Luetje, 1996; Williams and Robinson, 1984). The concentration of DH β E utilized blocks $\alpha 4\beta 2$ nAChR activity in the auditory gating paradigm (Simosky et al., 2003). DH β E prevented the 5-I A-85380 induced increase in CAMP and decrease in TC ratios, demonstrating that CAMP can be increased by stimulation of $\alpha 4\beta 2$ nAChRs with a resulting improvement in auditory gating and this improvement can be blocked via a $\beta 2$ nAChR antagonist.

Because the agonist, 5-I A-85380, was injected systemically, it may interact with $\alpha 4\beta 2$ nAChRs throughout the brain. Labeling with ^3H -nicotine, thought to bind primarily to $\alpha 4\beta 2$ nAChRs, has been observed within the molecular layer of the dentate gyrus (Clarke et al., 1985; Pauly et al., 1989). The mossy fiber axons of dentate granule cells synapse onto both pyramidal neurons and interneurons in the CA3 region of the hippocampus (Henze et al., 2000). Therefore, it is possible that 5-I A-85380 activates $\alpha 4\beta 2$ nAChRs within the dentate gyrus, thereby altering neuronal responses within the CA3 region. Binding of ^3H -nicotine has also been seen in the VTA which projects diffusely to the dentate gyrus (Clarke et al., 1985; Pauly et al., 1989). Because the dentate gyrus receives diffusely distributed dopaminergic projections from cells in the VTA (Swanson et al., 1987), it is also possible that activation of $\alpha 4\beta 2$ nAChRs on dopaminergic neurons in the VTA results in activation of neurons within the dentate gyrus.

The $\alpha 7$ antagonist α -BTX (1 μ l of 1.25 nM, ICV) was pre-administered 20 minutes prior to IP injection of 5-I A-85380 (1mg/kg). The injection of α -BTX alone had no effect on auditory gating and pre-administration failed to prevent the 5-I A-85380 induced increase in CAMP or decrease in TC ratio. Overall, the results from both the DH β E and α -BTX experiments indicate a lack of $\alpha 7$ nAChR involvement in 5-I A-85380 activation of high-affinity nicotine receptors.

A decrease in TC ratios may occur through either a decrease in TAMP, an increase in CAMP, or by an influence on both. The present study demonstrated that the decrease in TC ratios with 5-I A-85380 was produced entirely by an increase in CAMP mediated through activation of a population of $\alpha 4\beta 2$ nAChRs. The mechanism producing the increase in CAMP is unknown. Like 5-I A-85380, two nicotinic agonists, neither selective for $\alpha 4\beta 2$, have been found to produce a significant increase in CAMP. In fimbria-fornix lesioned rats ABT-418 produced a significant increase in CAMP as well as a significant decrease in TAMP (Stevens and Wear, 1997). Also, acute nicotine injections to DBA/2 mice resulted in a significant increase in CAMP (Radek et al., 2006). The 5-I A-85380 compound utilized in this study is much more selective for $\alpha 4\beta 2$ -type nAChRs than either ABT-418 or nicotine (Arneric et al., 1994; Mukhin et al., 2000). The blockade of the increase in CAMP by the $\beta 2$ nAChR antagonist DH β E strongly indicates that the increase in CAMP is through an $\alpha 4\beta 2$ dependent mechanism. The dopaminergic system may also play a role in regulation of the CAMP. In a paper from Stevens et al. (1991) SCH 23390, a D1-type receptor antagonist, produced a significant increase in CAMP of amphetamine-treated rats with an auditory gating deficit.

It does not appear that activation of the $\alpha 4\beta 2$ receptor results in an overall increase in hippocampal excitability. An overall increase in excitability should be reflected in an increase in both CAMP and TAMP. The fact that the TAMP does not change suggests that inhibition must also have increased since TC ratios decreased after 5-I A-85380 administration. Without an increase in inhibition there would be an increase in both wave amplitudes with no change in TC ratios, as is seen with haloperidol administration (Simosky et al., 2003). The lack of effect of α -BTX on TAMP in DBA/2 mice is probably related to their already reduced numbers of hippocampal $\alpha 7$ receptors (Stevens et al., 1996). Blockade of these receptors does not further increase TAMP, though previous studies have shown that stimulation reduces TAMP and corrects auditory gating (Stevens et al., 1998; Stevens and Wear, 1997).

