Lower core body temperature and attenuated nicotine-induced hypothermic response in mice lacking the β4 neuronal nicotinic acetylcholine receptor subunit

Ram Sack a,b,1, Alona Gochberg-Sarver a,b,1, Uri Rozovsky a, Merav Kedmi a,b, Serena Rosner a, Avi Orr-Urtreger a,b,∗

a The Genetics Institute, Tel-Aviv Sourasky Medical Center, 6 Weizmann St., Tel Aviv 64239, Israel
b Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Received 8 December 2004; received in revised form 16 February 2005; accepted 27 February 2005
Available online 22 April 2005

Abstract

Diverse physiological and pathological effects of nicotine, including the alteration of body temperature, are presumably mediated by neuronal nicotinic acetylcholine receptors (nAChRs). Previous studies have suggested the involvement of distinct nAChR subunits in nicotine-induced thermoregulation. We studied genetically manipulated knockout mice lacking the β7, β5 or β4 subunit genes, in order to assess the effects of subunit deficiency on temperature regulation. Using a telemetry system, core body temperature was monitored continuously prior to and following nicotine administration in mutant mice and in wild-type littermates. Mice lacking in the β4 nAChR subunit gene had significantly lower baseline core body temperature than all other mouse strains studied. β4 null mice also demonstrated a reduced nicotine-induced hypothermic response and impaired desensitization following repeat nicotine exposure. These findings suggest the involvement of the β4 nAChR subunit in both core body temperature homeostasis and nicotine-elicited thermo-alterations in mice.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Nicotinic acetylcholine receptors; β4 subunit; Temperature regulation; Nicotine-induced hypothermia; Knockout mice

1. Introduction

The neuronal nicotinic acetylcholine receptors (nAChRs) belong to the large super family of ligand-gated ion channels and are expressed throughout both the central and peripheral nervous systems, and in non-neuronal cells (reviewed by [2]). To date, 12 distinct genes encoding neuronal nAChR subunits have been identified, 9 α-type (α2–α10) and 3 β-type (β2–β4), generating an abundance of structurally and functionally distinct hetero- and homo-pentameric receptors (reviewed by [9]). The neuronal nAChRs mediate the effects of the endogenous neurotransmitter acetylcholine, and are the principal biological targets of the tobacco alkaloid, nicotine. Nicotine has diverse effects on the nervous system, altering behavior and cognition [16], inducing seizures [17], mediating analgesia [26] and regulating autonomic functions [6,41]. Nicotine also elicits changes in core body temperature. Early experiments demonstrated the induction of a hypothermic response following intracerebral nicotine administration in cats [10], monkeys [11,12] and rats [15]. The significant tolerance to the hypothermic effects of an acute nicotine challenge following chronic systemic nicotine infusion in mice [5,19,34] as well as reported time and dose dependencies, support a receptor mediated process [5,23,24]. While the molecular mechanisms underlying mammalian thermoregulation are largely unknown, data suggest that nicotine-induced alterations in body temperature are influenced by genetic factors. Differential sensitivity to dose dependent...
nicotine-induced hypothermia and acute tolerance has been identified in different strains of inbred mice [3,20,22,25]. Furthermore, the missense mutation Charonu A529T identified in the murine α4 nAChR subunit gene was associated with increased sensitivity to various effects of nicotine, including nicotine-induced hypothermia [35,37,39]. A recent study in knockout mice deficient in the β2 nAChR subunit reported a reduced hypothermic response to low doses of nicotine, suggesting that this subunit partially mediates nicotine-induced hypothermia [38].

The objective of the present study was to evaluate the possible involvement of various neuronal nAChR subunit genes in temperature homeostasis and in nicotine-induced hypothermia using mutant mice deficient for α7, α5 or β4 subunits (α7−/−, α5−/− and β4−/−, respectively). Significant alterations in both baseline core body temperature and in the hypothermic response normally induced by nicotine were observed in mice deficient in the β4 subunit gene, suggesting that this nAChR subunit plays an important role in mammalian core body temperature homeostasis and in the mediation of nicotine-induced temperature changes.

