α7-Nicotinic acetylcholine receptor subunit is not required for parasympathetic control of the heart in the mouse

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Submitted 6 April 2004; accepted in final form 16 March 2005

Deck, Jennifer, Steve Bibevski, Tomaso Gneckchi-Ruscone, Valentina Bellina, Nicola Montano, and Mark E. Dunlap.α7-Nicotinic acetylcholine receptor subunit is not required for parasympathetic control of the heart in the mouse. Physiol Genomics 22: 86–92, 2005. First published March 29, 2005; 10.1152/physiolgenomics.00085.2004.—Nicotinic acetylcholine receptors (nAChR) are assembled from a pool of nine α-subunits and three β-subunits into functional pentamers in peripheral autonomic neurons. The contribution of different subunits to native, physiologically important nAChR for synaptic transmission in autonomic ganglia is unclear. Here, we examined the importance of the α7-subunit for parasympathetic innervation of the heart. Normal Chrna7-deficient mice. These data support previous findings in vitro and highlight the important differences in function between nicotinic receptor subtypes because α7-deficient mice display major autonomic dysfunction. We conclude that the α7-subunit does not contribute critically to resting parasympathetic control of the heart.

SYNAPTIC TRANSMISSION across sympathetic and parasympathetic neuronal ganglia in mammalian species is mediated by nicotinic acetylcholine receptors (nAChR). nAChR have been divided into neuronal and nonneuronal subtypes, based initially on sensitivity to blockade by mecamethonium salts. More recently, it has become evident that neuronal nAChRs are assembled in vivo from a combination of nine different α-subunits and three different β-subunits that form a functional ligand-gated ion channels in various stoichiometries. An exception to this rule relevant to mammalian species has been the α7-subunit, which, in the nervous system, most likely forms a homomeric pentamer (6), although heteromeric pentamers containing α7 are possible (23). Currently, the exact subunit composition of nAChR responsible for ganglionic transmission in autonomic neurons to the mammalian heart remains unknown. We (2) have previously shown in the canine cardiac ganglion that nAChR containing α3/β2-subunits are the primary native receptor species that mediate synaptic transmission in efferent pathways to the heart with a possible smaller role for α7-containing receptors. Others have shown that α7-deficient mice may have altered baroreceptor function in the sympathetic efferent limb as well as altered sensitivities to hexamethonium despite normal parasympathetic function (7). Previous work has shown that α7-subunit-containing pentamers (which are sensitive to α-bungarotoxin) (1, 24) contribute a significant component of the excitatory postsynaptic current in chick ciliary ganglia (22) as well as rat (4), mouse (18), and chick sympathetic neurons (23). However, pharmacological blockade of α7-containing receptors with α-bungarotoxin does not block synaptic transmission, indicating the importance of other subunits in mediating ganglionic transmission. In addition, the localization of the α7-subunit has been shown to be at the periphery of the synaptic end plate and not in the main functional domain (8, 21), which has added to the ambiguous role of α7-containing receptors. A possible functional role for the α7-subunit was suggested by Chang and Berg (3), who showed that the α7-subunit was required for reliable synaptic transmission at higher stimulation frequencies. A role in ganglionic signaling for α7-containing receptors in cardiac neurons has been suggested by the presence of α7-mediated currents in rat intracardiac ganglia (4). These findings have indicated a possible role for α7-nAChR under artificial, in vitro conditions but have failed to demonstrate a putative role of these receptors in normal physiology. Here, we examined the contribution of the α7-subunit to autonomic function in both the resting physiological state as well as in direct nerve stimulation studies in vivo in normal and α7-deficient mice.

