Published in final edited form as:

Eur J Pharmacol. 2013 October 15; 718(0): . doi:10.1016/j.ejphar.2013.08.037.

Effects of the sazetidine-a family of compounds on the body temperature in wildtype, nicotinic receptor $\beta 2^{-/-}$ and $\alpha 7^{-/-}$ mice

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Abstract

Nicotine elicits hypothermic responses in rodents. This effect appears to be related to nicotinic receptor desensitization because sazetidine-A, an $\alpha 2$ nicotinic receptor desensitizing agent, produces marked hypothermia and potentiates nicotine-induced hypothermia in mice. To determine the specificity of sazetidine-A induced hypothermia to $\alpha 2$ subunit-containing nicotinic receptors, we tested its efficacy in $\alpha 2$ knockout ($\alpha 2^{-/-}$) mice. These effects were compared with wildtype (WT) and $\alpha 7$ knockout ($\alpha 7^{-/-}$) mice. Confirming our earlier results, sazetidine-A elicited a pronounced and long-lasting hypothermia in WT mice. In comparison, sazetidine-A induced a much attenuated and shorter hypothermic response in $\alpha 2^{-/-}$ mice. This indicates that the greater proportion of sazetidine-A induced hypothermia is mediated via actions on $\alpha 2$ -containing nicotinic receptors, while a smaller component of hypothermia induced by sazetidine-A is mediated by non- $\alpha 2$ nicotinic receptors. Similar to WT mice, $\alpha 7^{-/-}$ mice showed the full extent of the sazetidine-A effect, suggesting that the hypothermia produced by sazetidine-A did not depend on actions on $\alpha 7$ nicotinic receptor subtype. Three other novel nicotinic receptor desensitizing agents derived from sazetidine-A, triazetidine-O, VMY-2-95 and YL-1-127 also produced hypothermia in WT and $\alpha 7^{-/-}$ mice. Furthermore, unlike sazetidine-A, triazetidine-O and YL-1-127 did not show any hint of a hypothermic effect in $\alpha 2^{-/-}$ mice. VMY-2-95 like sazetidine-A did show a residual hypothermic effect in the $\alpha 2^{-/-}$ mice. These studies show that the hypothermic effects of sazetidine-A and the related compound VMY-2-95 are mainly mediated by nicotinic receptors containing $\alpha 2$ subunit, but that a small component of the effect is apparently mediated by non- $\alpha 2$ containing receptors.

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The novel compounds in the publication are covered by issued patent and patent applications. The following authors are listed as inventors: for sazetidine-A, KJK and YX; for triazetidine-O, MLB, PM, YX and KJK; for VMY-2-95, MLB, MP, YX, KJK, VMY, EDL and AHR; and for YL-1-127, YX, KJK, MLB, MP, YL, EDL and AHR. Georgetown University is the holder for issued patents.

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Keywords

Nicotinic; Temperature Regulation; sazetidine-A; Desensitization; Knockout

1. Introduction

Neuronal nicotinic receptors have been implicated in the regulation of body temperature in a variety of species including pigeons (Komarova, 2009), rodents (Gordon et al., 2002; Marks et al., 1984; Overstreet et al., 1998; Ruskin et al., 2008; Sack et al., 2005; Salminen and Ahtee, 2000; Zarrindast et al., 1995) and primates (Hall and Myers, 1972). Peripheral administration of nicotine induces hypothermia (Zarrindast et al., 2001; Zarrindast et al., 1995) while administration of mecamylamine, a non-specific nicotinic receptor antagonist, blocks or decreases nicotine-induced hypothermia in mice (Nordberg and Sundwall, 1983; Zarrindast et al., 2001). Studies in inbred mouse strains have demonstrated that the high-affinity nicotine-binding sites probably mediate the hypothermic effects of nicotine (Marks et al., 1989). Moreover, although not confirmed, it appears that the hypothermic effect of nicotine may be related to its nicotinic receptor desensitizing effects inasmuch as the selective $\alpha 4 \beta 2$ nicotinic receptor desensitizing agent sazetidine-A also produces hypothermia and potentiates nicotine-induced hypothermia in rodents (Rezvani et al., 2012). To better understand the involvement of nicotinic receptor subtypes in regulation of body temperature, we compared the effects of four selective $\alpha 4 \beta 2$ desensitizing compounds with different pharmacologic properties on body temperature in wildtype, $\alpha 2^{-/-}$ and $\alpha 7$ KO mice.

