Effects of Ginsenoside Rg2 on Human Neuronal Nicotinic Acetylcholine Receptors

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ABSTRACT
Ginseng saponins, major active components of ginseng root used by folk medicine in the treatment of various diseases, produce multiple pharmacological responses having many effects on the central and peripheral nervous system. Specifically, ginsenoside Rg2 has been shown to block the nicotinic acetylcholine receptors in bovine chromaffin cells. We have studied the effect of Rg2 on different types of human neuronal nicotinic acetylcholine receptors (nAChRs), both homomeric and heteromeric, expressed in Xenopus oocytes. Rg2 did not affect the acetylcholine (ACh)-induced currents in α7 human receptors, however Rg2 affected the peak currents, and mainly the desensitization of heteromeric receptors α3β4, α3β2, α4β2, and α4β2. Both effects, a diminution of peak current and an increase of desensitization, are dose-dependent and are very similar for all the receptors. The mechanism of action has been studied in more detail in α3β2 and α3β2 receptors where we found a negligible shift in the ACh dose-response curves and a persistence of the Rg2 effects at high ACh concentrations, indicative of a noncompetitive antagonism. A lack of voltage dependence on the reduction of the peak currents induced by ACh also suggests that Rg2 does not act as an open channel blocker of human nAChR. The results indicate that Rg2 acts specifically on heteromeric human nAChRs modulating their desensitization and suggest a possible mechanism by which this saponin contributes to the multiple therapeutic effects of ginseng.

Ginseng root, from Panax ginseng, has long been used by folk medicine in the treatment of various diseases. Ginseng’s effects include improved mental performance, learning, and memory and sensory awareness. Ginsenosides, the major active components of ginseng, are a diverse group of steroidal saponins that produce multiple pharmacological responses (Attele et al., 1999).

Ginsenosides have many behavioral effects. They have protective effects on scopolamine-induced memory impairment (Li et al., 1999), reduce aggression in mice (Yoshimura et al., 1988), attenuate anorexia (Fujimoto et al., 1989), inhibit nicotine-induced hyperactivity (Kim and Kim, 1999), and contribute to antinociception (Nah et al., 2000).

On the other hand, neuronal nicotinic receptors have been involved in many brain functions (Jones et al., 1999; Patserson and Nordberg, 2000), including synaptic function and plasticity (Albuquerque et al., 2000; Ji et al., 2001) and several physiopathological processes, including aging and dementia (Perry et al., 2001), schizophrenia (Breese et al., 2000), tobacco dependence (Picciotto et al., 1998), and neurological diseases associated with increased incidence of smoking (Leonard and Bertrand, 2001). Furthermore, there is a substantial interest in developing subtype-selective agonists and antagonists that may have potential as therapeutic agents (Gotti et al., 2000; Dwoskin and Crooks, 2001).

Ginsenosides can block many ionic channels. Ginseng extracts reversibly block α subunits of brain Na+ channels, and it has been suggested that this effect may contribute to its neuroprotective effect during ischemia (Liu et al., 2001). Ginsenosides also have been shown to reduce Ca2+ currents in chromaffin cells (Kim et al., 1998) and sensory neurons (Nah et al., 1995). Recently, it has also been shown that Ginseng saponins activate Ca2+-dependent Cl− currents in Xenopus oocytes (Choi et al., 2001). Ginsenoside Rg2, in particular, reduces the acetylcholine-evoked catecholamine release from bovine adrenal chromaffin cells (Tachikawa et al., 1995). This effect is produced by blocking the nicotinic ACh recep-
tors. Furthermore, it has also been suggested that ginsenosides at higher concentrations modulate the fluidity of the plasma membrane, which might contribute to the observed reduction in the secretion of catecholamines (Tachikawa et al., 2001).

In this work we have studied the effect of ginsenoside Rg2 on the function of different types of human neuronal nicotinic acetylcholine receptors, both homomeric and heteromeric, expressed in Xenopus oocytes.

