Nicotine-induced Up-regulation and Desensitization of α4β2 Neuronal Nicotinic Receptors Depend on Subunit Ratio*

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Desensitization induced by chronic nicotine exposure has been hypothesized to trigger the up-regulation of the α4β2 neuronal nicotinic acetylcholine receptor (nAChR) in the central nervous system. We studied the effect of acute and chronic nicotine exposure on the desensitization and up-regulation of different α4β2 subunit ratios (1α:4β, 2α:3β, and 4α:1β) expressed in Xenopus oocytes. The presence of α4 subunit in the oocyte plasmatic membrane increased linearly with the amount of α4 mRNA injected. nAChR function and expression were assessed during acute and after chronic nicotine exposure using a two-electrode voltage clamp and whole-mount immunofluorescence assay along with confocal imaging for the detection of the α4 subunit. The 2α:3β:2 subunit ratio displayed the highest ACh sensitivity. Nicotine dose-response curves for the 1α:4β:2 and 2α:3β:2 subunit ratios displayed a biphasic behavior at concentrations ranging from 0.1 to 300 μM. A biphasic curve for 4α:1β:2 was obtained at nicotine concentrations higher than 300 μM. The 1α:4β:2 subunit ratio exhibited the lowest ACh and nicotine-induced macroscopic current, whereas 4α:1β:2 presented the largest currents at all agonist concentrations tested. Desensitization by acute nicotine exposure was more evident as the ratio of β2:α4 subunits increased. All three α4β2 subunit ratios displayed a reduced state of activation after chronic nicotine exposure. Chronic nicotine-induced up-regulation was obvious only for the 2α:3β:2 subunit ratio; ACh, acetylcholine; ANOVA, analysis of variance.

Neuronal nicotinic acetylcholine receptors (nAChRs) belong to a superfamily of ligand-gated ion channels (e.g. γ-aminobutyric acid, glutamate, 5-hydroxytryptamine, among others) and play an important role in modulating neurotransmitter release in distinct areas of the central and peripheral nervous system (1–5). Nicotine is the active ingredient of tobacco and specifically binds to nAChRs in the brain (3). One of the most remarkable effects of chronic nicotine exposure is the up-regulation of the α4β2 subtype in the central nervous system (6–10). Another important effect of chronic nicotine exposure is the long lasting functional deactivation of nAChR receptor (11–17). Chronic nicotine exposure produces a loss of nicotinic functional activity as a result of rapid and persistent desensitization (11, 18–20). Desensitization induced by chronic exposure to nicotine has been hypothesized to trigger the up-regulation of the α4β2 nAChR (3, 6, 21–23). The effect of chronic nicotine exposure on the activity of nAChR subtypes may be related to symptoms associated with nicotine addiction (3, 24, 25) such as tolerance, dependence, and withdrawal. In contrast to the aforementioned studies, a recent work suggests that the α4β2 subtype expressed in the stable cell line K-177 functionally up-regulates with chronic nicotine exposure (26). The molecular mechanisms that underlie a possible link between desensitization, deactivation, and up-regulation of the α4β2 subtype induced by chronic nicotine exposure remain unclear.

Previous studies have proposed that the subunit stoichiometry of the α4β2 expressed in oocytes is (α4)2(β2)2 (27, 28). Various functional studies, however, suggest that (α4)2(β2)2 is not the only stoichiometry present in cells expressing this nAChR subtype. For instance, patch clamp recordings from oocytes expressing the α4β2 nAChR have demonstrated that single channel conductance depends on the α:β ratio of the mRNA injected into the oocyte (29). When the relative levels of expression of the α4 and β2 were varied by nuclear injection of three α:β ratios into Xenopus oocytes, different sensitivities to acetylcholine and d-tubocurarine were obtained using voltage clamp recording (30). These results suggest that the subunit stoichiometry of functional heteromeric α4β2 nAChRs is not limited to (α4)2(β2)2. Furthermore, a recent work by Nelson et al. (31) reported two functional types of α4β2 nAChRs expressed in human embryonic kidney cells. These investigators found that the predominant subunit stoichiometry of α4β2 nAChRs expressed in human embryonic kidney cells was (α4)2(β2)2, yet overnight nicotine exposure increased the proportion of nAChRs with a (α4)2(β2)2 stoichiometry.