METHODS

Telemetry Implantation

All experiments were performed in accordance with guidelines for the care and use of research animals at Case Western Reserve University and all protocols were approved by the Institutional Review Board at the Cleveland Veterans Affairs Medical Center. Ten-week-old, 25- to 30-g, male C57BL/6J and Chrma7 mice were purchased from Jackson Labs (Bar Harbor, ME). The α7-deficient mice were generated as described by Orr-Utregger et al. (18). Two control

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groups were used. Wild-type (C57BL/6-129s; +/+ ) littermates of the Chrna7 mice and regular C57BL/6J mice were studied to help detect any physiological differences between normal and α7-deficient mice. The comparison between wild-type and regular C57BL/6J mice was done to determine whether regular C57BL/6J mice could be used in exchange for wild-type littermates given the restricted availability of the wild-type littermates.

All animals were anesthetized with 0.1 ml/20 g body wt (intraperitoneally) of a rodent cocktail anesthetic composed of 150 mg ketamine (100 mg/ml), 30 mg xylazine (20 mg/ml), and 5 mg acepromazine (10 mg/ml). Physiotel TA10EA-F20 telemetry devices (Data Sciences; St. Paul, MN), which were used to capture ECG signals in conscious, unseathed, freely moving mice, were placed into the peritoneal cavity under sterile conditions without disruption to the viscera and sutured with a nonabsorbable suture to the peritoneal wall. Leads were tunneled subcutaneously and anchored to the muscle in a lead II configuration. For the duration of the implant procedure, body temperature was maintained at 37°C via Deltaphase Isothermal pads (Baintree, MA), and heart rate (HR) was monitored via ECG electrodes placed under the skin. Marcaine (0.25%, 0.01 mg/kg) was injected at the incision sites to provide postsurgical analgesia. Before surgery, animals were housed individually in plastic cages fitted with sterile nesting material, allowed free access to tap water, and fed standard mouse chow.

**Recordings in Conscious Animals**

All experimental recordings were performed on weekdays during the hours of 8 AM and 3 PM. Animals were not used for recordings on adjacent days and were selected for recordings randomly. After adequate recovery from the surgical procedure (2 wk), mice were placed into a round (15 cm diameter) plethysmograph chamber containing bedding from their own cage 5 h daily for an additional week to acclimatize them to the new environment. ECG signals were recorded from within the plethysmograph chamber for two primary reasons. First, the chamber was large enough so that the animal was able to move freely without restraint, and, second, it provided a controlled means by which the animal’s respiratory pattern could be recorded. Before recordings, mice were permitted to acclimatize to the chamber until resting comfortably (~ 1 h). Five-minute baseline recordings were collected, followed thereafter by administration of either atropine sulfate (0.04 mg/kg), propranolol (1 mg/kg), or hexamethonium bromide (25 mg/kg) intraperitoneally. The animal was given 10 min to settle down after the injection, and a 5-min recording was repeated.

**Protocol.** Animals underwent single and combined autonomic blockade with three different experimental drug administrations selected randomly (see Fig. 1). All dosages were based on reported amounts in the literature where total blockade of the respective division was required and was confirmed in pilot studies in our lab before the protocol was undertaken. Once a successful recording was collected, the next drug in the protocol was administered to the animal. In the case where hexamethonium was selected first, this was the only drug given on that day. The time from which the first drug was given was carefully monitored because recordings under subsequent drugs needed to be obtained during the active half-life of the previous drug. If there were issues that prevented obtaining recordings within the appropriate time frame, the experiment was discontinued and repeated at another date. Double-blockade experiments were usually completed within 30 min for atropine and 45 min for propranolol when they were given as the first drugs, respectively. These time limits were well short of expected and tested washout of drug effectiveness.