2. Methods

2.1. Animals

All mice used in this study were from congenic lines that were backcrossed onto a C57BL/6J background for at least six generations after germline transmission. Mutant mice were homozygous for a null mutation in the α7 (n = 11) [29], α5 (n = 10) [32] or β4 nAChR subunits (n = 21) [43]. Wild-type littermates from the mutant strains were used as controls (n = 15). Experiments were performed on a total of 57 mice (31 males and 26 females). Average age and weight were 13 ± 4.9 weeks and 24 ± 3.8 g, respectively. Prior to the experiments, 2–5 mice were housed in each cage, under a 12-h light/12-h dark cycle in temperature-regulated rooms (22–24 °C), with food and water ad libitum. All procedures were approved by the institutional animal and care committee, in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Each mouse was genotyped twice, once before and once following the experiment. The genotyping of α5 and β4 null mice and WT mice was as described previously [14]. α7 null mice were genotyped using three-way PCRs with primers sequences as follows: α7 forward, 5′-GGTTTCTCCTGGTCTCGTGTGTTA-3′; α7 reverse, 5′-TGCCTGOGAAATCTGCAAGCACACTTGGCG-3′; α7 mutant reverse, 5′-GGCCAGAGGTACCCAGCCAGTTTTGC-3′. All PCR reactions were performed in a total volume of 25 µL using 1.25 units of Taq DNA polymerase (Sigma–Aldrich, St. Louis, MO, USA) and 500–1000 ng genomic DNA as a template, in a T3 Thermocycler (Biometra® Göttingen, Germany). The PCR conditions for α7 genotyping included an initial denaturation step of 95 °C for 2 min followed by 35 cycles, each of 94 °C for 45 s, 62 °C for 45 s and 72 °C for 45 s, and a final extension step of 72 °C for 5 min.

2.2. Telemetry

The telemetry system (DATA Sciences’ PhysioTel® Telemetry, St. Paul, MN, USA) was used to measure physiological parameters including temperature and heart rate. The system includes an implantable transmitter chip (radiofrequency transducer model TA10ETA-F20), a receiver panel (RPC-1), a consolidation matrix and a personal computer with accompanying Dataquest Data Analysis software (Dataquest A.R.TM, Version 2.10, St. Paul, MN, USA), allowing continuous recording in conscious, unrestrained and uninterrupted stress-free animals.

2.3. Surgical procedures

Mice were anaesthetised by an intra-peritoneal (i.p.) injection of Ketamine HCl/Rumpon (FORT DODGE®, Iowa, USA), and the telemetry transmitter chip was secured in the intra-peritoneal cavity. Positive and negative leads were directed subcutaneously to the left groin and right shoulder areas, respectively, and securely sutured. Incisions were closed and the animals were returned to individual cages where they recovered in a warm environment until fully awakened. For the remainder of the experiment, mice were caged individually.

2.4. Temperature recording

Immediately following the surgical procedure continuous data sampling was initiated, with 10’s fragments recorded every 10 min. After a designated 3-day recovery period, baseline temperature values were recorded for 3 consecutive days (4th, 5th and 6th post-operative days, a total of 72 h).

2.5. Drug administration

A single dose of 1 mg/kg body weight (−)-Nicotine (Sigma–Aldrich®, Rehovot, Israel), dissolved in sterile isotonic saline (B. Braun, Melsungen, Germany), was injected intraperitoneally at volume of 0.01 ml/g body weight once daily on the 7th, 8th and 9th post-operative days, and temperature values were recorded in 10’s fragments each minute for 3h, 1 h prior to and 2 h following i.p. nicotine injection. Saline administration at the same volume per body weight served as a control.