The widely characterized property of nicotinic receptor desensitization involves the inability of the receptor channel to open when bound by either the endogenous ligand acetylcholine or an exogenous drug. This is an inherent property of nicotinic receptors, as well as several other ligand-gated channels. In the case of nicotinic receptors, the agonist activity of nicotine and other nicotinic agonists is relatively short-lived (seconds to minutes), whereas the desensitization effects last much longer (minutes to hours). The actions of a nicotinic agonist to both activate and desensitize nicotinic receptors have been known for more than 50 years (Katz and Thesleff, 1957). The important contributions of desensitization to effects of nicotinic ligands in vivo and in vitro have been widely characterized (Buccafusco et al., 2009; Hulihan-Giblin et al., 1990; Picciotto et al., 2008; Quick and Lester, 2002; Sharp and Beyer, 1986; Xiao et al., 2006). It has been shown that a single injection of nicotine desensitizes the response for several hours (Hulihan-Giblin et al., 1990; Sharp and Beyer, 1986). We reported previously that the selective $\alpha 4 \beta 2$ nicotinic receptor antagonist DH E did not reduce the hypothermia effect of sazetidine-A; instead, it significantly prolonged the induced hypothermia (Rezvani et al., 2012). It is clear that the desensitization plays an important role in hypothermia reduced by nicotinic agents.

Sazetidine-A has a very high binding affinity for rat $\alpha 4 \beta 2$ nicotinic receptors and, more importantly, it is >10,000-fold and 3,500-fold selective for $\alpha 4 \beta 2$ receptors compared to its affinity at rat $\alpha 3 \beta 4$ and $\alpha 7$ nicotinic receptors, respectively (Xiao et al., 2006; Xiao et al., 2008). Consistent with its high selectivity for $\alpha 4 \beta 2$ receptors in binding assays, sazetidine-A is a much weaker desensitizer of $\alpha 3 \beta 4$ or $\alpha 7$ nicotinic receptor subtypes (Liu et al., 2010; Xiao et al., 2006; Xiao et al., 2010). Thus, sazetidine-A is a highly selective and potent $\alpha 4 \beta 2$ nicotinic receptor desensitizer (Xiao et al., 2006).

Recently, three analogues of sazetidine-A have been synthesized. These analogues are triazetidine-O (Xiao et al., 2009), VMY-2-95 (Yenugonda et al., 2013) and YL-1-127 (Liu et al., 2013). Similar to sazetidine-A, in binding assays these three compounds have more than 10,000 fold selectivity for rat $\alpha 4 \beta 2$ receptors over rat $\alpha 3 \beta 4$ or $\alpha 7$ receptors. In

functional studies in cell models, these three sazetidine-A analogs potently and selectively desensitize $\alpha 4 \beta 2$ receptors. Although similar to sazetidine-A, these compounds have important medicinal and pharmacological properties that are different from those of sazetidine-A.

To determine the roles of specific nicotinic receptor subtypes in the hypothermia induced by sazetidine-A and its novel analogs, we studied effects of these compounds on body temperature in wildtype (WT) mice, $\alpha 2$ knockout ($\alpha 2^{-/-}$) mice and $\alpha 7$ knockout ($\alpha 7^{-/-}$) mice. Based on the current literature regarding the role of neuronal nicotinic receptors on regulation of body temperature and our previous findings (Rezvani et al., 2012), it was hypothesized that the desensitization of nicotinic receptors containing $\alpha 2$ subunit would significantly reduce body temperature and that the induced hypothermia would be absent in $\alpha 2^{-/-}$ mice.

