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The enhancement of contextual fear conditioning by ABT-418

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Abstract

Activation of nicotinic acetylcholine receptors (nAChRs) is known to modulate various forms of learning and memory, including contextual fear conditioning. Although numerous studies have shown that high-affinity $\beta 2$ -containing nAChRs are necessary for the nicotine-induced enhancement of contextual fear conditioning, it is unknown whether other high-affinity nAChR agonists are capable of enhancing this learning. To examine this issue, ABT-418, a high-affinity nAChR agonist with greater selectivity for high-affinity receptors than nicotine, was administered before acquisition and/or recall of contextual fear memories. ABT-418 enhanced acquisition of contextual fear memories in a dose-dependent manner.

Keywords

ABT-418; acetylcholine; learning; memory; mouse; nicotinic acetylcholine receptors; nicotine

Introduction

Nicotinic acetylcholine receptors (nAChRs) have long been known to modulate synaptic plasticity, learning, and memory (Kenney and Gould, 2008a; Placzek *et al.*, 2009). A variety of nAChR subtypes present throughout the central nervous system may contribute to the effects of nicotine on learning and memory. Central nAChRs are pentameric ligand-gated ion channels that are composed of either α ($\alpha 2$ – $\alpha 10$) subunits or a combination of α and β ($\beta 2$ – $\beta 4$) subunits (Hogg *et al.*, 2003; Millar and Gotti, 2009). Various subunit combinations confer different functional properties such as affinity for nicotine, rate of desensitization, and calcium permeability (McGehee and Role, 1995; Albuquerque *et al.*, 1997). The differences in functional properties of nAChR subtypes may contribute to the varying effects of nicotine on learning and memory.

High-affinity $\beta 2$ -subunit-containing nAChRs have been shown to be critical for the nicotine-induced enhancement of contextual fear conditioning (Wehner *et al.*, 2004; Davis and Gould, 2007) where contextual learning refers to the conjunctive representation of a particular space. Although these studies suggest that high-affinity $\beta 2$ -subunit-containing nAChRs are necessary for the nicotine-induced enhancement of contextual fear learning, it is unknown whether preferential activation of this class of nAChRs is sufficient for such enhancement. To explore this possibility, this study examined the effect of (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole (ABT-418), a novel high-affinity nAChR agonist with greater selectivity towards high-affinity nAChRs than nicotine (Arneric *et al.*, 1994; Holladay *et al.*, 1997), on contextual and cued fear conditioning.

Methods

Subjects

Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine, USA) aged 8–12 weeks were group housed. A 12-h light/dark cycle was maintained with experiments conducted during the light cycle. All experimental procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Apparatus

Mice were trained and tested for contextual conditioning in four identical clear Plexiglas chambers and tested for cued fear conditioning in four different chambers housed in a separate room. These chambers have been described in detail elsewhere (Kenney and Gould, 2008b).

Drugs

ABT-418 hydrochloride (Sigma, St Louis, Missouri, USA) was dissolved in physiological saline and was administered intraperitoneally at a dose of 0.026, 0.26, or 2.6 mg/kg 15 min before training and/or testing; doses based on preliminary experiments.

Fear conditioning

Training and testing for fear conditioning and measurement of freezing was performed as described earlier (Gould *et al.*, 2004). Briefly, the mice used in studies presented in Fig. 1 were trained using two conditioned stimulus (CS)–unconditioned stimulus (US) (30 s, 85 dB white noise) (2 s, 0.57mA footshock) pairings separated by a 120-s intertrial interval. Twenty-four hours later, mice were tested for contextual conditioning and an hour later tested for generalized freezing (Pre-CS) and cued fear conditioning in an altered context. One group of mice was retested for contextual freezing 7 days after training. In an additional experiment mice were trained with one CS–US pairing with a 15 s CS.

Shock sensitivity

Shock sensitivity testing (0.10–0.60 mA) with measurements of vocalizations and motor movements was performed as described earlier (Gulick and Gould, 2009).