The magnitude of nicotine-induced hypothermic response in each mouse was determined by calculating the area under the curve (AUC) 5–120 min after i.p. nicotine injection. The following formula calculated AUC based on the area generated by the differences between the baseline temperature (before nicotine injection) and the temperature nadir at each minute: \[ \text{AUC} = 115a - \frac{(b + c + 2d)2}{2} \] (AUC, arbitrary units), where a is the average baseline temperature...
5–60 min prior to nicotine injection, \( b \) the temperature value 5 min post-nicotie injection, \( c \) the temperature value 120 min post-nicotie injection and \( d \) is the sum of temperature values 6–119 min post-nicotie injection. The values of the first 5 min after nicotine injection were not included in the calculation in order to avoid artifactual measurements resulting from handling the mice during the injection.

2.6. Statistical analysis

The basic statistical model was analysis of variance (ANOVA). When necessary, we used planned comparison to analyze a specific hypothesis by constructing a contrast, and post hoc analysis to locate a source of significance, using the Tukey algorithm. A \( p \)-value of less than 0.05 was considered statistically significant. Pearson correlation was calculated between variables on an interval scale and the Fisher exact test was applied for a parametric comparison of groups that included less than 25 mice. Statistical analysis was performed using SPSS V12 software (SPSS Inc., Chicago, IL, USA). Graphs were constructed with Prism software (Prism, GraphPad 4 Software, San Diego, CA, USA). Data are expressed as means and standard deviations.

3. Results

3.1. Diurnal/nocturnal core body temperature periodicity is maintained in WT, \( \alpha7^{−/−} \), \( \alpha5^{−/−} \) and \( \beta4^{−/−} \) mice

Following the designated 3-day recovery period, baseline core body temperatures (\( T_c \)) were recorded continuously for a 72 h period (4th, 5th and 6th post-operative days) in WT (\( n = 10 \)), \( \alpha7^{−/−} \) (\( n = 10 \)), \( \alpha5^{−/−} \) (\( n = 9 \)) and \( \beta4^{−/−} \) (\( n = 17 \)) mice (Fig. 1). Since age, weight and gender may influence body temperature, we examined the possibility of a potential confounding effect of each of these variables. No significant difference was found between the male to female ratio in each group of mice tested (44–60% males, \( p = 0.874 \)). In a detailed analysis of all data recorded on the 5th post-operative day, no correlation was found between age and weight and the mean \( T_c \) (\( r_p = 0.205 \) and \( 0.266 \), respectively, Pearson Correlation test). Interestingly, a significantly lower mean core body temperature was found in all males tested when compared to all females (36.23 ± 0.60 and 36.73 ± 0.46 °C, \( n = 25 \) and 21, respectively, \( p = 0.002 \)) independent of the genotype (WT or mutants). The gender of mice was therefore entered as a covariate in all statistical analyses and all results are reported after controlling for this variant.

The diurnal/nocturnal \( T_c \) pattern was maintained in all strains of mice tested during the 72 h recording with day \( T_c \) lower than night \( T_c \) (Fig. 1). The average day (all data points from 07.00 to 19.00) and night (all data points from 19.00 to 07.00) \( T_c \) were evaluated on the 5th post-operative day (Fig. 2). Differences between these average temperatures were analyzed using ANOVA in a mixed inter-intra subject model with two independent variables: time (day versus night) as a repeated (within) subject variable, and strain of mice (WT, \( \alpha7^{−/−} \), \( \alpha5^{−/−} \) or \( \beta4^{−/−} \) mice) as an inter (between) subject variable. Day \( T_c \) was significantly lower than night \( T_c \) (\( F(1,41) = 43.55, p < 0.0001 \)) in all groups of mice tested (Fig. 2). The difference between day and night \( T_c \) in WT mice was more pronounced when compared to the differences observed in \( \alpha7 \), \( \alpha5 \) and \( \beta4 \) null mice (0.88, 0.70, 0.52 and 0.59 °C, respectively, \( F(3,41) = 3.235, p = 0.032 \)).