**Data Capture and Analysis**

ECG signals were captured at 10 kHz and stored on a personal computer using Ponemah, a digital data-acquisition and analysis system (Gould Instruments; Valley View, OH). Respiratory signals were obtained with a differential pressure transducer connected on one side to the recording chamber and on the other side to a sealed reference chamber. The chamber was custom built from Plexiglas (dimensions = 15 cm diameter × 12 cm high) and was attached to a regulated vacuum source adjusted to provide continuous air flow at 1 l/min. The recording chamber rested on a PhysioTel telemetry receiver (Data Sciences) for detection of the transmitted ECG signal. All recordings were saved to hard drive and later replayed and inspected to ensure appropriate detection of ECG and respiratory signals. Assessment of resting autonomic tone to the heart in both control and Chrna7 mice was done with spectral analysis of HR variability using autoregressive algorithms. The theoretical and analytical procedures have been described by us and others (5, 14, 20). An automated routine generated a time series (tachogram) from the ECG signal. The respiratory signal was sampled once per cardiac cycle to obtain a time series synchronous with the tachogram. On stationary segments of R-R interval time series (250–500 beats), autoregressive parameters were estimated via Levinson-Durbin recursion, and the order of the model was chosen according to Akaike’s criterion (14, 17, 20). An autoregressive spectral decomposition was then performed. This procedure permitted us to automatically quantify the center frequency and power of each relevant component in absolute as well as in normalized units (NU). The normalization procedure was performed by dividing the power of the low-frequency (LF) or high-frequency (HF) component by the total spectral power from which the power of the very-LF (VLF) component had been subtracted and multiplying the result by 100 (5, 14, 20). As previously reported (10), we selected <0.15, 0.15–1.5, and 1.5–5 Hz as the frequency ranges for the VLF, LF, and HF oscillations in mice (respectively). The monitoring of respiratory activity in our experiments allowed us to carefully evaluate the correspondence between respiratory rate and the HF component of R-R variability.

**Vagus Nerve Stimulation Protocol**

Animals were anesthetized with a rodent cocktail solution at a dosage of 0.2 ml/20 g body wt ip. This cocktail was composed of 150 mg ketamine (100 mg/ml), 30 mg xylazine (20 mg/ml), and 5 mg acepromazine (10 mg/ml). A tracheotomy was performed, and the animal was ventilated using a MiniVent (Hugo Sachs Electronik) at a stroke volume of 300 μl and at 250 breaths/min. ECG signals were monitored via subcutaneous electrodes. The right vagus nerve was dissected and isolated at the cervical level through a single midline incision. The nerve trunks were ligated, sectioned at the cranial end, and soaked with mineral oil. The caudal remnant of the nerve was then

![Fig. 1. Protocol for conscious studies with autonomic blockade. Animals were randomly selected to undergo either protocol 1, 2, or 3 on the testing day. Only one protocol was performed in any given day with at least a 48-h rest period between repeat studies. Hexameth, hexamethonium.](https://www.physiolgenomics.org/fig/1.png)
laid on the exposed ends of Teflon-coated stainless steel wire electrodes with bared ends for contact to the nerve. Three sets of vagus nerve stimulations were performed at 5, 10, 15, and 20 Hz at 1 mA and a pulse width of 1 ms (Grass SD9 Stimulator). ECG signals were recorded for 15 s of baseline, during 10 s of continuous electrical stimulation, and 15 s of recovery for each stimulation level performed. Before subsequent stimulations, ample time was given to allow HR to return to resting levels. The vagal response was taken from the average R-R period during the entire stimulation period, which was instantaneous and stable for the duration of stimulation.

**RESULTS**

**HR Changes With Pharmacological Blockade**

Resting HR was similar in control, wild-type, and Chrna7 animals under baseline conditions and after atropine, propranolol, and hexamethonium when each drug was given as the first drug only (single blockade; see Table 1; all \( P > 0.05 \) by \( t \)-test). The change in HR in response to autonomic blockade using atropine (A), propranolol (B), and hexamethonium (C) is shown in Fig. 2. Atropine caused a significant increase in HR in all three groups, confirming prominent parasympathetic tone at rest in all three groups (Fig. 2A). Hexamethonium resulted in similar changes in HR compared with atropine, suggesting that, like most other mammals studied to date, parasympathetic tone predominates at rest and the resting HR is determined primarily by vagal modulation (Fig. 2C). There was an expected bradycardia in response to propranolol in the C57BL/6J control group (decreased by 27 beats), which was not observed in the wild-type (increased by 8 beats) or Chrna7 group (increased by 5 beats), but the differences were not statistically significant (\( P > 0.05 \)) across all groups (Fig. 2B).