Statistical analyses

Data were analyzed using one-way analyses of variance (ANOVAs), independent-samples *t*-tests or repeated-measures ANOVAs as appropriate and followed up with Tukey's honestly significant difference post-hoc tests. Any animal that was 2.5 standard deviations from the mean was considered an outlier and excluded from data analyses (2 mice).

Results

To determine an effective enhancing dose of ABT-418 on contextual fear conditioning, saline or one of three doses of ABT-418 (0.026, 0.26, or 2.6 mg/kg) was administered before training of contextual fear conditioning (Fig. 1a). A one-way ANOVA revealed a trend towards an effect of treatment on contextual fear conditioning [$F(3,58)=2.62$, $P=0.059$] and a significant effect on cued fear conditioning [$F(3,58)=3.24$, $P<0.05$]. Post-hoc tests revealed that mice administered 0.26 mg/kg ABT-418 froze significantly more than saline-treated mice in contextual fear conditioning ($P<0.05$) and mice administered 2.6 mg/kg ABT-418 froze significantly less than mice administered 0.26 mg/kg ABT-418 in cued fear conditioning ($P<0.05$). No other pair-wise comparisons were significant. To confirm the enhancement of contextual fear conditioning by ABT-418, the effect was replicated [context

freezing levels: saline=39.8%, 0.26 mg/kg ABT-418=53.9%; $t(30)=2.67$, $P<0.02$]. These data suggest that 0.26mg/kg ABT-418 administered before training is sufficient to enhance contextual fear conditioning. Next, ABT-418 was administered before either training and testing or testing only to determine whether ABT-418 affects the recall of contextual fear memories (Fig. 1b). Interestingly, unlike the administration of 0.26 mg/kg ABT-418 before training only, administration of the drug before training and testing or before testing only was without effect on contextual [$F(2,28)=1.08$, NS] or cued fear conditioning [$F(2,28)<1$].

To determine whether the enhancement of contextual fear conditioning by ABT-418 was long lasting, mice were administered 0.26mg/kg of ABT-418 before training in fear conditioning, and tested in contextual fear conditioning 1 and 7 days after training (Fig. 1c). Although ABT-418 enhanced contextual fear conditioning 1 day after training [$t(27)=2.14$, $P<0.05$], there was only a trend towards an effect at 7 days [$t(27)=1.75$, $P=0.091$]. Thus, the longer-term enhancement of contextual fear conditioning by ABT-418 does not seem to be particularly robust.

The lack of an effect of ABT-418 on cued fear conditioning may be because of ceiling levels of freezing during the testing of cued fear conditioning after training with two CS-US pairings. To decrease overall cued conditioning levels a modified fear conditioning procedure was used in which the CS was shortened to 15 s and only one CS-US presentation was used (Gould *et al.*, 2004). In this modified conditioning paradigm, ABT-418 had no effect on cued fear conditioning [Fig. 2a; $F(3,28) <1$].

The effect of ABT-418 on contextual fear conditioning may be because of altered shock sensitivity as increased sensitivity to a foot shock because of drug administration could explain the enhanced contextual fear conditioning. To test this possibility, a shock-sensitivity test was performed using three doses of ABT-418 (Fig. 2b and c). A 4×8 (drug $\times$ shock level) repeated-measures ANOVA found a significant main effect of shock level for both the motor score [$F(7,140)=112$, $P<0.001$] and vocalizations [$F(7,140)=67.7$, $P<0.001$] but no significant main effect of drug [$F(3,20) <1$] or shock by drug interaction [$F(21,140) <1$] on either measure, showing that ABT-418 did not affect shock sensitivity.

Discussion

The high-affinity nAChR agonist ABT-418 selectively enhances the acquisition of contextual fear conditioning but not cued fear conditioning. One of the primary differences between contextual and cued fear conditioning is that contextual fear conditioning depends on the hippocampus whereas cued fear conditioning does not; both are amygdala dependent (Kim and Fanselow, 1992; Rudy *et al.*, 2004). The expression of nAChR subunits in the hippocampus and amygdala differs, with the hippocampus having slightly higher expression levels for most subunits (Wada *et al.*, 1989; Dineley-Miller and Patrick, 1992). Together, this suggests that hippocampal nAChRs specifically contribute to information processing critical to the effects of cholinergic ligands on hippocampus-dependent cognition.

