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Partial Agonist Properties of Cytisine on Neuronal Nicotinic Receptors Containing the β2 Subunit

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SUMMARY

As previously reported by Luetje and Patrick [*J. Neurosci.* 11:837-845 (1991)], the nicotine-like alkaloid cytisine is relatively ineffective in evoking current responses from nicotinic receptors containing the β2 subunit. In our experiments, the responses of α4β2- and α3β2-injected oocytes to the application of 1 mM cytisine were only 14.7 ± 4% and 2.5 ± 0.8% of the responses to 1 mM acetylcholine (ACh), respectively. Concentration-response relationships for ACh were examined in the presence and absence of cytisine. Although cytisine was relatively ineffective in stimulating current, the coapplication of cytisine and ACh reduced the responses to ACh. For α4β2 receptors, 3 μM cytisine shifted the dose-response curve for ACh to the right, resulting in a 60-fold increase in the apparent EC_{50} for ACh. For α3β2 receptors, 30 μM cytisine shifted the apparent EC_{50} for ACh from ≈150 μM to 1 mM. Although the efficacy of cytisine for α3β2 receptors was very low, cytisine could effectively inhibit the responses of these receptors, with an IC_{50} of ≈10 μM. The efficacy of cytisine for α4β2 receptors was greater than that for α3β2 receptors, and it was possible to evaluate the partial agonist properties of cytisine for these receptors. Although the EC_{50} of cytisine for stimulating current through α4β2 receptors was about 1 μM, concentrations of cytisine as low as 20 nM were able to inhibit 50% of the response to 1 μM ACh. The inhibitory effects of cytisine were reversible over a period of 5 min. Our analysis suggests that cytisine is a true partial agonist for β2-containing ACh receptors and as such can inhibit the response of these receptors to ACh through a competitive mechanism. In the case of α4β2 receptors cytisine binds with high apparent affinity and low efficacy to a site shared with ACh, and for α3β2 receptors both the apparent affinity and efficacy of cytisine are relatively low.

The cloning in recent years of genes coding for neuronal nicotinic ACh receptor subunits (for review, see Ref. 1) has made possible new approaches to the study of nicotinic function in the nervous system. Immunohistochemical studies and analysis of the patterns of RNA expression using techniques such as *in situ* hybridization make it possible to suggest the existence of particular subunit combinations in particular tissues. The ectopic expression of cloned subunits in defined cell systems makes it possible to study the physiological properties of those specific subunit combinations.

To date, five α subunit genes and two β subunit genes have been cloned from the nervous system and shown to code for functional nicotinic ACh receptor subunits. Three of the neuronal α subunits (α2, α3, and α4), which have been cloned from both rats and chicks (2-5), form functional receptors in pairwise combinations with either of the neuronal β subunits (β2 or β4). We have shown that different subunit combinations give rise to receptors with unique single-channel properties (6, 7). The different combinations of α and β subunits have also been shown to have distinct pharmacological profiles for agonists and antagonists (8, 9).

Because nicotinic drugs interact in unique ways with different types of nicotinic receptors, based on the specific subunit composition of the receptor, a systemically applied drug may interact with neuromuscular junction receptors, ganglionic receptors, or different subtypes of brain receptors. By studying cloned receptor subunits we can model the effects of specific pharmacological agents on subunit combinations that are likely to represent particular receptor subtypes found in these different tissues. For example, α4β2 receptors may be a common nicotinic receptor subtype in the brain. These subunits have the widest pattern of expression (10) and immunoprecipitation studies have confirmed that the large majority of high affinity nicotine binding sites in brain membranes are associated with receptor complexes containing α4 and β2 subunits (11-13). Most of this high affinity nicotine binding in brain tissue is

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ABBREVIATIONS: ACh, acetylcholine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

associated with presynaptic receptors (14). Because presynaptic receptors are difficult to study with conventional electrophysiological methods in the brain, we have studied these receptors by expressing cloned $\alpha 4$ and $\beta 2$ subunit RNAs in *Xenopus* oocytes.

In this paper we report data regarding the effects of cytisine, which is an alkaloid extracted from the seeds of *Cytisus laburnum*. Its physiological actions were first described by Dale and Laidlaw (15) as "qualitatively indistinguishable from nicotine." Of all commonly used nicotinic ligands, cytisine has the highest affinity for nicotinic receptors in the brain (16–18). It is a potent ganglionic stimulant and is as potent as nicotine for stimulating dopamine release from synaptosomal preparations (14). However, some published observations suggest that cytisine is qualitatively different from nicotine with regard to its apparent efficacy for brain nicotinic receptors. Cytisine is only 20–30% as effective as nicotine for stimulating rubidium efflux from synaptosomal preparations (19), and Luetje and Patrick (8) reported that cytisine is a much less potent agonist than nicotine for $\alpha 4\beta 2$ receptors expressed in *Xenopus* oocytes. Among the agonists tested by Luetje and Patrick (cytisine, nicotine, ACh, and 1,1-dimethyl-4-phenylpiperazinium) with the six known pairwise combinations of neuronal nicotinic α ($\alpha 2$, $\alpha 3$, and $\alpha 4$) and β ($\beta 2$ and $\beta 4$) subunits, cytisine was the most potent agonist for all $\beta 4$-containing receptors and the least potent for $\beta 2$-containing receptors. Luetje and Patrick also showed that coapplication of cytisine and ACh to $\alpha 3\beta 2$ receptors reduced the response to ACh. Although their data showed that the inhibition by cytisine was not voltage dependent, those authors suggested that cytisine inhibited the response to ACh through some form of channel block. In this paper we report results that demonstrate that cytisine is a partial agonist and inhibits the responses of $\alpha 3\beta 2$ and $\alpha 4\beta 2$ receptors to ACh by competing with ACh for the agonist binding site.