Table 2 shows power spectral analysis data for the C57BL/6J control, wild-type, and Chrna7 groups. HF and LF NUs were similar across all three groups (\( P > 0.05 \) by \( t \)-test), although the wild-type group had a large value in the LF component. This high value in the LF domain did not reach statistical significance compared with the other groups due to high interindividual variability within the wild-type group. Figure 3 shows a representative power spectrum (PSD) from recordings obtained in mice under baseline conditions from each group. Animals displayed a significant HF component that correlated tightly to the respiratory frequency indicative of significant vagal modulation at rest. A LF component was also identified in each group at a similar frequency and power. Notably, the respiratory frequency was the same in each group of animals. Under conditions of autonomic blockade, the variance of R-R interval was abolished with atropine and hexamethonium and dramatically reduced with propranolol [atropine:

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline HR</th>
<th>Atropine HR</th>
<th>Propranolol HR</th>
<th>Hexamethonium HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>471 ( \pm ) 21</td>
<td>667 ( \pm ) 16</td>
<td>444 ( \pm ) 32</td>
<td>626 ( \pm ) 21</td>
</tr>
<tr>
<td>Wild type</td>
<td>446 ( \pm ) 24</td>
<td>630 ( \pm ) 31</td>
<td>530 ( \pm ) 31</td>
<td>636 ( \pm ) 27</td>
</tr>
<tr>
<td>Chrna7</td>
<td>442 ( \pm ) 22</td>
<td>606 ( \pm ) 24</td>
<td>442 ( \pm ) 27</td>
<td>579 ( \pm ) 31</td>
</tr>
</tbody>
</table>

Data are means \( \pm \) SE; \( n \), no. of recordings from \( N = 5 \) control (C57BL/6J), 4 wild type (C57BL/6-129S), and 8 Chrna7 mice. Values are from recordings taken when each drug was given first in the protocol. HR, heart rate (in beats/min).

Fig. 2. Change in heart rate (HR) from baseline (in beats/min) after autonomic blockade. Atropine sulfate (A), propranolol (B), or hexamethonium (C) treatments in control (C57BL/6J), wild-type (C57BL/6-129S), and Chrna7 mice are shown. \( n \), No. of different mice. There were no statistically significant differences (\( P > 0.05 \) by \( t \)-test) between the groups in HR responses to any of the pharmacologic agents tested.
control 0.7 ± 0.3, wild-type 1.3 ± 0.6, and Chrna7 2.0 ± 1.7 for reductions from baseline of 98.8, 99, and 97.6%, respectively (P > 0.05); propranolol: control 9.6 ± 3.9, wild-type 1.7 ± 0.7, and Chrna7 30.2 ± 19.3 for reductions from baseline of 79, 94, and 42%, respectively (P > 0.05); hexamethonium: control 3.5 ± 2.8, wild-type 0.9 ± 0.4, and Chrna7 0.7 ± 0.2 for reductions from baseline of 97, 99.7, and 99.3%, respectively (P > 0.05)]. Under this low-variance state, specific frequencies could not be reliably detected, and power spectral analysis after autonomic blockade was not performed.

Cumulative drug data obtained by sequentially adding the next drug in the protocol during the effective period of the preceding drug is shown in Fig. 4, A and B. When propranolol was given as the second drug after atropine, there was a prolongation in R-R interval, as expected. When propranolol was given as the first drug, however, there was only a very small effect on R-R interval (P > 0.05), suggesting that vagal tone predominates at rest and that sympathetic activity to the sinoatrial node is more apparent after blockade of vagal activity.