Although Rg2 did not affect the ACh-induced currents in α7 receptors, it reduced slightly the peak currents, and increased the desensitization of heteromeric receptors α2β4, α3β2, α4β4, and α4β2. Both effects, the diminution of peak current and the increase of desensitization rate, are shown to be dose-dependent. We also have investigated the mechanism of action, by studying the effect on dose-response and current-voltage curves for α2β4 and α4β2 receptors. The results suggest that the ginsenoside Rg2 blocks the ACh-induced ionic currents by means of a noncompetitive mechanism.

Materials and Methods

Oocyte Expression. cDNAs of human neuronal nicotinic acetylcholine receptor subunits (α, α, α, β, and β) were inserted into the pSP64T vector (Krieg and Melton, 1984). Capped mRNA was synthesized in vitro using SP6 RNA polymerase. Defolliculated Xenopus oocytes were injected with 5 ng of total RNA in 50 nl of sterile water. The two subunits of heteromeric receptors were injected in an equimolar ratio. All measurements were made within 3 to 6 days after injection.

Electrophysiological Recordings. Two-electrode voltage-clamp recordings were obtained as described previously (Stühmer, 1998) using a standard two-electrode voltage-clamp amplifier (Oocyte Clamp OC-725B; Warner Instrument, Hamden, CT). Oocytes were perfused (10–12 ml/min) with a modified frog Ringer’s solution containing 82.5 mM NaCl, 2.5 mM KCl, 2.5 mM BaCl2, 1 mM MgCl2, and 5 mM HEPES, pH 7.4. With barium as the permeant divalent cation no calcium-activated chloride currents were detected, because no significant difference was found when BAPTA was injected into the oocytes. Holding potential was −80 mV unless otherwise indicated. Currents were filtered at 50 Hz with a low pass eight-pole Bessel filter (Frequency Devices, Haverhill, MA), sampled at 100 to 500 Hz and stored on hard disk for later analysis.

Low-resistance electrodes, filled with 2 M KCl, were used to get good voltage-clamp recordings, even with currents as large as 50 μA. The quality of the voltage clamp was always assessed by monitoring the output of the voltage electrode in an oscilloscope, and only the current records for which the voltage was completely flat were stored. Data acquisition and agonist application were controlled by a DigiData 1200 interface driven by pClamp 6.0 software (Axon Instruments, Union City, CA).

Rg2 powder, obtained from Korea Ginseng and Tobacco Research Institute (Taejon, Korea) with a purity greater than 98%, was dissolved in dimethyl sulfoxide as a stock solution of 200 mM. Solutions containing ACh were freshly prepared every day directly in the recording solution. Control experiments showed that dimethyl sulfoxide alone, at the levels present in the solutions containing Rg2 did not affect the ACh-induced currents.

Drug application was controlled by an electromechanical valve, by which the flow was fed under gravity through a 1-mm-diameter tube positioned very close to the oocyte. This system allowed a rapid application of the drugs, but in many cases, the impetus of the flow mechanically deformed the oocyte and gave rise to some irregularities in the current traces (Fig. 2). Solution exchange time, as assessed by changes in junction potentials of the voltage electrode in control current-clamp experiments, was in the range of 0.1 and 0.3 s (activation time measured as the time from 20 to 80% of the peak current in α2 receptors was less than 75 ms). However, our system did not allow us to achieve a fast removal of the applied drugs, which was probably due to the large volume, approximately 1 ml, of the recording chamber. This prevented us from being able to measure reliably the closing kinetics of the currents.

We usually applied the drugs only for 2 s because this period of time was usually enough to reach the peak current in control conditions even for the slower heteromeric receptors (Figs. 1 and 2). Also, for this time scale the desensitization of the heteromeric receptors, with the exception of αβ2 receptors, was very small or nonexistent, thus allowing to see more clearly the effect of Rg2 on the kinetics of the currents.