3.2. \( \beta4 \) nAChR subunit null mice have a significantly lower baseline core body temperature

The average core body temperature of \( \beta4^{−/−} \) mice was lower than the average \( T_c \) of WT control mice and of \( \alpha7 \)
Wild-type vs. hypothermia was calculated for each group of mice, using the difference between area under the baseline curve (AUC) prior to and following the 1st nicotine injection (see text for detailed explanation). Differences in AUC between the four different groups calculated shows that T deficiency was detected between T of wild-type, α7/−/− and α5/−/− mice (ANOVA followed by Tukey post hoc analysis). The magnitude of nicotine-induced hypothermia was calculated for each group of mice, using the difference between area under the baseline curve (AUC) prior to and following the 1st nicotine injection (see text for detailed explanation). Differences in AUC between the four different groups calculated shows that β4/−/− mice have a significantly attenuated nicotine-induced hypothermic response compared to wild-type mice (ANOVA followed by Tukey post hoc analysis).

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Average differences in Tc</th>
<th>Difference</th>
<th>p-Value</th>
<th>Average differences in AUC</th>
<th>Difference</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type vs. α7/−/−</td>
<td>0.35</td>
<td>0.37</td>
<td></td>
<td>20.89</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Wild-type vs. α5/−/−</td>
<td>−0.08</td>
<td>0.98</td>
<td></td>
<td>37.93</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Wild-type vs. β4/−/−</td>
<td>0.82</td>
<td>&lt;0.0001</td>
<td></td>
<td>88.19</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>β4/−/− vs. α7/−/−</td>
<td>−0.47</td>
<td>0.075</td>
<td></td>
<td>−67.36</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>β4/−/− vs. α5/−/−</td>
<td>−0.89</td>
<td>&lt;0.0001</td>
<td></td>
<td>−50.26</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>α7/−/− vs. α5/−/−</td>
<td>−0.42</td>
<td>0.22</td>
<td></td>
<td>17.04</td>
<td>0.93</td>
<td></td>
</tr>
</tbody>
</table>

On the 5th post-operative day, mean core body temperatures were evaluated for each group of mice, and differences were compared. Significant Tc differences were detected between the α7/−/− and α5/−/− mice, whereas no differences were found when comparing β4/−/− and α7/−/− mice. There were no differences between the Tc of wild-type, α7/−/− and α5/−/− mice (ANOVA followed by Tukey post hoc analysis). The magnitude of nicotine-induced hypothermia was calculated for each group of mice, using the difference between area under the baseline curve (AUC) prior to and following the 1st nicotine injection (see text for detailed explanation). Differences in AUC between the four different groups calculated shows that β4/−/− mice have a significantly attenuated nicotine-induced hypothermic response compared to wild-type mice (ANOVA followed by Tukey post hoc analysis).

and α5 null mice during a 72 h recording (Fig. 1). The average day (07:00–19:00) and night (19:00–07:00) Tc evaluated on the 5th post-operative day revealed significant differences between genotypes (F(3,41) = 10.85, p < 0.0001, Table 2). The average 24h core body temperature of β4/−/− mice was significantly lower than that of WT control mice and α5 null mice and nearly significantly lower than α7 deficient mice (p = 0.0001, p = 0.0001 and p = 0.075, respectively; Table 1 and Fig. 2).

A significantly lower Tc was also evident in β4/−/− mice when day and night temperatures were compared independently (F(3,41) = 10.19, p = 0.0001 and F(3,41) = 10.07, p = 0.0001, respectively), and when male and female mice were analyzed separately (F(2,31) = 6.23, p = 0.003 and F(2,31) = 7.78, p = 0.002, respectively).