Until recently, improved auditory gating was conceived of as a decrease in TAMP inhibition mediated indirectly via the $\alpha 7$ nAChR which resulted in a decreased TC ratio. The current study, along with data from Radek et al. (2006), indicate that improved auditory gating may also occur through an increase in CAMP mediated via a pathway involving the $\alpha 4\beta 2$ nAChR. Although the $\alpha 7$ receptor may mediate the hippocampal response to the test stimulus, the $\alpha 4\beta 2$ receptor appears to play a modulatory role in hippocampal response to the conditioning stimulus. These findings implicate the involvement of more than one subtype of nAChR in the auditory gating of DBA/2 mice.

4. Experimental Procedure

Animals

Male DBA/2 mice (20–25 g) were obtained from Harlan SD (Indianapolis, IN) and housed five to a cage in the Center for Comparative Medicine at the University of Colorado Denver, School of Medicine (UCD-SOM). The mice were provided water and food (Harlan Teklad, Indianapolis, IN) ad libitum. Lighting was cycled at 12 hour intervals (lights on at 0600 hours). Rodent procedures were performed in accordance with the Principles of Laboratory Animal Care (Institute of Laboratory Animal Research, 1996) with approval from the Institutional Animal Care and Use Committee of UCD-SOM.

Surgery

As previously described (Stevens et al., 1996), mice were anesthetized with chloral hydrate (400 mg/kg) IP administration followed by IP administration of pyrazole (400 mg/kg) to retard the metabolism of the chloral hydrate. During recording, the anesthetic and pyrazole were supplemented as necessary (5 mg/kg, IP) to maintain a surgical plane of anesthesia as evidenced by lack of reflexive limb withdrawal in response to toe pinch. Once anesthetized the mouse was placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA) and hollow earbars, attached to miniature earphones connected to a sound amplifier, were placed adjacent to the externalization of the aural canal. A stable core temperature was maintained at 35°C by a heating pad.

The scalp was incised and a burr hole was drilled over the dorsal CA3 region of the hippocampus (−1.8 mm posterior from bregma, +2.7 mm lateral from midline (Paxinos, 2001)). A Teflon-coated stainless-steel cut wire recording electrode (0.127 mm diameter) was inserted 1.5 to 1.7 mm ventral from the dorsal brain surface into the CA3 pyramidal cell layer of the hippocampus. Final placement was determined by the presence of complex action potentials typical of hippocampal pyramidal neurons (Miller et al., 1992). A second burr hole was drilled anterior to bregma and contralateral to the recording electrode for placement of the reference electrode on top of the dura. Electrical responses were amplified 1000× with analog to digital conversion (SciWorks DataWave, Berthoud, CO) for averaging by computer.

Experimental Protocols

Auditory stimuli in the form of tones (3000 Hz, 10 ms, 70 dB) generated as a sine wave were presented in pairs with a 500 ms interval between the paired tones and a 10 s interval between pairs of stimuli. Responses to 16 pairs of tones were averaged at 5-minute intervals and digitally filtered with a bandpass between 10 and 5000 Hz. The maximum negativity between 20 and 60 ms after the stimulus was selected as the N40 wave and measured relative to the proceeding positivity, the P20 wave. The ratio of amplitudes of the response to the second tone, known as the test amplitude (TAMP), to the response of the first tone, known as the conditioning amplitude (CAMP), provides a measure of the animal's auditory inhibition. A decreased TC ratio indicates improved inhibition of auditory processing. Five baseline records were obtained prior to test drug administration. For agonist studies 5-I A-85380 was dissolved in 0.9 % NaCl and administered IP at four doses (0.03 mg/kg, n = 12; 0.1 mg/kg, n = 12; 0.3 mg/kg, n = 11; 1 mg/kg; n = 10). After injection, recordings continued for 40 minutes, at five minute intervals.