Analysis. Desensitization of currents for heteromeric receptors was quantified as the quotient of the current after 2 s of pulse and the peak current. Dose-response curves were fitted with the

![Fig. 1. Currents elicited by pulses of 1 mM ACh in human neuronal nicotinic receptors. The application bars indicate pulses of 2 s for α7 receptors and 10 s for heteromeric receptors. For the heteromeric receptors, two current records are shown. The current records on the left have a duration of 40 s, and the current records on the right show the initial part of the previous record at a time scale 10-fold smaller; their duration is 4 s, showing with more detail the kinetics of activation of the currents.](image-url)
Results

**Rg$_2$ Affects Ionic Currents through Heteromeric Human Nicotinic Acetylcholine Receptors.** Application of 1 mM ACh to −80 mV voltage-clamped Xenopus oocytes expressing human neuronal nicotinic acetylcholine receptors elicits inward currents of different magnitude and kinetics depending on the particular receptor (Fig. 1). Homomeric $\alpha_7$ receptors produce the fastest activating and inactivating currents. On the other hand, heteromeric $\alpha_3\beta_4$, $\alpha_4\beta_4$, $\alpha_2\beta_2$, and $\alpha_3\beta_2$ receptors produce slowly desensitizing currents. Although the rate in desensitization is variable, usually heteromeric $\alpha_3\beta_2$ receptors produce faster desensitizing currents. In a smaller time scale it can be seen that the currents reach their peak values usually before 2 s, and that, for the heteromeric receptors, 2 s after the application of ACh the currents have decayed very little. Therefore, we decided to use pulses of ACh of 2 s because this was a convenient short duration where the peak of the current could still be reached and, except for $\alpha_3\beta_2$, very little decay of the current had yet developed. On the other hand, recovery from desensitization was complete after 5 min of washing, and for that reason we always spaced by 5 min the application of agonist pulses.

Figure 2 shows currents elicited by 2-s pulses of 1 mM ACh. Coapplication of 100 $\mu$M Rg$_2$ did not produce any effect on the size or kinetics of the currents through $\alpha_7$ receptors. In contrast, the effect of Rg$_2$ on the magnitude of the peak currents through heteromeric receptors was rather small, but the degree of desensitization was increased. This last effect was especially evident on the currents of receptors that desensitize very slowly in control conditions, i.e., in $\alpha_3\beta_4$ and $\alpha_4\beta_2$ receptors. The effect was completely reversible, and after the usual 5-min washing period the control current was completely recovered. Similar results were obtained with nicotine (data not shown). Thus, after these first results we decided to investigate the effect of Rg$_2$ only on heteromeric receptors.

**Coapplication of Rg$_2$ Affects Mainly Desensitization of Receptors.** The statistics of the effect of coapplication of 100 $\mu$M Rg$_2$ on the peak current and desensitization proportion are shown in Fig. 2. Peak currents obtained in control conditions, i.e., 1 mM ACh and −80 mV membrane voltage, were measured 5 min before and 5 min after the coapplication of 1 mM ACh and 100 $\mu$M Rg$_2$, and the interpolated value of the peak control currents was used to normalize the peak current obtained in the presence of Rg$_2$ (Fig. 3A). Coapplication of Rg$_2$ has no effect on the peak currents of $\alpha_7$ receptors, and the effect on the peak currents of all heteromeric receptors, although statistically significant, is small, less than 30% reduction.