In this study, we examine the effect of acute and chronic exposure to nicotine on the desensitization and up-regulation of different subunit ratios of α4β2 expressed in Xenopus oocytes. The desensitization and up-regulation of three subunit ratios of α4β2 nAChR were assessed using a two-electrode voltage clamp in Xenopus oocytes, and whole-mount immunofluorescence assay and confocal imaging were used for surface detec-
tion of α4 neuronal subunits. Our data suggest that different α4β2 subunit ratios expressed in Xenopus oocytes can produce differential rates of desensitization and up-regulation, thus implying that subunit assembly of this nAChR is critical in these two processes.

EXPERIMENTAL PROCEDURES

In Vitro Synthesis of mRNA and Oocyte Microinjection—Subunit mRNA was synthesized in vitro from linearized pGEMHE plasmid templates of Rattus norvegicus cDNA coding for α4 and β2 nAChR subunits using the mMessage mMachine RNA transcription kit (Ambion, Austin, TX). mRNA mixtures of α4 and β2 subunits were prepared at a 3:2 or 2:3 ratio, and one batch of each different ratio was microinjected using a displacement injector (Drummond Instruments, Broomhall, PA) into stage V and VI oocytes that had been extracted, incubated in collagenase Type 1A (Sigma), and defolliculated by manual dissection. The injected oocytes were incubated at 19 °C for 3 days in 0.5× Leibovitz’s L-15 medium (Invitrogen) supplemented with 400 μg/ml bovine serum albumin, 119 mg/ml penicillin, 290 mg/ml streptomycin, and 110 mg/ml pyruvic acid. Electrophysiological experiments were performed after the third day of mRNA injection.

Membrane Isolation from Xenopus Oocytes for Immunoblot Assay—Four batches of oocytes (~30–40 oocytes/batch) were used for each immunoblot assay. Three batches were microinjected with different α4 and β2 subunit ratios, and one batch that was not microinjected served as a control. The number of oocytes was kept constant for each experiment. Membrane isolation was performed 3 days after microinjection as described by Ohlsson et al. (32) but with minor modifications. Briefly, a two-phase sucrose gradient was prepared with 1.5 and 0.3 M sucrose buffers (20 mM Tris, pH 7.6, 137 mM NaCl, and 0.1% Tween 20). Membranes were microinjected with the mMessage mMachine RNA transcription kit (Ambion Software, San Diego, CA). mRNA mixtures of neuronal subunits using the mMessage mMachine RNA transcription kit (Ambion Software, San Diego, CA). mRNA mixtures of neuronal subunits using the mMessage mMachine RNA transcription kit (Am-
disappearance of the band produced by the blocking peptide (data not shown). The results for three independent experiments were normalized with respect to the 1α4:4β2 subunit ratio (left lane in Fig. 1A). The mean and S.E. of the optical density ratios were 1.0 ± 0.0, 1.8 ± 0.3, and 3.7 ± 0.9 for 1α4:4β2, 2α4:3β2, and 4α4:1β2, respectively. Fig. 1B shows that the optical density magnitudes increased linearly in proportion to the ratio of α4:β2 injected into oocytes. A one-way ANOVA with a Bonferroni post-test showed that the percent values of the normalized optical densities of the three subunit ratios were significantly different (Fig. 1C). A 0.92 ± 0.04 slope ($R^2 = 0.998$) was obtained from linear regression analysis, indicating that the presence of the α4 subunit in the plasmatic membranes increased linearly with the amount of the α4 mRNA subunit injected into the oocytes.