Materials and Methods

In vitro synthesized cRNA transcripts and oocyte injection were as described by Boulter *et al.* (2). The oocytes were injected on the day after harvesting, and recordings were made 2–6 days after injections. Normal injections consisted of 5 ng of cRNA for each subunit per oocyte.

The data were obtained by means of two-electrode voltage-clamp recording, using an Axoclamp 2A amplifier. Recordings were made in frog Ringer solution (115 mM NaCl, 10 mM HEPES, 2.5 mM KCl, 1.8 mM $CaCl_2$, pH 7.3) with 1 μM atropine, at room temperature (21–24°). The voltage electrode was filled with 3 M KCl, and the current electrode solution contained 250 mM CsCl, 250 mM CsF, and 100 mM EGTA, pH 7.3. Fresh ACh (Sigma) stock solutions were made daily in Ringer solution and diluted. (−)-Cytisine was obtained from Sigma, and stock solutions of 100 mM were made up in Ringer solution, aliquoted, and kept frozen until used. Electrodes were fabricated from Dagan FLG 15 glass capillary tubes. The resistance of voltage electrodes was on the order of 3–9 MΩ, and current electrodes were between 0.5 and 3 MΩ, usually approximately 1 MΩ.

Bath solution and drug applications were delivered through a linear perfusion system to oocytes placed in a Lucite chamber with a total volume of 0.5 ml. Drug delivery involved preloading a 1.8-ml length of tubing at the terminus of the perfusion system, and a Mariotte flask filled with Ringer solution was used to maintain a constant hydrostatic pressure for drug deliveries and washes. With this system the agonist and antagonist application pulses were consistent in both volume and duration. Current responses were recorded on a Gould chart recorder, and peak currents were measured with a digital meter in parallel with the amplifier output. Recordings were made at a holding potential of −70 mV or at −50 mV when responses were too large to be effectively clamped at −70 mV. Current responses were in the range of 0.2–5 μA and could be measured accurately to the nearest 0.001 μA (i.e., 1 nA). Data represent the means ± standard errors of at least three oocytes (three to eight) for each experimental condition. Data were normalized to the initial responses of each oocyte to a control application of ACh. A small amount of rundown (i.e., progressive diminution of response) is occasionally observed when repeated agonist applications are made to neuronal nicotinic receptors. In these experiments the amount of rundown varied between batches of oocytes, but for any particular batch of oocytes the variability (i.e., standard deviation) of the rundown in control groups was normally <10%. Rundown may represent some form of progressive desensitization or inactivation that is intrinsic to the properties of the receptors or may be a consequence of the ion flux through open channels (e.g., local changes in internal Ca^{2+} concentrations). There may also be changes in the calcium buffering of the cells over time as a result of fluid exchange with the current electrode, and this could affect calcium-dependent chloride channels that may contribute to the inward currents. Although responses were not specifically corrected for rundown, in direct comparisons between ACh and cytisine the agonists were applied in alternating order and the order had no significant effect on the relative amplitudes of the responses.

Results

Cytisine as an agonist. Fig. 1 illustrates that the ability of cytisine to activate neuronal nicotinic receptors depends strongly on the presence of the $\beta 4$ subunit. Cytisine is a relatively potent agonist for the $\alpha 3\beta 4$ receptor but a poor agonist for $\alpha 3\beta 2$. At high concentrations of agonist (1 mM) the