**Hill equation using nonlinear fitting routines in Origin 5.0 (Microcal Software, Inc., Northampton, MA). Averaged data are presented as means ± S.E.M. (number of cases). Statistical calculations were done with Statistica 5.0 (StatSoft, Tulsa, OK). Statistical significance is indicated by * (p < 0.05), ** (p < 0.01), or *** (p < 0.001).**

![Fig. 2. Chemical structure of Rg$_2$ and effect of 100 $\mu$M Rg$_2$ on ionic currents elicited by 1 mM ACh in different types of human neuronal nicotinic acetylcholine receptors. The structure shown is common for ginsenosides. The side chains for Rg$_2$ are R$_1$ and R$_2$, -OH, and R$_3$, -O-Glc$_2$-Rha, where abbreviations for carbohydrates are Glc for glucopyranoside and Rha for rhamnopyranoside. Currents were recorded under voltage-clamp conditions at −80 mV. Dashed lines are control currents during a 2-s pulse of 1 mM ACh; solid lines are currents in the presence of 100 $\mu$M Rg$_2$; and dotted lines are recovery control currents, 5 min after washing with the perfusion solution. The small discontinuity seen more clearly in the control records of $\alpha_3\beta_4$ and $\alpha_4\beta_2$ at the end of the pulse of ACh corresponds to an artifact due to the closing of the application valve.**

![Fig. 3. Effect of 100 $\mu$M Rg$_2$ on peak currents and desensitization for different receptors. A, average normalized peak currents in the presence of 100 $\mu$M Rg$_2$. Currents were normalized at the peak level obtained in control conditions: 1 mM ACh, membrane voltage held at −80 mV. B, average desensitization of the currents after 2 s of initiating the pulse of agonist. $I_{2,peak}/I_{2,peak}$ is the quotient between the current at the end of a 2-s duration pulse and the peak current. White columns correspond to control conditions, and black columns correspond to the coapplication of 100 $\mu$M Rg$_2$ and 1 mM ACh. Numbers in parentheses indicate number of different oocytes measured.](http://aspetjournals.org/atps/)
The desensitization of the heteromeric receptors, quantified as the quotient between the current level at the end of a 2-s pulse and the peak current, $I_{\text{des}}/I_{\text{pk}}$, is however strongly affected by the coapplication of Rg$_2$. In control conditions the currents of $\alpha_3\beta_4$ and $\alpha_4\beta_4$ hardly desensitize after 2 s (Fig. 2), and $I_{\text{des}}/I_{\text{pk}}$ is close to 93 and 82%, respectively (Fig. 3B). However the coapplication of Rg$_2$ increases the desensitization, giving $I_{\text{des}}/I_{\text{pk}}$ values close to 50 and 40%, respectively. $\alpha_4\beta_4$ receptors usually gave less steady control currents during the pulse (Fig. 2), but statistically the desensitization at the end of the pulse was rather small, with an $I_{\text{des}}/I_{\text{pk}}$ value close to 83% and the effect of Rg$_2$ on the desensitization of these receptors was very large, giving $I_{\text{des}}/I_{\text{pk}}$ close to 18%. Finally, the currents through $\alpha_4\beta_2$ receptors are the ones that usually desensitize faster under control conditions, and are also strongly affected by the coapplication of Rg$_2$, giving $I_{\text{des}}/I_{\text{pk}}$ close to 52 and 13%, respectively.

**Effect of Coapplication of Rg$_2$ on Desensitization Is Dose-Dependent.** Figure 4 shows dose-response curves for the effect of coapplication of Rg$_2$ on the desensitization of the different receptors. Data obtained at $-80$ or at $-40$ mV have been pooled. Data points have been fitted with the Hill equation with the $y_{\text{max}}$ fixed to the value of desensitization obtained in control conditions. Only $\alpha_4\beta_4$ receptors have IC$_{50}$ greater than 100 $\mu$M, the maximum concentration of Rg$_2$ measured, whereas the other receptors have IC$_{50}$ values around 20 or 40 $\mu$M. The Hill coefficients are less than 1 for all receptors except for $\alpha_3\beta_3$, the receptor that has the faster desensitization in control conditions. Insets in Fig. 4 show representative current traces for the kinetics of the currents with 1, 10, and 100 $\mu$M Rg$_2$ coapplication, where the dose dependence is more apparent.