As seen in Fig. 1, hexamethonium was given after the administration of either atropine or propranolol in two different sequences. When hexamethonium was administered after propranolol as the second drug (Fig. 4A), there was an additional bradycardia of ~20–30 beats/min (P < 0.05), which suggested that a small degree of sympathetic tone remained. This may indicate a degree of incomplete blockade with the doses of atropine or propranolol used or a degree of washout of the previously administered drug. In comparison, when hexamethonium was administered after atropine (Fig. 4B), with propranolol as the first drug in the sequence, a smaller degree of bradycardia was observed (P < 0.05). This suggested that the effect was not related to washout because there should have been more washout when propranolol was given earlier in the protocol. Because the doses of propranolol used were adequate for blockade of sympathetic activity, it is possible this small

Table 2. Spectral analysis of HR variability summary data for control, wild type, and Chrna7 mice

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Mean</th>
<th>Variance</th>
<th>LF Component, Hz</th>
<th>LF</th>
<th>LFNU</th>
<th>HF Component, Hz</th>
<th>HF</th>
<th>HFNU</th>
<th>LF/HF</th>
<th>Respiratory Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mean</td>
<td>141.3</td>
<td>124.3</td>
<td>0.4</td>
<td>49.2</td>
<td>40.4</td>
<td>2.0</td>
<td>48.8</td>
<td>41.4</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>8.8</td>
<td>68.6</td>
<td>0.1</td>
<td>27.4</td>
<td>14.8</td>
<td>0.2</td>
<td>40.6</td>
<td>2.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Wild type</td>
<td>Mean</td>
<td>143.3</td>
<td>237.6</td>
<td>0.4</td>
<td>148</td>
<td>55.3</td>
<td>2.2</td>
<td>60.4</td>
<td>28</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>16.8</td>
<td>99.8</td>
<td>0</td>
<td>98.7</td>
<td>15.1</td>
<td>0.2</td>
<td>36.6</td>
<td>11.6</td>
<td>9.2</td>
</tr>
<tr>
<td>Chrna7</td>
<td>Mean</td>
<td>127.2</td>
<td>121.9</td>
<td>0.4</td>
<td>50</td>
<td>39.5</td>
<td>2.2</td>
<td>34.8</td>
<td>36.6</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>11.1</td>
<td>49.8</td>
<td>0.1</td>
<td>30.6</td>
<td>17.4</td>
<td>2.2</td>
<td>27.5</td>
<td>14.8</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Data are given as measurements of sinus cycle length in mean ± SE; N = 5 control (C57BL/6J), 4 wild type (C57BL/6-129s), and 8 Chrna7 mice. LF, low frequency; LFNU, LF normalized units; HF, high frequency; HFNU, HF in normalized units.

Fig. 3. Power spectral analysis for control, wild-type, and α7-deficient (knockout) mice. Note the tight coupling of the high-frequency (HF) component with the respiratory frequency in each group, representing parasympathetic tone, and a low-frequency (LF) component, representing sympathetic tone at rest. There were no statistical differences between any of the groups (P > 0.05 by t-test) [n = 6 control (C57BL-6J), 4 wild-type (C57BL/6-129s), and 8 Chrna7 mice].
bradycardia resulted from decreased cotransmitter release from postganglionic nerve terminals after hexamethonium.

**Electrical Vagal Stimulation**

Vagal stimulation was done to elicit differences between the groups in a controlled, dose-response manner across a wide spectrum of intensities. Vagal stimulation resulted in an instantaneous onset of R-R prolongation, which was stable for the duration of stimulation. There was similar change in RR interval between the control and Chrna7 groups (Table 3). There was no observed difference between control and Chrna7 responses to vagal stimulation throughout the frequency range used ($P > 0.05$ by ANOVA), suggesting that the $\alpha_7$-subunit did not play an obligatory role at either spectrum of parasympathetic function (resting or high activity; see Fig. 5).