**Subunit Ratio and Nicotine-induced Desensitization**—Fig. 2 illustrates the effect of acute nicotine exposure on the response to six consecutive applications of 300 μM ACh to oocytes injected with three α4β2 mRNA subunit ratios (1α4:β2, 2α4:3β, or 4α4:1β). For the experimental conditions, nicotine (0.3, 1.0, or 10.0 μM) was acutely applied between each ACh application as described under “Experimental Procedures.” Fig. 2A (from left to right) illustrates representative current traces of control experiments with nicotine-free washes between ACh pulses for the three subunit ratios. In the absence of acute nicotine, ACh does not produce a significant current loss in the 1α4:4β2 and 4α4:1β2 subunit ratios; however, in the 2α4:3β2 subunit ratio, ACh produces a mild desensitization (30% of current lost) after six consecutive ACh applications (Fig. 2F). Fig. 2, B–D, depicts the results of acute nicotine exposure on the ACh-induced currents. The nicotine concentrations used were 0.3 (B), 1.0 (C) and 10 μM nicotine (D). At all nicotine concentrations tested, 1α4:4β2 (green) displayed the largest progressive loss in macroscopic current during acute nicotine exposure, whereas 4α4:1β2 (red) displayed the lowest loss of macroscopic current (Fig. 2, E and F). For example, at 1.0 μM acute nicotine exposure the percentages of current lost from their original macroscopic current were 72% for 1α4:4β2 (green), 66% for 2α4:3β2 (blue), and 51% for 4α4:1β2 (red) (Fig. 2F). The mean values for the amount of current lost after acute nicotine exposure using 1.0 μM nicotine were significantly different ($p < 0.05$) for the three α4β2 subunit ratios. Fig. 2, E and F, demonstrate that the...
ACh-induced response decreases as the nicotine concentration increases. The current loss during acute exposure was probably caused by nAChR desensitization.

The amount of recovery from acute exposure (see “Experimental Procedures”) was measured for the three subunit ratios at each nicotine concentration (Fig. 2G). One-way ANOVA showed that the means of the amount of current recovered after acute nicotine exposure were not significantly different between subunit ratios. After acute exposure to 1.0 μM nicotine, the percentages of current recovered were 94 ± 10, 87 ± 25, and 102 ± 12 for 1α:4β2, 2α:3β2, and 4α:1β2, respectively. Statistical analysis showed that the mean values for recovered current were not significantly different between subunit ratios. Therefore, these data suggest that the percent recovery from acute nicotine exposure is independent of subunit ratio.

Activation Properties of the Different α4β2 Subunit Ratios before Chronic Nicotine Incubation—The activation properties of oocytes expressing different α4β2 subunit ratios were evaluated using two-electrode voltage clamp recording. To this end, we measured the current response to seven ACh and nicotine concentrations. In all oocytes tested, the 1α:4β2 subunit ratio showed the lowest ACh (Fig. 3A, left traces) and nicotine-induced macroscopic currents (Fig. 3B, left traces). In contrast, 4α:1β2 presented the largest currents at all agonist concentrations tested. The average ACh- and nicotine-induced macroscopic peak currents before chronic nicotine incubation are presented in Fig. 3C. One-way ANOVA (Bonferroni post-test) results demonstrate that the average macroscopic peak currents for the three subunit ratios studied are significantly different, with 4α:1β2 being the subunit ratio displaying the largest ACh- and nicotine-induced responses. The corresponding dose-response curves for ACh and nicotine are shown in Fig. 4 in blue. The EC50 values obtained for ACh and nicotine are very consistent with those reported previously for the wild type receptor (33). ACh dose-response curves were fitted using a single Hill equation and a two-component Hill equation. A better fit was obtained using the two-component equation for the 4α:1β2 subunit ratio (Fig. 4, top panel). The ACh EC50
values determined for 1α4:4β2 and 2α4:3β2 subunit ratios were 1.4 ± 1.1 μM and 0.9 ± 1.5 μM, respectively (Table I, see Controls). The estimated ACh EC50 values for the 4α4:1β2 subunit ratio were 4.0 ± 5.2 and 52.2 ± 0.2 μM (Table I, see Controls). The ACh EC50 value for the 4α4:1β2 subunit ratio was significantly different from the other two subunit ratios (p = 0.0001). The dose-response curves for nicotine showed a bell-shaped profile for the 1α4:4β2 and 2α4:3β2 subunit ratios at nicotine concentrations ranging from 0.1 to 300 μM (Fig. 4, lower panel in blue); however, the 4α4:1β2 did not exhibit a biphasic response at these concentrations. 4α4:1β2 exhibited a biphasic behavior when the nicotine concentration was increased to 3 mM (Fig. 4, panel insert). One-way ANOVA analysis showed no significant difference among the mean EC50 values for nicotine for the three subunit ratios (Table I). No significant difference in the Hill slope was observed between the three subunit ratios when using either ACh or nicotine as agonists (Table I, see Controls).