**Preapplication of Rg$_2$ Decreases Peak Currents in a Dose-Dependent Manner.** The effect of coapplication of 100 $\mu$M Rg$_2$ with 1 mM ACh on the peak current, although significant, was small for all the receptors, thus the dose-response curves, measured with concentrations from 0.1 to 100 $\mu$M Rg$_2$ show little concentration dependence (Fig. 5, open circles, dotted lines). However, when Rg$_2$ was preapplied for 5 min before the pulse of agonist, a significant dose-dependent effect was observed, (Fig. 5, solid circles, solid lines). The dependence is not very steep, only $\alpha_4\beta_4$ receptors have an absolute value of the $n$ of Hill greater than 1. Preapplications of 5 min 100 $\mu$M Rg$_2$ produced a reduction of the peak current of more than 85% in $\alpha_4\beta_4$, $\alpha_3\beta_4$, and $\alpha_3\beta_2$ receptors, but only a reduction of about 40% in $\alpha_3\beta_2$ receptors. Insets in Fig. 5 show representative current traces for 5-min preapplication of Rg$_2$ at concentrations of 1, 10, and 100 $\mu$M, where the dose dependence is more clearly seen. Preapplication of 10 $\mu$M Rg$_2$ also produced a small decrease in the peak current of $\alpha_7$ receptors of approximately 10% (data not shown), compared with reductions of 15, 30, 50, and 60% for $\alpha_3\beta_4$, $\alpha_3\beta_4$, $\alpha_3\beta_2$, and $\alpha_3\beta_3$, respectively.

**Effect of Rg$_2$ on ACh Dose-Response Curves of $\alpha_4\beta_4$ and $\alpha_3\beta_2$ Receptors.** To further investigate the mechanism by which Rg$_2$ inhibits nAChRs we have chosen to pursue our study in more detail with $\alpha_3\beta_2$ and $\alpha_4\beta_4$ receptors, the most abundant in the central and peripheral nervous system, respectively.

We obtained ACh dose-response curves, measured at the peak of the currents, with and without coapplication of 10 $\mu$M Rg$_2$ for $\alpha_4\beta_2$ and $\alpha_3\beta_2$ receptors, as shown in Fig. 6, top. In these experiments, a smaller concentration of 10 $\mu$M Rg$_2$ was chosen instead of 100 $\mu$M because, as indicated in Fig. 5, for $\alpha_4\beta_2$ and $\alpha_3\beta_2$ receptors the same reduction of the peak current is obtained with either 10 or 100 $\mu$M Rg$_2$ coapplication. Dose-response data were fitted with the Hill equation with an inverse error weight-fitting routine. In both recep-

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**Fig. 4.** Dose-response curves of the effect of Rg$_2$ on the desensitization for heteromeric human nAChR. Rg$_2$, at the doses indicated was coapplied with 1 mM ACh. Membrane potential was held either at $-80$ or $-40$ mV. When differences with the control are significant, the number of cases in parentheses and the significance level are indicated. Solid lines through data points represent fits to the Hill equation with the $y_{\text{max}}$ fixed to the value $I_{\text{des}}/I_{\text{pk}}$ in control conditions. Fit parameters ($y_{\text{max}}$, IC$_{50}$, and $n_H$) are $\alpha_3\beta_4$ (0.90, 106 $\pm$ 78 $\mu$M, and $-0.65 \pm 0.30$), $\alpha_3\beta_4$ (0.54, 36 $\pm$ 9 $\mu$M, and $-1.13 \pm 0.28$), $\alpha_3\beta_4$ (0.85, 17 $\pm$ 4 $\mu$M, and $-0.80 \pm 0.13$), and $\alpha_3\beta_4$ (0.82, 34 $\pm$ 23 $\mu$M, and $-0.42 \pm 0.16$). Insets show representative current traces normalized to the peak value for 1, 10, and 100 $\mu$M Rg$_2$ coapplication.
tors, Rg2 coapplication produced a reduction of the maximum response of a little more than 10%, without affecting much neither the EC50 nor the nH.