2. Methods

2.1. Animals

Adult male WT, $\alpha 2^{-/-}$ and $\alpha 7^{-/-}$ mice weighing 25 ± 2 (S.E.M.) g were used in these studies. The nicotinic $\alpha 7$ and $\alpha 2$ receptor knockout mouse breeding pairs and wildtype controls (C57/BL6) were generously provided by Dr. John Dani and colleagues at Baylor University. Animals were bred in our laboratory. They were housed in groups of three for one week before surgery and were single housed after the surgery. Wood chip bedding was used throughout. Animals had free access to food and water in an ambient temperature of $22 \pm 1^\circ\text{C}$ and 12L:12D photoperiod (lights on at 1800 h). Injections were given s.c. in a volume of 10 ml/kg during the dark phase when animals were active.

2.2. Surgery and Telemetry System

To measure core body temperature in mice, an automated telemetry system was used. Animals were anesthetized with a dose of 60 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, IA, USA) mixed with 15 mg/kg domitor (Pfizer Animal Health, New York, NY, USA) injected IP. The fur over the ventral abdominal cavity was shaved and the area was cleaned with beta iodine first and a solution of 70% (v/v) alcohol. After the alcohol dried, a 2-cm longitudinal incision was made along the midline of the abdomen about 1 cm below the sternum, and a transmitter weighing about 3.5 g (Model TA-F20) was inserted into the abdominal cavity. The incision was sutured and the outer skin was closed with wound staples. After two weeks of recovery, each cage was positioned on a receiver board, which decoded the temperature of the transmitter within the body cavity. The body temperature was collected automatically every 5 min and stored in a computer using the Data Quest IV software (Data Sciences, Inc., St. Paul, MN, USA). The data for analysis were collected for three hours after drug administration.

2.3. Experimental Protocol

Dose effect functions of four novel $\alpha 4 \beta 2$ nicotinic desensitizing compounds with slightly different properties were tested on body temperature of WT, $\alpha 2^{-/-}$ and $\alpha 7^{-/-}$ mice using a repeated measures counterbalanced design for each study. In experiment one, mice were injected subcutaneously with 0.3, 1 or 3 mg/kg of sazetidine-A or an equal volume of saline (N=7 WT, N=7 $\alpha 2^{-/-}$ and N=8 $\alpha 7^{-/-}$). In another set of mice, a dose-response function for triazetidine-O was established. Mice were injected s.c. with a dose of 1.5, 3, 6 or 18 mg/kg triazetidine-O or saline (N=11 WT, N=11 $\alpha 2^{-/-}$ and N=8 $\alpha 7^{-/-}$). Three weeks after the last triazetidine-O administration, a dose-response function was established for VMY-2-95 in the same mice. Mice were injected s.c. with a dose of 0.3, 1, or 3 mg/kg VMY-2-95 or vehicle (N=11 WT, N=11 $\alpha 2^{-/-}$ and N=8 $\alpha 7$ KO). In another group of mice the acute effects of

YL-1-127 on body temperature were assessed. Mice were injected with a dose of 0.3, 1, 3, 6, 12 and 18 mg/kg of YL-1-127 or the vehicle (N=11 WT, N=9 $2^{-/-}$ and N=7 $7^{-/-}$). All animals in each group received all treatments and the intervals between injections were at least 3 days.

2.4. Drug Preparation

Sazetidine-A HCl was synthesized at RTI, (Research Triangle, NC) and supplied by the National Institute on Drug Abuse; triazetidine-O, VMY-2-95 and YL-1-127 were synthesized as the free base by methods reported previously (Brown et al., 2009; Liu et al., 2013; Xiao et al., 2006; Yenugonda et al., 2013). The compounds were >98.5% pure. Sazetidine-A and triazetidine-O were dissolved in saline solution, while VMY-2-95 and YL-1-127 were dissolved in a solution of 100 mM HCl. All of the drugs were diluted in saline to needed concentrations. The pH of the drug solutions were adjusted to 6.5–7.0. All drugs doses are referred to as the salt. Drug solutions were prepared weekly and kept refrigerated between experiments. The drug was injected (s.c.) in volume of 10 ml/kg body weight.