**Chronic Nicotine Exposure and Activation of Different α4β2 Subunit Ratios**—To evaluate the effect of chronic nicotine exposure on the functional activation of oocytes expressing different α4β2 subunit ratios, their response to several concentrations of ACh and nicotine (0.1–300 μM) were measured after 24 h of incubation in 1.0 μM nicotine (Fig. 3, A and B, right panel).
traces). After chronic nicotine treatment all three subunit ratios exhibited a reduced state of activation as evidenced by a significant reduction in their respective peak currents (Table I). Furthermore, after chronic nicotine exposure the 2α4:3β2 subunit ratio displayed a significant increase in the EC_{50} value for ACh, whereas 1α4:4β2 and 4α4:1β2 subunit ratios showed a significant increase in the nicotine EC_{50} values (Fig. 4 and Table I). The 2α4:3β2 subunit ratio was the only one that displayed a statistically significant reduction in the Hill coefficient after chronic exposure to nicotine.

### Table I

<table>
<thead>
<tr>
<th>Subunit ratio</th>
<th>1α4:4β2</th>
<th>2α4:3β2</th>
<th>4α4:1β2</th>
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<tr>
<td>Peak current at 300 μM ACh (nA ± S.E.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.4 ± 1</td>
<td>0.9 ± 1.5*</td>
<td>4.0 ± 5.2</td>
</tr>
<tr>
<td>1 μM nicotine incubation</td>
<td>2.7 ± 1.6</td>
<td>4.1 ± 1.3*</td>
<td>7.4 ± 12.3</td>
</tr>
<tr>
<td>EC_{50} ACh (μM ± S.E.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.3 ± 1.4*</td>
<td>2.6 ± 1.3</td>
<td>5.6 ± 1.5</td>
</tr>
<tr>
<td>1 μM nicotine incubation</td>
<td>13.3 ± 1.1*</td>
<td>5.6 ± 1.5</td>
<td>0.5 ± 2.0</td>
</tr>
<tr>
<td>Hill slope ACh</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.3</td>
<td>0.7 ± 3.3</td>
</tr>
<tr>
<td>1 μM nicotine incubation</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.7 ± 3.3</td>
</tr>
<tr>
<td>Hill slope nicotine</td>
<td>1.6 ± 0.7</td>
<td>1.5 ± 0.7*</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>1 μM nicotine incubation</td>
<td>1.4 ± 0.2</td>
<td>1.1 ± 0.4*</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

* indicates p < 0.05, n indicates the number of oocytes tested, 300 μM ACh and 30 μM nicotine were used to estimate peak currents.