For /H92514/H9252 receptors data points obtained at low concentrations of ACh suggest the presence of a high-affinity site (Covernton and Connolly, 2000), although not enough data were obtained to perform a two-site fit.

Effect of Rg2 on Desensitization at Different ACh Concentrations. The effect of coapplication of Rg2 on the desensitization of the currents was also dependent on the

Fig. 5. Dose-response curves of the effect of co- or preapplication of Rg2 on the peak currents for heteromeric human nAChR. Open circles: Rg2 at the doses indicated was coapplied with 1 mM ACh, points are connected with a dotted spline line. Solid circles: Rg2 at the doses indicated was preapplied for 5 min before the application of 1 mM ACh, when the normalized values are significantly different from one the number of cases in parentheses and the significance level are indicated. Solid lines are fits to the Hill equation with ymax fixed to 1. Fit parameters (IC50 and nH) are α,β4 (162 ± 138 μM and −67 ± 0.39), FoW2 (4.4 ± 2.7 μM and −0.46 ± 0.14), α,β2 (22 ± 12 μM and −1.3 ± 0.7), and α,β2 (3.9 ± 2.3 μM and −0.46 ± 0.13). Membrane potential was held either at −80 or −40 mV. Insets show representative current traces for 5 min, at 1, 10, and 100 μM Rg2 preapplication. The currents have been normalized to the peak value obtained in control conditions.

Fig. 6. Effect of Rg2 on dose-response curves of ACh for α,β2 and α,β4 receptors. Top, ACh dose-response curves for peak currents in the absence (○) or presence (●) of 10 μM Rg2. Data have been normalized to the 1 mM ACh concentration peak current. Lines are fits to the Hill equation with the following parameters (ymax, EC50, and nH): α,β2 control, dotted line (1.04 ± 0.05, 40 ± 11 μM, and 0.96 ± 0.28); coapplication of 10 μM Rg2, solid line (0.90 ± 0.18, 30 ± 19 μM, and 1.00 ± 0.67); α,β4 control, dotted line (1.04 ± 0.03, 76 ± 6 μM, and 1.51 ± 0.14); and coapplication of 10 μM Rg2, solid line (0.90 ± 0.05, 78 ± 12 μM, and 1.68 ± 0.34). Bottom, ACh dose-response curves for desensitization after 2 s in the absence (○, dotted spline line) or presence (solid circles, solid spline line) of 10 μM Rg2. For the values with Rg2 that are significantly different from the control the number of cases in parentheses and the significance level are indicated. Insets show representative current traces for 10 μM Rg2 coapplied with 100, 300, and 1000 μM ACh. The currents have been normalized to their peak values to compare the desensitization kinetics.
ACh concentration used to elicit the current as shown in Fig. 6, bottom. Although in control conditions no dependence of desensitization on the ACh concentration was seen, in the presence of 10 μM Rg2 the level of desensitization was almost constant for low concentrations of ACh, but then started to increase with the ACh concentration. Insets in Fig. 6 show representative currents for α4β2 and α4β2 receptors, normalized at their peak values to compare the kinetics, at three ACh concentrations, 100, 300, and 1000 μM with 10 μM Rg2 coapplication, where the concentration dependence of the desensitization is apparent.

**Voltage Dependence of Rg2 Effect.** To further investigate the mechanism of action of Rg2 we studied the voltage dependence of its effect both on the peak currents and on the desensitization. Figure 7, top, shows peak current-voltage relationships for α4β2 and α4β2 receptors obtained with 1 mM ACh, with or without 100 μM Rg2 coapplication. The effect of Rg2 coapplication is small, although the relative reduction of the peak current seems to be larger at more depolarized potentials. See also insets in Fig. 7 where representative records, obtained at −100 and +40 mV are shown for α4β2 and α4β2 receptors both in the absence and in the presence of Rg2 coapplication.