Different combinations of nAChR subunits confer varying functional properties to the resulting pentameric receptor. For example, $\alpha 7$ homomeric nAChRs have fast rates of desensitization, high calcium permeability, and low affinity for nicotine, whereas $\alpha 4\beta 2^*$ (* indicates other receptor subunits may be included) nAChRs have slower desensitization rates, lower calcium permeability but higher affinity for nicotine (McGehee and Role, 1995; Albuquerque *et al.*, 1997). ABT-418 has higher selectivity towards the activation of high-affinity $\alpha 4\beta 2^*$ nAChRs over lower-affinity $\alpha 7$ nAChRs than nicotine (Holladay *et al.*, 1997), and both nicotine and ABT-418 are most potent at $\alpha 2\beta 2$ and $\alpha 4\beta 2$ nAChRs with less potency at $\alpha 3\beta 2$ nAChRs (Luetje and Patrick, 1991; Covernton *et al.*, 1994; Papke *et al.*,

1997). This suggests that $\alpha 2$, $\alpha 4$, or $\beta 2$ -containing nAChRs contribute to the mnemonic effects of nicotinic receptor ligands on contextual learning.

ABT-418 has been found earlier to enhance other forms of learning, such as inhibitory avoidance in rodents (Decker *et al.*, 1994) and attention in both rodents (McGaughy *et al.*, 1999; Hahn *et al.*, 2003) and nonhuman primates (Prendergast *et al.*, 1997, 1998). In addition, ABT-418 has been found to be effective in decreasing cognitive deficits in patients with attention deficit hyperactivity disorder and Alzheimer's disease (Potter *et al.*, 1999; Wilens *et al.*, 1999). Thus, modulation of high-affinity nAChRs seems to be an effective strategy for modifying a variety of cognitive processes.

This study showed that ABT-418 is capable of enhancing contextual fear conditioning. As both nicotine and ABT-418 have high affinities for $\alpha 2$, $\alpha 4$, and $\beta 2$ -containing nAChRs these subunits may mediate the effects of these drugs on cognitive processes. These nAChR subunits deserve further attention with regard to their role in diseases that involve concurrent changes in both cholinergic and cognitive function.

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Fig. 1.

ABT-418 enhances the acquisition of contextual fear conditioning. (a) Administration of 0.26 mg/kg ABT-418 before training enhanced contextual fear conditioning; mice administered 2.6 mg/kg of ABT-418 were impaired in cued fear conditioning relative to mice that received 0.26 mg/kg ABT-418; $n=13-20$. (b) 0.26 mg/kg ABT-418 administered before training and testing or testing only had no effect on contextual or cued fear conditioning; $n=9-11$. (c) 0.26 mg/kg ABT-418 enhanced contextual fear conditioning 24 h after training but had only a marginal effect at a one week re-test; $n=14-15$. * $P<0.05$ compared with saline-treated mice; # $P<0.05$ compared with 0.26 mg/kg ABT-418 treated mice; † $P<0.10$ compared with saline-treated mice. Error bars represent SEM.

Fig. 2.

ABT-418 had no effect on cued fear conditioning or shock sensitivity. (a) No dose of ABT-418 administered before training had an effect on a cued fear conditioning task in which the CS was reduced to 15 s and only one CS–US pairing was presented; $n=8$. (b) No dose of ABT-418 had an effect on the motor score measure of shock sensitivity (defined as 0, no response; 1, hop; 2, jump; 3, run; 4, horizontal jump; 5, vertical jump); $n=6$. (c) No dose of ABT-418 had an effect on the mean number of vocalizations measure of shock sensitivity; $n=6$. Error bars represent SEM.