**DISCUSSION**

Previous studies have provided both direct and indirect evidence for a functional role of $\alpha_7$-containing receptors in autonomic function at the cellular level. Studies in $\alpha_7$-deficient mice have indicated that baroreflex stimulation of the parasympathetic limb does not display altered parasympathetic function but that stimulation of the sympathetic limb demonstrates abnormal sympathetic responses (7). The possibility has remained, however, that $\alpha_7$-subunits play a role in resting autonomic tone rather than in stimulated activity, and the possibility of some modulatory role on autonomic function has remained. In this study, we have shown that the $\alpha_7$-nAChR subunit is not required for parasympathetic modulation of HR in the resting state or under artificial controlled stimulation of vagal pathways to the heart throughout a wide stimulation range.

Resting HR and PSD profiles were not significantly different between the groups of mice, suggesting that either the $\alpha_7$-subunit does not play a role in resting autonomic tone or that its role is replaced by a different subunit due to redundancy of nAChR subunits. We believe the latter is not a likely explanation because, despite the fact that many different subunits can form functional pentamers, changes in single subunits result in significantly different biophysical properties of the functional

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**Table 3. Baseline and vagal stimulation RRI in control vs. Chrna7 mice**

<table>
<thead>
<tr>
<th>Stimulation Level</th>
<th>Control</th>
<th>Chrna7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline RRI</td>
<td>213±9</td>
<td>280±26</td>
</tr>
<tr>
<td>Stimulation RRI</td>
<td>323±32</td>
<td>338±35</td>
</tr>
<tr>
<td>5 Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Hz</td>
<td>215±8</td>
<td>275±23</td>
</tr>
<tr>
<td></td>
<td>397±30</td>
<td>422±26</td>
</tr>
<tr>
<td>15 Hz</td>
<td>216±9</td>
<td>275±24</td>
</tr>
<tr>
<td></td>
<td>474±38</td>
<td>486±38</td>
</tr>
<tr>
<td>20 Hz</td>
<td>216±10</td>
<td>288±23</td>
</tr>
<tr>
<td></td>
<td>523±42</td>
<td>525±44</td>
</tr>
</tbody>
</table>

Data are given as mean R-R intervals (RRI; in ms) ± SE; $N = 7$ control (C57BL/6J) and 6 Chrna7 mice. Change in RRI is shown graphically in Fig. 5 ($P > 0.05$) for all stimulations.
channel, as reviewed by Role and Berg (19). A change in subunit composition should therefore manifest itself in different “dose–response” curves such as those performed in the vagus nerve stimulation studies. Furthermore, it is unlikely that the α7-subunit forms heteropentamers with other subunits at neuronal synapses because in the autonomic nervous system, α7-subunits seem to form homomeric pentamers (6). However, we cannot exclude entirely the possibility that otherwise “redundant” α-subunits might be utilized in the absence of α7-subunits.

An intriguing finding in our study is the lack of difference between normal and α7-deficient mice in HR responses after single and double autonomic blockade. Despite abnormal baroreflexes in the sympathetic limb, as described previously (7), there was no detectable difference in resting HR or the responses to autonomic blockade in α7-deficient mice. Although blood pressure was not recorded in our studies and we cannot make any conjectures regarding blood pressure, HR at rest and after individual and combined autonomic blockade was similar in both normal and α7-deficient mice. Specifically, propranolol did not change HR. This suggests that the α7-subunit is not required for sympathetic tone at rest or that there is very little sympathetic tone at rest in the mouse. The former interpretation is commensurate with previous findings by others showing that blockade of α7-containing receptors results in functional changes only at high levels of stimulation (3). This also fits with the anatomic studies that have shown that the α7-subunit is located at the periphery of synapses, where it may play a role in saturation of the synaptic cleft with neurotransmitter at high levels of stimulation (3, 8, 21). The interpretation that there was low sympathetic tone at rest in our mice is possible, however. The failure to induce bradycardia after propranolol supports the notion that there is little resting sympathetic tone to the heart in the mouse at rest. This interpretation is reinforced by the finding that there was significant bradycardia with propranolol after blockade of parasympathetic tone with atropine first (see Fig. 4A). In this situation, propranolol prolonged R-R by ~20 ms, suggesting blockade of parasympathetic tone uncovers/disinhibits sympathetic outflow, possibly by removal of vagal inhibition of sympathetic nerve terminals, as described by Levy and Zieske (12). It also confirmed that we were able to detect changes when they were present.