Figure 7, bottom, shows the voltage dependence of desensitization for α4β2 and α4β2 receptors both in control conditions and in the presence of 100 μM Rg2 coapplication. For α4β2 receptors the linear fit to the data points indicates that there is no significant voltage dependence, neither in control nor with Rg2 coapplication conditions, because in both cases the slope of the fitted straight lines is not statistically different from zero. For α4β2 receptors, however, the voltage dependence of desensitization can be fitted with a straight line with a very statistically significant nonzero slope, both in control (p < 0.05) and with Rg2 coapplication (p < 0.001) conditions. The desensitization increases at more depolarized potentials and this trend is increased by the presence of Rg2.

**Discussion**

In this work we have studied the effect of Rg2, a major active component of ginseng, on the function of human nAChR expressed in *Xenopus* oocytes.

The function of the receptors was assessed by measuring the ionic current elicited by ACh pulses in voltage-clamp conditions. The general characteristics of the human nAChR currents recorded in *Xenopus* oocytes, i.e., the size of the peak current and the kinetics of activation and desensitization measured in control conditions, were similar to those reported by Chavez-Noriega et al. (1997). With the short pulse used in this work, our current readings usually show complex kinetics. This is probably due to a mixture of perfusion artifacts and an irregular distribution of receptors on the oocyte surface, which result in nonsynchronized activation.

Coapplication of 100 μM Rg2 produced a small reduction of peak current in all the heteromeric receptors investigated but not in homomeric α4 receptors. A similar differential blockade by ω-conotoxin MVIIIC, ω-conotoxin GVIA, and diltiazem has been reported for rat α4β2 and α4 neuronal nicotinic receptors (Herrero et al., 1999).

The effect of Rg2 on the peak currents was small and not very dose-dependent when coapplied; however, the main effect of Rg2 coapplication was to increase the rate of decay of the currents of heteromeric receptors. This result could indicate an increase of the desensitization rate or could simply reflect the progression of the channel block by Rg2.

Desensitization is a general property of ligand-gated ion channels and it has been known that the subunit composition of heteromeric nAChRs determines the degree of desensitization. This has also been shown to be the case for human nAChRs (Gerzanich et al., 1998). The factors by which desensitization is modulated include receptor phosphorylation, calcium, and noncompetitive blockers (Ochoa et al., 1989), and although the molecular mechanism of desensitization is
not known it has been shown recently that some regions of the N-terminal domain of the β2 subunit determine the rate of desensitization (Bohler et al., 2001). The modulatory effect of Rg2 on desensitization was dose-dependent, and could not be easily related to the presence of the β2 subunit. Thus, although IC50 was smaller and nH was larger for αβ2 compared with α3β2, the reverse was true for α4β2 compared with α4β4.

A lack of dose dependence for the reduction of the peak currents together with a clear dose dependence of the value of desensitization could suggest that the mechanism of action of Rg2 could be similar to a slow open channel blocker, so that currents together with a clear dose dependence of the value of be easily related to the presence of the /H9252 concentration between 1 and 50 /H9251 can ginseng extract containing a mixture of ginsenosides at a lar range. These concentrations are similar to the EC50 value of Rg2 on the peak currents when coapplied, the reduction of (Buisson and Bertrand, 1998).

Contrasting with the lack of dose dependence of the effect of Rg2 on the peak currents when coapplied, the reduction of the peak currents was clearly dose-dependent when preincubated for 5 min, reaching 90% with 100 μM, the largest concentration applied. These results suggest that Rg2 is acting on resting state of the receptor, either stabilizing it or promoting desensitization from it. The receptors with the β2 subunit had smaller IC50 values than the corresponding receptors with the β4 subunits. However, the differences are small, and taking into account the statistical errors of the fit, those values overlap considerably.

On the other hand, the receptors with the β4 subunit also had more shallow inhibition curves with Hill coefficients less than 1. In our case, this cannot be due to heterogeneity in the receptor population, thus suggesting that more than one molecule of Rg2 binds to those receptors with negative cooperativity.