#### Fig. 4

Dependence of the functional response to ACh and nicotine on the mRNA subunit ratio injected into Xenopus oocytes. Agonist-induced responses were normalized to the maximum response (I/I_{max}). Top panels show the dose-response relationships using ACh as agonist for each subunit ratio, and the nicotine dose-response relationships are presented in the bottom panels (0.1, 1, 3, 10, 30, 100, and 300 μM for each agonist). ACh dose-response curves were fitted using a single Hill equation and a two-component Hill equation. A better fit was obtained using the two-component equation for the 4α4:1β2 subunit ratio. The inset in the ACh dose-response for 4α4:1β2 (top panel) corresponds to the one-component fitting. Nicotine dose-response demonstrates a biphasic behavior for 1α4:4β2 and 2α4:3β2 using 0.1, 1, 3, 10, 30, 100, and 300 μM concentrations. 4α4:1β2 exhibited a biphasic behavior when the nicotine concentration was increased to 3 mM (lower panel inset). Data from control oocytes (in the absence of chronic nicotine incubation) are shown in blue, whereas the results after 24 h 1.0 μM nicotine incubation are shown in red. Dose-response curves for both agonists represent the average and standard error of the mean for 6–11 oocytes.

#### α4β2 Up-regulation and Subunit Ratio

Whole-mount immunofluorescence assays for the detection of surface α4 subunit and confocal imaging were performed to determine the effect of chronic nicotine exposure on the up-regulation of oocytes injected with different α4β2 subunit mRNA ratios. Oocytes were paired according to their similar current profile (i.e. no more than a 400-nA difference); oocytes incubated for 24 h with 1.0 μM nicotine were compared with oocytes without nicotine exposure. Fig. 5 illustrates representative confocal sections of immunolabeled α4 subunit on the surface membranes

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* indicates p < 0.05, n indicates the number of oocytes tested, 300 μM ACh and 30 μM nicotine were used to estimate peak currents.
Up-regulation of the α4β2 Nicotinic Receptor, Subunit Ratios

Fig. 5. Confocal images of the immunofluorescence surface detection of the α4 subunit after chronic nicotine exposure. A, fluorescence intensity of oocytes injected with 1α4:4β2 subunit ratio (Gain 840). The initial responses at 300 μM ACh for the incubated and non-incubated oocytes were 228 nA and 190 nA, respectively. B, fluorescence intensity of oocytes injected with 2α4:3β2 ratio (Gain 840). The initial response at 300 μM ACh for the incubated oocyte was 3333 nA and 2834 nA for the non-incubated oocyte. C, fluorescence intensity of oocytes injected with 4α4:1β2 ratio (Gain 840). The initial responses at 300 μM ACh for the incubated and non-incubated oocytes were 2240 nA and 3027 nA, respectively. D, fluorescence intensity of oocyte not injected with any α4/β2 subunit ratio but treated with primary and secondary antibodies (left). Oocyte injected with 1α4:4β2 (160 nA) but not treated with primary antibody (right). E, the average n-fold changes are presented as bar graphs for each α4/β2 subunit ratio (1α4:4β2 in green, 2α4:3β2 in blue, and 4α4:1β2 in red). *, p < 0.05.
and their corresponding fluorescence intensity profiles for each of the three subunit ratios studied. The total fluorescence intensity for each condition was calculated as explained under “Experimental Procedures.” Table II shows the $\alpha_4$ fluorescence intensity originating from the plasma membrane of oocytes before and after chronic nicotine exposure for the three subunit ratios. The $n$-fold values presented in Table II correspond to the intensity after chronic nicotine exposure divided by the intensity before chronic nicotine exposure. $n$-fold values did not reveal a consistent increase or decrease for the $1\alpha_4:2\beta_2$ and $4\alpha_4:1\beta_2$ subunit ratios, thus indicating that there was no obvious change in the fluorescence intensity of the $\alpha_4$ subunit before and after chronic nicotine exposure (Fig. 5, A and C, respectively). A plot of the average $n$-fold values for the three subunit ratios studied is shown in Fig. 5E. The average $n$-fold values were $1.0 \pm 0.2$ and $1.1 \pm 0.1$ for the $1\alpha_4:2\beta_2$ and $4\alpha_4:1\beta_2$ subunit ratios, respectively. In contrast, the average $n$-fold value for the $2\alpha_4:3\beta_2$ subunit ratio of $2.1 \pm 0.4$ was significantly different from the other two subunit ratios ($p = 0.0119$). This significant increase in the average $n$-fold was consistent with the robust increment in the fluorescence intensity observed for the $\alpha_4$ subunit (Fig. 5B, right). These results indicate that $2\alpha_4:3\beta_2$ was the only subunit ratio that clearly up-regulated after chronic nicotine exposure and suggest that the subunit ratio of $2\alpha_4:2\beta_2$ nAChR is critical for the nicotine-induced up-regulation.