For antagonist experiments involving DH β E or α -BTX, a third burr hole was drilled over the anterior lateral ventricle (+0.8 mm anterior from bregma, −0.5 mm lateral from midline (Paxinos, 2001)) ipsilateral to the recording electrode. A 26-gauge needle attached to a 10 μ l Hamilton syringe (Hamilton, Reno, NV) was inserted into the ventricle 2.0 mm below the dura for intracerebroventricular (ICV) administration of antagonist. A 1 μ l volume of either 1.25 nM α -BTX or 30 nM DH β E was administered following baseline recordings (Simosky et al., 2003). After injection of antagonist, recordings proceeded for 20 minutes at five minute intervals, after which the optimal dose of 5-I A-85380 (1 mg/kg) was administered IP and an additional 40 minutes of records obtained. Eleven mice per antagonist were studied. Following each antagonist experiment the mouse was decapitated under anesthesia and the brains were removed to verify electrode and needle placement.

Compounds

5-I A-85380 dihydrochloride, α -BTX, and DH β E hydrobromide were obtained from Tocris (Ellisville, MO). Both 5-I A-85380 and DH β E dosing were based on salt weights. α -Bungarotoxin dosing was according to the free base weight. All compounds were dissolved in 9% NaCl.

Statistical Analysis

The time course of 5-I A-85380 alone or in conjunction with antagonist were analyzed, for each dose, using repeated measures MANOVA. Where appropriate Fisher's protected least-significant difference (PLSD) a posteriori analysis was used to compare individual post injection time points to collapsed average baseline values.

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Fig. 1.

Effect of 5-I A-85380 on TC ratio. The first five time points (b) refer to baseline recordings obtained prior to drug injection. 5-I A-85380 injection (IP) occurred at the dashed line. Records were obtained at 5 minute intervals following injection. *P<0.05, **P<0.01 compared to average baseline as determined by Fisher's PLSD. Data are mean $\pm$ SEM; n=12 for 0.03 and 0.1 mg/kg; n=11 for 0.3 mg/kg; n=10 for 1mg/kg.

Fig. 2. Effect of 5-I A-85380 on the amplitudes of the conditioning (●) and test (○) responses. The first five time points (b) refer to baseline recordings obtained prior to drug injection. Records were obtained at 5 minute intervals following injection. * $P < 0.05$, ** $P < 0.01$ compared to an average baseline as determined by Fisher's PLSD. Data are mean $\pm$ SEM; $n = 12$ for 0.03 and 0.1 mg/kg; $n = 11$ for 0.3 mg/kg; $n = 10$ for 1 mg/kg.

Fig. 3. Inhibition of 5-I A-85380 (1 mg/kg, IP) induced improvement of auditory gating by DHβE (1 μl of 30 nM, ICV). The first five time points (b) refer to baseline recordings obtained prior to drug injection. Injection of the $\alpha 4\beta 2$ antagonist DHβE (dashed line) occurred 20 minutes prior to injection of 5-I A-85380 (dotted line). (●) Represents CAMP values and (○) represents the TAMP values at each time point. Data are mean $\pm$ SEM; n=11.

Fig. 4.

Effects of α -BTX (1 μ l of 1.25 nM, ICV) on 5-I A-85380 (1 mg/kg, IP) induced improvement in auditory gating. The first five time points (b) refer to baseline recordings obtained prior to drug injection. Injection of the $\alpha 7$ antagonist α -BTX (dashed line) occurred 20 minutes prior to injection of 5-I A-85380 (dotted line). (●) Represents CAMP values and (○) represents the TAMP values at each time point. Data are mean $\pm$ SEM; n=8. * P <0.05; ** P <0.01 compared to an average baseline as determined by Fisher's PLSD compared to pre-drug baseline.

Fig. 5.

Representative P20-N40 waveforms averaged from individual DBA/2 mice. Waveforms obtained prior to drug administration (Baseline), after 5-I A-85380 injection (1 mg/kg, IP), after DHβE injection alone or prior to 5-I A-85380 administration and after α-BTX injection alone or prior to 5-I A-85380 administration. 5-I A-85380 alone and in the presence of α-BTX resulted in decreased TC ratios indicating improvement in auditory gating. Tick marks denote the P20-N40 wave and stimulus onset is denoted by arrows. Calibration 20 μV/10 ms.