In general, the concentrations at which Rg2 affects these human neuronal nicotinic receptors was in the low micromolar range. These concentrations are similar to the EC50 value (around 30 μM) for the increase of Ca2+ activated Cl− currents in Xenopus oocytes by Rg2 (Choi et al., 2001). Also, the concentrations of Rg2 that reduce ACh-evoked Ca2+ and Na+ influx in bovine chromaffin cells are in the same range (Tachikawa et al., 1995). Other ginsenosides, such as Rf for example, also affect Ca2+ channels with an IC50 of 40 μM approximately (Nah et al., 1995). Similarly, 1 mg/ml American Ginseng extract containing a mixture of ginsenosides at a concentration between 1 and 50 μM also blocks brain Na+ channels.

We studied the mechanism by which Rg2 inhibits nAChRs, measuring the effect of 10 μM Rg2 on different concentrations of ACh for αβ2 and α3β2 receptors. Dose-response curves obtained in control conditions were similar to those reported previously (Chavez-Noriega et al., 1997) but, in control conditions the desensitization did not depend much on the agonist concentration. In both receptors, a negligible shift in the ACh dose-response curves was produced, the inhibition of the current was not relieved at high ACh concentrations, and the increase produced by Rg2 on desensitization was larger at high ACh concentrations. All these results suggest a noncompetitive mechanism with no subtype selectivity. The effect of the noncompetitive antagonists mecamylamine and d-tubocurarine, on agonist-stimulated intracellular calcium concentration elevations has also been shown to be nonselective between α2β4, α3β4, and α4β4 receptors. In contrast, the effect of the competitive antagonist dihydro-β-erythroidine was highest with αβ4 receptors expressed in human embryonic kidney 293 cells (Stauderman et al., 1998). Other drugs have an action mechanism dependent on the receptor type (Chiodini et al., 2001).

Finally, we investigated the possibility that Rg2 could act as an open channel blocker by measuring its effect on current-voltage relationships. Current-voltage relationships show a strong inward rectification for both receptors either in control conditions or in the presence of 100 μM Rg2. It has been shown that ginseng extracts and ginsenoside Rb1 block Na+ channels in a voltage-dependent manner (Liu et al., 2001). In contrast, Rg2 reduction of peak currents of αβ2, and α3β2 receptors does not show a substantial voltage dependence, thus suggesting that Rg2 does not act as an open channel blocker. On the other hand, we found that the voltage dependence of the desensitization, and its modification by Rg2 coapplication, were different for αβ2 and α3β2 receptors. Thus, although αβ2 did not show any appreciable voltage dependence of desensitization neither in control conditions nor in the presence of 100 μM Rg2, the currents of αβ4 receptors desensitized faster at more depolarized potentials and this dependence was increased by the presence of Rg2. This is different from the behavior of rat α3β2 receptors stably transfected in human embryonic kidney cells (Zhang et al., 1999) where the time constant of desensitization of nicotine-activated current was concentration-dependent, but not voltage-dependent. Unfortunately, we were unable to wash out the drugs fast enough to be able to resolve tail currents that would have provided information about the recovery from a possible open channel block.

In this work, we have used the term desensitization to indicate the reduction of the current during steady application of ACh, either alone or together with Rg2. No mechanistic implications were intended. It is possible that during coapplication of Rg2, the “intrinsite” mechanism of the desensitization of the receptors is proceeding normally, i.e., is not being affected by Rg2. However, the kinetics of onset of block by Rg2 produces an increase in the decay rate of the current that is seen as an “apparent” increase in desensitization rate. As discussed above, our data do not allow us to rule out this last possibility.

In conclusion, our results show that ginsenoside Rg2 has several effects on human heteromer NaAChR, principally altering its desensitization characteristics, while leaving unaffected homomeric α7 receptors. These differential inhibitory effects of one of the main components of ginseng could be one of the mechanisms of its many pharmacological and therapeutic properties.

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References