2.5. Statistical Analysis

The temperature data were assessed with analysis of variance for repeated measures. The between subjects factor was genotype and the within subjects factors were drug treatment dose and five-minute block over 3-hours. The studies were run in repeated cohorts, each containing all of the genotypes and cohort was included as a control factor. The threshold for significance for the main effects and interactions was $p < 0.05$. Comparisons of the treated groups to control were done using the Bonferroni correction. With three comparisons of each dose vs. control we have tightened to $p < 0.01$ as the threshold for significance for the comparisons between treatment conditions.

3. Results

3.1. Sazetidine-A

With sazetidine-A there was a significant main effects of sazetidine-A treatment ($F(3,57)=36.37$, $P<0.0005$), genotype ($F(2,19)=6.21$, $P<0.01$) and time ($F(35, 665)=12.79$, $P<0.0005$). There were significant interactions of sazetidine-A x genotype ($F(6,57)=7.72$, $P<0.0005$), sazetidine-A x time ($F(105, 1995)=5.41$, $P<0.0005$) and sazetidine-A x genotype x time ($F(210, 1995)=6.94$, $P<0.0005$). In the wildtype mice, the sazetidine-A main effect was significant ($F(3,18)=37.38$, $P<0.0005$), with an mean decrease from 37.0 ± 0.03 averaged over the three hours after injection of saline to 28.9 ± 0.2 after injection of 3 mg/kg of sazetidine-A. In the $2^{-/-}$ mice the hypothermic effect of sazetidine-A was much diminished, but the sazetidine-A main effect was still significant ($F(3,18)=13.18$, $P<0.0005$) with the $2^{-/-}$ mice showing a decline from 36.6 ± 0.1 after saline injections to 35.2 ± 0.1 after 3 mg/kg of sazetidine-A averaged over the three hours after administration. The $7^{-/-}$ mice showed a relatively undiminished hypothermic effect of sazetidine-A with a significant main sazetidine-A effect ($F(3,21)=7.82$, $P<0.0005$) with temperature declining from 36.7 ± 0.04 after saline to 31.9 ± 0.2 after 3 mg/kg of sazetidine-A averaged over the three hours after administration. Within the three hours after administration, the maximum drop in temperature with the high dose of sazetidine-A (3 mg/kg) in wildtype mice was approximately 10 °C, while this value for the $2^{-/-}$ mice and the $7^{-/-}$ mice was approximately 4 and 7 °C, respectively (Fig. 1). In the $2^{-/-}$ mice the late phase of the sazetidine-A induced hypothermia was eliminated, but the early phase was left intact.

3.2. Triazetidine-O

Similar to sazetidine-A, triazetidine-O also caused significant hypothermia ($F(5, 120)=14.57$, $P<0.0005$). There was a significant main effect of genotype ($F(2,24)=9.05$, $P<0.0005$) and a significant genotype x triazetidine-O interaction ($F(10,120)=5.26$, $P<0.0005$). In the WT mice there was a significant main effect of triazetidine-O ($F(5,45)=12.02$, $P<0.0005$), but the extent of hypothermia was much less than seen with sazetidine-A (-10°C with 3 mg/kg sazetidine-A vs. -4°C with 18 mg/kg triazetidine-O). Furthermore, in contrast to sazetidine-A the $2^{-/-}$ mice showed no indication of hypothermia with triazetidine-O. The $7^{-/-}$ mice showed a significant ($F(5,30)=3.73$, $P<0.01$) hypothermic effect of triazetidine-O (Fig. 2), indicating that as with sazetidine-A, the 7 receptor subtype is not essential for the hypothermic response.