### DISCUSSION

The results presented here demonstrate that the functional state of the $\alpha_4\beta_2$ nAChR in the surface membrane of Xenopus oocytes can be manipulated by injecting different subunit ratios of mRNAs encoding for these two nAChR subunits. Our data are consistent with previous studies (29–31) and clearly demonstrate that the functional properties of the $\alpha_4\beta_2$ neuronal nAChR depend on the heteropentamer subunit ratio.

#### Functional and Structural Implications of Subunit Ratios

The optical density data presented in Fig. 1 indicate that the proportion of a subunit in the oocyte membrane increases as the ratio of $\alpha/\beta$ injected increases. In addition, the electrophysiological data shown establish a marked difference in function for each of the subunit ratios studied. It is thus reasonable to hypothesize that functional heteropentamers of different stoichiometries, $4\alpha_4:1\beta_2$, $3\alpha_4:2\beta_2$, $2\alpha_4:3\beta_2$, and $1\alpha_4:4\beta_2$, were assembled in the plasma membrane of oocytes injected with different $\alpha/\beta$ subunit ratios. Moreover, it is possible that a heterogeneous population of the previously mentioned stoichiometries is present on any given oocyte injected with either of the subunit ratios. Although we recognize that such a plausible scenario based on the increase in $\alpha_4\beta_2$ on the oocyte plasma membrane as a function of $\alpha_4$ subunit injected and the distinct electrophysiological data for each subunit ratio injected, we hypothesize that at least one $\alpha_4\beta_2$ stoichiometry is favored for each of the subunit ratios injected.

It has been proposed that $(\alpha_4)^2(\beta_2)_2$ is the predominant stoichiometry in the central nervous system (27, 28). Previous studies have shown that $\alpha_4$ expressed alone does not form active nAChR channels (30); therefore, functional heteropentamer $\alpha_4\beta_2$ nAChRs presumably contain at least two agonist binding sites and are likely to be located at the $\alpha_4:2\beta_2$ subunit interface. For these putative heteropentamers, the number of agonist binding sites (i.e., $\alpha_4:2\beta_2$ subunit interfaces) may control the degree of ion channel activation. We found that the amount of ACh-induced macroscopic current increased proportional to the amount of $\alpha_4$ subunit detected in the plasma membrane of the oocyte. This result was consistent for the $1\alpha_4:4\beta_2$ and $2\alpha_4:3\beta_2$ subunit ratios given that the corresponding hypothetical numbers of symmetric agonist binding sites ($\alpha_4:2\beta_2$ sites) are 1 and 2, respectively, and their corresponding peak currents are $211 \pm 28$ nA and $522 \pm 99$ nA. It is noteworthy that the $4\alpha_4:1\beta_2$ subunit ratio displayed the largest macroscopic current and is the only subunit ratio displaying two EC$_{50}$ values for ACh (4.0 and 52.2 $\mu$M). These results lead to the hypothesis that the large currents observed for $4\alpha_4:1\beta_2$ could be caused by an increase in the fraction of functional receptors or a larger unitary conductance when compared with the other subunit ratios. Interestingly, the EC$_{50}$ value for nicotine activation was not significantly affected as the amount of $\alpha_4$ subunit was increased, yet there was a significant increase in the peak currents as a function of $\alpha_4$ subunit. These results suggest that the properties of agonist binding for $\alpha_4\beta_2$ channel activation might have distinct dynamics or perhaps structural requirements for ACh and nicotine. In contrast to the nicotine-induced activation of the $\alpha_4\beta_2$ nAChR, which appears to be independent of the subunit ratio expressed on the oocyte surface, desensitization was remarkably affected by acute nicotine exposure. The aforementioned results suggest that activation and desensitization of the $\alpha_4\beta_2$ nAChR by nicotine could be triggered by two independent mechanisms, which in turn suggest the possibility of at least two distinct binding sites for nicotine.