3.3. Mice with β4 nAChR subunit deficiency demonstrate an attenuated nicotine-induced hypothermic response

WT (n = 15), α7/−/− (n = 9), α5/−/− (n = 10) and β4/−/− (n = 9) mice received nicotine (1 mg/kg) once a day for 3 consecutive days (7th, 8th, 9th post-operative days). Fig. 3 shows the core body temperature response of the four groups of mice tested following the 1st nicotine injection. In all groups of mutant and control mice tested, there were no statistical differences between the average baseline temperatures (5–20 min prior to nicotine injection) and the average temperatures 105–120 min after nicotine injection (WT: 36.51 ± 0.84 and 36.12 ± 0.48; α7: 36.05 ± 0.71 and 35.94 ± 0.55; α5: 36.48 ± 0.68 and 36.83 ± 0.82; β4: 35.84 ± 0.53 and 35.50 ± 1.24, respectively).

One way ANOVA with the AUC as the dependent variable and the genotypes of mice as the independent variable revealed a differential response to nicotine (F(3,42) = 3.88, p = 0.016). Tukey post hoc analysis comparing mutants and WT mice identified the source of significance as the reduced hypothermic response of β4/−/− mice to nicotine (p = 0.009, Table 1).

The Tc response of mice to daily nicotine injections for 3 consecutive days was also examined. Fig. 4A–E demonstrates the Tc responses for each group of mice following both days 1 and 3 nicotine injections as determined by calculating the AUC (area under the curve). Repeated nicotine exposure diminished the hypothermic response in all strains (F(1,39) = 5.23, p = 0.028). Although no interaction was found between the genotype of mice and repeated nicotine injections (1st versus 3rd injection, F(1,39) = 1.33, p = 0.28), the absence of an attenuated response following the 3rd nicotine injection was apparent in β4/−/− mice relative to WT mice (p = 0.005). Contrast analysis comparing all null mutants to WT controls supported the observation that β4/−/− mice showed a reduced response to recurrent nicotine injections.

Fig. 3. Nicotine-induced hypothermia in wild-type, α7/−/−, α5/−/− and β4/−/− mice. Core body temperatures were measured 1 h prior to and 2 h following nicotine injection. Saline was administered to β4/−/− mice as a control. Graph represents the average temperatures of wild-type (n = 15), α7/−/− (n = 9), α5/−/− (n = 10) and β4/−/− (n = 9) mice. Arrow represents the time of nicotine/saline administration.
Fig. 4. Nicotine-induced hypothermia in wild-type, α7−/−, α5−/−, and β4−/− mice following the 1st and 3rd nicotine injections. Graph represents the average temperatures 1 h prior to and 2 h following the 1st (represented by black solid line) and the 3rd (represented by dotted gray line) nicotine injections. (A) Wild-type mice (n = 15), (B) α7−/− mice (n = 9), (C) α5−/− mice (n = 10) and (D) β4−/− mice (n = 9). Arrow represents the time of nicotine administration. (E) Bar histogram represents AUC ± S.D. following the 1st and 3rd nicotine injections in each of the groups of mice tested (1st nicotine injection: wild-type = 249.43 ± 89.34, α7 = 228.54 ± 30.85, α5 = 211.50 ± 45.32 and β4 = 161.24 ± 41.40, and 3rd nicotine injection: wild-type = 178.02 ± 72.58, α7 = 176.62 ± 65.04, α5 = 185.91 ± 38.53 and β4 = 146.46 ± 46.03). The difference in the magnitude of nicotine-induced hypothermia between the 1st and 3rd nicotine injections in β4−/− mice was significantly less than that of wild-type mice (*p = 0.005).

4. Discussion

The central nervous system and a range of peripheral autonomic mechanisms maintain mammalian core body temperature within a narrow range [1]. Although molecular mechanisms underlying temperature homeostasis are poorly understood, they likely involve multiple genes.

A wide variety of pharmacological agents elicit alterations in core body temperature. The hypothermic effects of nicotine have been demonstrated in numerous animal studies and differential sensitivity to nicotine-induced hypothermia documented in several inbred strains of mice, suggests that a genetic component influences nicotine-induced responses [20,22,25]. Presuming that the effects of nicotine on body temperature are mediated through nAChRs, our objective was to study the association between specific neuronal nAChR subunit genes and temperature regulation, aiming to further clarify the genetic mechanisms underlying this complex process. We studied knockout mice deficient in α7, α5, or β4 subunit genes, comparing these null mutants to
WT littermates. To the best of our knowledge, our results represent the first evidence of a role for a nAChR subunit gene in baseline homeostasis of core body temperature with β4 null mice exhibiting significantly lower core body temperatures.