3.3. VMY-2-95

With VMY-2-95 there was a significant main effect of VMY-2-95 treatment ($F(3,66)=18.46$, $P<0.0005$) and genotype ($F(2,22)=3.85$, $P<0.05$) and time ($F(35, 770)=1.88$, $P<0.005$). Significant interactions were seen for time x genotype ($F(70,770)=1.57$, $P<0.005$), VMY-2-95 x time ($F(105,2310)=4.52$, $P<0.0005$) and VMY-2-95 x genotype x time ($F(210,2310)=1.41$, $P<0.0005$). Follow-up tests of the simple main effects were made for the highest order significant interaction VMY-2-95 x genotype x time. Figure 3 shows the effects of VMY-2-95 on body temperature over the course of 3 hours after injection in WT, $2^{-/-}$ and $7^{-/-}$ mice. The WT mice showed a significant effect of VMY-2-95 ($F(3,27)=5.19$, $P<0.01$) with the 3 mg/kg VMY-2-95 dose causing an overall significant hypothermia ($F(1,27)=12.55$, $P<0.005$). With the WT mice there was a significant interaction of VMY-2-95 x time ($F(105,945)=1.28$, $P<0.05$). Tests of the dose-effect for the simple main effects at each time point showed for the wildtype mice that the 3 mg/kg dose caused significant ($F(1,945)$, $P<0.0005$) hypothermia for each time point starting 10 minutes post injection and continuing throughout the rest of the 3-hour period after injection. The 1 mg/kg doses caused a more modest but still significant ($F(1,945)$, $P<0.01-0.0005$) hypothermia from 25 minutes through the 3-hour period after injection. The 0.3 mg/kg dose had no discernable effect on body temperature.

With the $2^{-/-}$ mice there was also a significant main effect of VMY 2-95 ($F(3,24)=4.53$, $P<0.05$), but the magnitude of hypothermia was much less than with the wildtype mice. There was a significant VMY 2-95 x time interaction ($F(105,840)=2.07$, $P<0.0005$). The 3 mg/kg dose caused significant hypothermia starting 10 minutes post-injection and lasting throughout the rest of the 3-hour period after injection ($F(1,840)$, $P<0.01-0.0005$). The lower doses caused modest but significant episodes of hyperthermia with the 1 mg/kg doses causing significant hyperthermia 140–150 minutes post-injection and the lower 0.3 mg/kg dose causing significant hyperthermia 75–80 minutes post-injection ($F(1,840)$, $P<0.01-0.005$).

With the $7^{-/-}$ mice, there was a significant main effect of VMY-2-95 ($F(3,15)=35.07$, $P<0.0005$) with the 3 mg/kg VMY-2-95 dose causing significant hypothermia ($F(1,15)=77.98$, $P<0.0005$). There was a significant VMY 2-95 x time interaction ($F(105,525)=8.85$, $P<0.0005$). In the $7^{-/-}$ mice the 3 mg/kg VMY-2-95 dose caused a significant degree of hypothermia starting 10 minutes post injection and lasting throughout the rest of the 3-hour period after injection ($F(1,525)$, $P<0.0005$). The lower doses caused lesser and shorter extents of hypothermia, The 1 mg/kg dose caused modest but significant hypothermia 10–50 minutes post injection ($F(1,525)$, $P<0.005-0.0005$) and at the end of the 3-hour post-injection period ($F(1,525)$, $P<0.0.05$).

3.4. YL-1-127

The next experiment tested the hypothermic effects of YL-1-127. The lower dose range of YL-1-127 (0.3, 1 and 3 mg/kg) did not produce any significant hypothermia (Fig. 4). Even the higher dose range of YL-1-127 (6, 12 and 18 mg/kg) did not produce significant hypothermia (Fig. 5), although there was a trend toward a hypothermic effect in the WT and the $\alpha 7^{-/-}$ mice (Fig. 5).