ACh produced a small activation of $1\alpha_4:4\beta_2$ subunit ratio; this is consistent with the presence of a predominant stoichiometry $(\alpha_4)(\beta_2)_4$ or a problem of receptor assembly. It is note-
worthy that the dose-response curve for nicotine shows a biphasic profile for the 1:4:2 subunit ratio, whereas higher nicotine concentrations resulted in a monophasic curve for the 2:2 subunit ratio. In contrast, the proposed model is consistent with a previous study (14). This previous study suggested that the subunit ratio is critical for the nicotine-induced activation of the neuronal α4β2 nAChR.

Subunit Ratio Versus Desensitization and Chronic Up-regulation

Desensitization of the α4β2 nAChR induced by acute exposure to nicotine (0.3, 1.0, or 10 μM) increased as the ratio of the α2/α4 subunit increased in the oocyte surface membrane. These results are consistent with previous work suggesting that the α2 subunit controls the desensitization of the α4β2 nAChR (14). In theory, if the proportion of subunits expressed in the surface membrane is linearly proportional to the most abundant stoichiometry, only the 1:4:2 and 4:3:6β2 subunit ratios will contain both α4-β2 and β2-β2 subunit contacts. Along the same theoretical assumption, the 4:1:2β2 subunit ratio, which produced the largest macroscopic currents, would not contain β2-β2 contacts but only one α4-β2 contact. The present data are consistent with these theoretical assumptions given that the 1:4:2β2 and 4:3:6β2 subunit ratios, which have β2-β2 contacts, displayed a remarkable nicotine-induced desensitization (Fig. 2E) and a distinct biphasic dose-response curve for nicotine. In contrast, the 4:1:2β2 subunit ratio shows a biphasic dose-response curve for nicotine at higher nicotine concentrations and displays a remarkable resistance to desensitization. Based on these results we hypothesize that intersubunit contacts involving β2 subunits control the allosteric interactions, which govern the conformational transition to the desensitized state. In theory, this biphasic profile could be consistent with the presence of two different binding sites for nicotine: one activation site and a second binding site promoting receptor desensitization. In this theoretical model, the site responsible for nicotine-induced activation of the α4β2 nAChR has different dynamics and/or structural requirements from the site that promotes receptor desensitization. Nevertheless, more experimental data are needed to further test this theoretical model.

After chronic nicotine exposure there was a loss in function in all three subunit ratios, shown by the decrease in peak current and the shift of EC50 values toward higher agonist concentrations. The most significant increase in EC50 for ACh was observed in oocytes expressing 2:2 subunit ratio, whereas the 4:1:2β2 and 4:4:1β2 subunit ratios showed a significant increase in their EC50 values (Table 1). Remarkably, only oocytes expressing the 2:2 subunit ratio clearly up-regulated after chronic nicotine exposure (Fig. 5), suggesting that the subunit ratio is critical for the nicotine-induced up-regulation of this neuronal nAChR.

The results presented here clearly indicate that the functional state of the α4β2 nAChR is regulated by subunit ratio. Previous studies addressing α4β2 up-regulation did not consider the importance of subunit stoichiometry (10, 13, 16, 22, 26, 35–37). Our results also suggest that nicotine-induced desensitization and up-regulation of this neuronal nAChR appears to be remarkably defined by very specific interactions between the neuronal nicotinic subunits. Given that the α4β2 nAChR is widely spread in brain presynaptic terminals, it is reasonable to consider that control of the subunit ratios of these heteropentamer receptors could regulate neurotransmitter release in the central nervous system and nicotine sensitivity in humans.

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REFERENCES

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