The lack of bradycardia after propranolol in our study is in disagreement with a number of previous studies (9, 15), which have shown a moderate bradycardia of ~80 beats after the same dose of propranolol. The discrepancy between our findings and those in earlier literature may lie in the fact that previous studies looking at HR control in rodents were under dissimilar conditions involving different awake/sleep states of the animals and less acclimatization to the recording environments. Under these conditions, animals would have higher resting sympathetic tone due to the normal stress/activity response. A review of reports measuring baseline HR in male C57BL/6J mice by the means of telemetry shows an average HR of 577 beats/min, well above what we have recorded. A possible explanation for this difference is that our animals were recorded while at rest and after environmental conditioning for extended periods to ensure a “low-stress” environment. The lower HR in our mice compared with those previously studied (470 vs. 650 beats/min) (9), using the same dose of propranolol, support the notion of a low-stress environment. Nonetheless, in our studies, there were no consistent differences in HR responses to sympathetic blockade between normal and α7-deficient mice. It is important to consider, however, that, although our recording conditions were of low sympathetic tone, abnormalities may exist in the α7-deficient mice that were not detectable by our methods. This point is highlighted by previous reports that demonstrate sympathetic dysfunction in α7-deficient mice undergoing baroreflex stimulation (7). In our study, there was a decrease in R-R variance that was seen after propranolol administration that was attenuated in the Chrna7 mice. This difference did not reach statistical significance but may represent a real difference between the two groups. In this scenario, there would be no significant difference in HR or variance after propranolol because resting sympathetic tone is low, but, under conditions of increased sympathetic tone, we may be able to detect larger differences. The finding of a small decrement in variance after propranolol in the Chrna7 mouse group may point to defective sympathetic modulation in this group and should be interpreted cautiously. We also did not detect any differences between the wild-type and C57BL/6J mice in resting HR, response to medications administered, or PSD, suggesting that C57BL/6J mice can be used as a control for α7-deficient mice for studies of resting HR.

These findings are important for a number of reasons. These studies show that it may be possible to differentiate between sympathetic and parasympathetic pathways to the heart at the ganglionic level rather than at the end-organ receptor (β-adrenergic vs. muscarinic receptors). Blockade of sympathetic baroreflex function in the sympathetic limb may be possible by specific antagonists against α7-subunits because α7-deficient mice display abnormal sympathetic baroreflexes (7). We have demonstrated here that blockade of α7-subunits would not be likely to affect parasympathetic function. The implications for such findings are potentially very exciting for clinical relevance. If pharmacological discrimination between sympathetic and parasympathetic function is possible at the ganglion, one could theoretically block sympathoexcitation while leaving parasympathetic function and end-organ receptors in both limbs intact. Furthermore, stimulation of parasympathetic pathways in conditions ranging from heart failure, to aging, to hypertension may provide means to alter sympathovagal balance and reduce the risk of sudden cardiac death because chronic sympathoexcitation and vagal withdrawal have been associated with an increased risk of malignant arrhythmias (11, 13, 16). Discrimination of different subunits at each ganglion could provide a target for the development of agonists to stimulate parasympathetic function while blocking sympathetic activity. Perhaps more exciting is the possibility that different organ targets utilize different subsets of nAChR subunits in efferent pathways. This would enable one to stimulate parasympathetic function to the gastrointestinal tract to increase motility while not affecting HR. Such differentiation of ganglionic nAChR is completely theoretical at this stage; however, the diversity of nAChR subunits and their dramatic plasticity in response to a plethora of cellular signals makes such a concept feasible and worthy of further investigation.

GRANTS

This study was supported by a Department of Veterans Affairs Merit Review Grant (to M. E. Dunlap) and a University of Milan FIRST 2002 grant (to N. Montano).
REFERENCES