4. Discussion

The results of this study confirm our earlier finding (Rezvani et al., 2012) that sazetidine-A causes a robust and long lasting hypothermia in wildtype mice. The threshold for this effect is between 0.3 and 1 mg/kg. This dramatic hypothermic effect was certainly truncated in the $\alpha 2^{-/-}$ mice, however there was a smaller and shorter hypothermic effect of sazetidine-A in these mice during the early phase (0 – 90 min) despite the absence of $\alpha 4 \beta 2$ receptors. Thus, sazetidine-A must have produced this effect through a non- $\alpha 4 \beta 2$ nicotine receptor, either some other subtypes of the nicotinic receptor or a non-nicotinic receptor. The identity of this receptor is not currently known. Compared with sazetidine-A, triazetidine-O was considerably less potent in producing hypothermia in WT and $\alpha 7^{-/-}$ mice. It only produced significant hypothermia at a high dose of 18 mg/kg. This effect was not seen in the $\alpha 2^{-/-}$ mice even at the high dose of 18 mg/kg. VMY-2-95 cause much less hypothermia than sazetidine-A. There was some hypothermic effect also seen with the $\alpha 2^{-/-}$ and $\alpha 7^{-/-}$ mice but the temperature decrease was much less in extent than was seen with sazetidine-A. In contrast to the other drugs, YL-1-127 produced no significant hypothermia up to an acute high dose of 18 mg/kg. There was a non-significant trend toward hypothermia with the highest dose tested in the WT and $\alpha 7^{-/-}$ mice, which was not apparent in the $\alpha 2^{-/-}$ mice.

The differential hypothermic effects of these four $\alpha 4 \beta 2$ desensitizing drugs provide important information concerning possible clinical use when laid upon the information concerning the potency and efficacy of these drugs for reducing nicotine self-administration. Sazetidine-A significantly reduces nicotine self-administration at a dose of 3 mg/kg, but not lower (Levin et al., 2010). At this dose sazetidine-A causes dramatic hypothermia, which may be a useful effect if hypothermia is somehow related to the mechanism mediating reduction of nicotine self-administration, but if not it could be an undesired side effect. In rats a much smaller magnitude hypothermic effect is seen with the 3 mg/kg dose but it is still statistically significant (Rezvani et al., 2012). Importantly, the hypothermia induced by sazetidine-A is reduced in $\alpha 2^{-/-}$ mice but it is not eliminated, indicating that sazetidine-A might have actions on other receptors involved in regulation of body temperature.

The hypothermia induced by triazetidine-O and VMY-2-95 is much less robust than with sazetidine-A. Triazetidine-O in our other studies was not found to significantly reduce nicotine self-administration in rats in doses up to 18 mg/kg (unpublished data), so the lack of a hypothermic side effect at this dose range is not relevant to possible development for smoking cessation. In contrast, VMY-2-95 has been shown in our other studies to significantly reduce nicotine self-administration at 3 mg/kg to a similar extent as sazetidine-A (Slade et al., 2012). Sazetidine-A induced hypothermia might be considered an unsought side effect in its use for smoking cessation. The finding that VMY-2-95 causes much less hypothermia than sazetidine-A at this dose, which effectively reduces nicotine self-administration points to the possible superiority of this drug for development as a smoking cessation treatment. The finding that the modest hypothermic effect of VMY-2-95 was greatly minimized (though not eliminated) in $\alpha 2^{-/-}$ mice demonstrates the greater specificity of this compound compared with sazetidine-A. Finally, YL-1-127 had the least effect at producing hypothermia. Although no significant hypothermic effects were seen at doses up to 18 mg/kg, there was a modest trend toward hypothermia seen at 18 mg/kg in WT and

$7^{-/-}$ mice. No hint of an effect was seen in the $2^{-/-}$ mice. YL-1-127 was previously shown in our studies (Liu et al., 2010; Xiao et al., 2006; Xiao et al., 2010) to have a potency to desensitize $\alpha 4 \beta 2$ nicotinic receptors is 24-fold lower than that of sazetidine-A. As shown in Figure 4, YL-1-127 did not elicit significant hypothermia, at dose up to 3 mg/kg). However, as shown in Figure 5, YL-1-127 produced a trend of hypothermia when the doses were increased. The potency of producing hypothermia correlates well with the potency of desensitizing the receptor.