We initiated the study of temperature homeostasis in WT and mutant mice by observing diurnal/nocturnal temperature variability. Rodents maintain low diurnal and higher nocturnal core body temperatures, regulated by the hypothalamic supraoptic nucleus (SCN) [27]. The abundant expression of nAChR subunits, particularly α7, in the SCN [28], suggests their possible involvement in the maintenance of periodicity of body temperature. We hypothesized a potential disturbance in periodicity in mutant mice, specifically α7 null mice, but this was not confirmed. The pattern of temperature recorded demonstrated the conservation of low diurnal and higher nocturnal temperatures demonstrated in all groups of mutant mice tested, implying that the deficiency of either the α7, α5 or β4 nAChR subunit gene does not change the diurnal/nocturnal variation of murine body temperature. However, mice deficient for the β4 nAChR subunit gene demonstrated diurnal and nocturnal core body temperatures that were significantly lower than those of WT controls, suggesting that nicotine receptors assembled with the β4 subunit play a role in the maintenance of core body temperature.

Given the established association between body temperature and heart rate [8], we compared heart rate in all groups of mice tested, as a possible explanation for the significantly lower baseline body core temperature in β4 null mice. Paradoxically, the β4 null mice had significantly higher heart rates (data not shown) when compared to WT controls, and to α7 and α5 null mutant mice, dismissing bradycardia as the cause of hypothermia in β4−/− mice, and suggesting that the increased heart rate might possibly attempt to compensate for the lower core body temperature in β4−/− mice.

Our study also included the administration of 1 mg/kg body weight of nicotine to WT, α7−/−, α5−/− and β4−/− mice. β4 null mice exhibited a significantly attenuated nicotine-induced hypothermic response, when compared to WT mice and to α7 and α5 null mice, further supporting a role for the β4 nAChR subunit in murine thermoregulatory mechanisms.

Previous studies suggest that the β4 subunit largely influences nAChR affinities and sensitivities to agonists and antagonists [4,18,30,40], and dictates nAChR activation kinetics, conductance and channel open time [13,30,36,42]. The absence of the β4 subunit likely induces conformational changes in nAChRs, altering receptor function and possibly modifying sensitivity to receptor ligands [40]. In fact, studies in β4 null mice have demonstrated decreased sensitivity to both central and peripheral effects of acute nicotine or nicotine agonists, including significantly reduced sensitivity to nicotine-induced seizures [14,31], bladder contractility [7,43] and focal contractile responses [40]. We suggest that in the current experiment, as in the above reports, the attenuated nicotine-induced hypothermic response in β4−/− mice is the result of reduced receptor sensitivity to nicotine due to the deficiency of the β4 nAChR subunit.

Decreased sensitivity to the hypothermic effects of nicotine following repeated administration has been well documented in animal studies [5,21]. We examined nicotine-induced temperature changes in mice injected with nicotine for 3 consecutive days. While repeated nicotine administration ameliorated the nicotine-induced hypothermic response in WT, α7−/− and α5−/− mice, no significant alteration in nicotine-induced hypothermia was witnessed in β4−/− mice, suggesting the possible involvement of this subunit in receptor desensitization.

In summary, we have presented the first evidence for the involvement of the β4 nAChR subunit in molecular mechanisms underlying core body temperature homeostasis and nicotine-induced hypothermia in rodents, and possibly in humans as well.

Acknowledgements

This work was supported by the M.K. Humanitarian Fund. This work was performed in partial fulfillment of the requirements for a Ph.D. degree of Alona Gochberg-Sarver, Sackler Faculty of Medicine, Tel Aviv University, Israel.

References