In summary, sazetidine-A, which significantly reduces nicotine self-administration, also produces robust hypothermic effects. Furthermore, part of the hypothermic effect of sazetidine-A appears to derive from actions at non- $\alpha 4 \beta 2$ nicotinic receptors (either other nicotinic receptors or non-nicotinic receptors). It is interesting to note that the hypothermic effect of nicotine was reported to be mediated both by nicotinic receptors containing $\alpha 4$ subunits and those without $\alpha 4$ subunits (Tapper et al., 2007). The sazetidine-A analog VMY-2-95, which also seems to have a very good profile for reducing nicotine self-administration, produces only modest hypothermia. YL-1-127 is the least effective compound in causing hypothermia but its efficacy in reducing nicotine self-administration has not been fully explored.

In conclusion, given the hypothermic effects induced by sazetidine-A in $2^{-/-}$ mice, it is apparent that sazetidine-A probably has some non- $\alpha 4 \beta 2$ nicotinic receptor actions. However, the long duration of sazetidine-A's hypothermic effects seems to require $\alpha 2$ -containing nicotinic receptors since the persistent hypothermia induced by sazetidine-A was markedly truncated in $2^{-/-}$ mice.

Acknowledgments

This project was supported by NIH/NIDA grants U19DA027990 and R03DA025947.

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Sazetidine-A Effects on Body Temperature in Wildtype, Beta2 KO and Alpha7 KO Mice

Figure 1.

The hypothermic effect of sazetidine-A in wildtype mice and mice with knockouts of $\alpha 7$ and $\beta 2$ -containing nicotinic receptors (mean $\pm$ S.E.M.), Wildtype N=7, $\beta 2^{-/-}$ N=7, $\alpha 7^{-/-}$ N=8.

Triazetidine-O Effects on Body Temperature in Wildtype, Beta2 KO and Alpha7 KO Mice

Figure 2.

The hypothermic effect of triazetidine-O in wildtype mice and mice with knockouts of $\alpha 7$ and $\alpha 2$ -containing nicotinic receptors (mean $\pm$ S.E.M.), Wildtype N=11, $\alpha 2^{-/-}$ N=11, $\alpha 7^{-/-}$ N=8.

Figure 3.

The hypothermic effect of VMY-2-95 in wildtype mice and mice with knockouts of $\alpha 7$ and $\alpha 2$ -containing nicotinic receptors (mean $\pm$ S.E.M.), Wildtype N=11, $\alpha 2^{-/-}$ N=11, $\alpha 7^{-/-}$ N=8.

YL-1-127 Effects on Body Temperature in Wildtype, Beta2 KO and Alpha7 KO Mice

Figure 4.

Body temperature and the low dose range of YL-1-127 in wildtype mice and mice with knockouts of $\alpha 7$ and $\beta 2$ -containing nicotinic receptors (mean $\pm$ S.E.M.), Wildtype N=11, $\beta 2^{-/-}$ N=9, $\alpha 7^{-/-}$ N=7.

YL-1-127 Effects on Body Temperature in Wildtype, Beta2 KO and Alpha7 KO Mice

Figure 5. Body temperature and the high dose range of YL-1-127 in wildtype mice and mice with knockouts of $\alpha 7$ and $\beta 2$ -containing nicotinic receptors (mean $\pm$ S.E.M.), Wildtype N=11, $\beta 2^{-/-}$ N=9, $\alpha 7^{-/-}$ N=7.