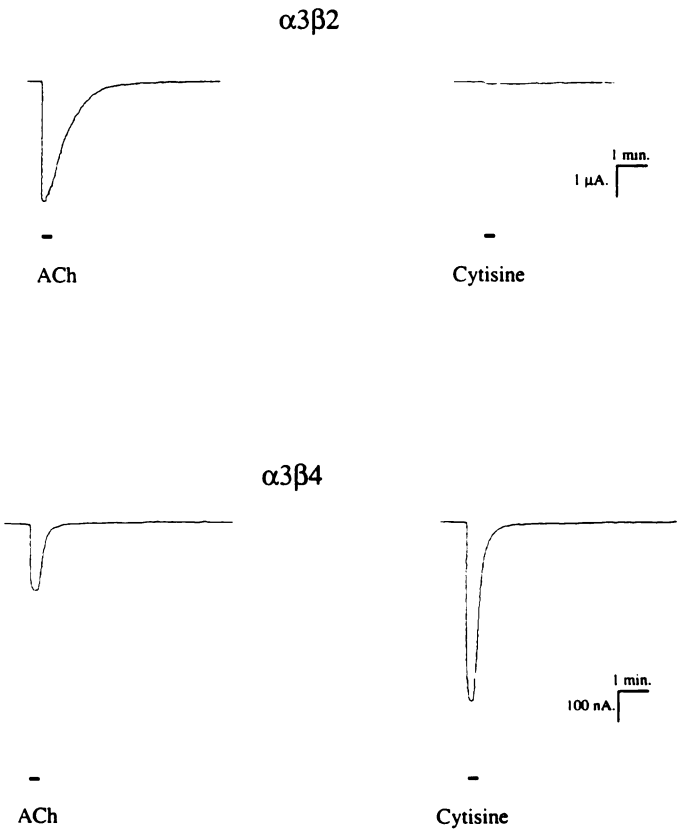

Fig. 1. Responses of $\alpha 3\beta 2$-injected oocytes and $\alpha 3\beta 4$-injected oocytes to ACh and cytisine. Voltage-clamp recordings of the responses of $\alpha 3\beta 2$-injected oocytes or $\alpha 3\beta 4$-injected oocytes to the bath application of 30 μM ACh or 30 μM cytisine are shown.

responses of $\alpha3\beta2$-injected oocytes to a brief application of cytisine were only 2.5 ± 0.8% of the response to ACh ($n = 4$). The responses of $\alpha3\beta4$-injected oocytes were 76 ± 7% of the response to 1 mM ACh. $\alpha4\beta2$-injected oocytes also responded poorly to cytisine as an agonist, with responses to the application of 1 mM cytisine being only 14.7 ± 4.4% of the response to 1 mM ACh (see Fig. 6).

Cytisine inhibition of $\alpha3\beta2$ responses. The current responses of $\alpha3\beta2$-injected oocytes to cytisine were too low to obtain a reliable estimate of the relative efficacy of cytisine for this receptor; however, it was possible to characterize the antagonism by cytisine of the ACh responses of these receptors. The concentration-response relationships were examined for $\alpha3\beta2$-injected oocyte responses to the application of ACh in the absence of cytisine or in the presence of 30 μM cytisine (Fig. 2A). The EC_{50} for ACh was 150 μM in the absence of cytisine and 1 mM in the presence of 30 μM cytisine. The clear shift to the right indicates that inhibition of the ACh response by cytisine occurs through a competitive mechanism. When both sets of data were normalized to initial responses to 10 μM ACh, the peak currents with 5 mM ACh with or without cytisine were not different. The response to 5 mM ACh alone was 17.6 ± 9.7 times the response to 10 μM ACh alone, whereas the response to 5 mM ACh plus 30 μM cytisine was 15.0 ± 4.0 times the response to 10 μM ACh alone. Note that responses to 5 mM ACh were taken as the saturating responses, because application of higher concentrations of ACh produced smaller responses (data not shown), presumably due to desensitization or channel block by ACh (20, 21). Fig. 3A shows the inhibition curve for cytisine effects on the response of $\alpha3\beta2$-injected oocytes to the application of 30 μM ACh. The IC_{50} for cytisine was 10 μM. As shown in Fig. 3B, the effects of cytisine at all concentrations were reversible after a 5-min wash period.

In their report of the effects of cytisine on the ACh responses of $\alpha3\beta2$ receptors, Luetje and Patrick (8) suggested that cytisine may act as a channel blocker, and their analysis must be reexamined in light of the present study. Luetje and Patrick measured ACh responses in the presence and absence of 1 μM cytisine and made a somewhat unconventional presentation of their data. Rather than plotting their data as in our Fig. 2A, they plotted each point as the percentage of the control ACh response obtained at each ACh concentration in the absence of cytisine. In Fig. 2B we show a similar plot of ACh responses obtained in the presence of 3 μM cytisine, normalized to the control ACh responses at each concentration. As shown in Fig. 3, the inhibitory effects in this range of cytisine concentrations (1–3 μM) are relatively weak (10–20% inhibition of the responses to 30 μM ACh). At these concentrations, however, cytisine stimulates small but detectable currents, and these currents activated by cytisine contribute to the total response. When the responses to the coapplications of ACh and cytisine are normalized to the responses to ACh alone at each concentration, the contribution of cytisine to the total current is initially high but then decreases as the ACh concentration (and control ACh current) increases. This accounts for the U-shaped curve in Fig. 2B, which is similar to the one published by Luetje and Patrick. Although Luetje and Patrick suggested that cytisine-induced current contributed to the response they obtained with the coapplication of cytisine and 200 nM ACh, they misinterpreted the decrease in the ratio of the cytisine current to the control ACh current at higher concentrations as increased

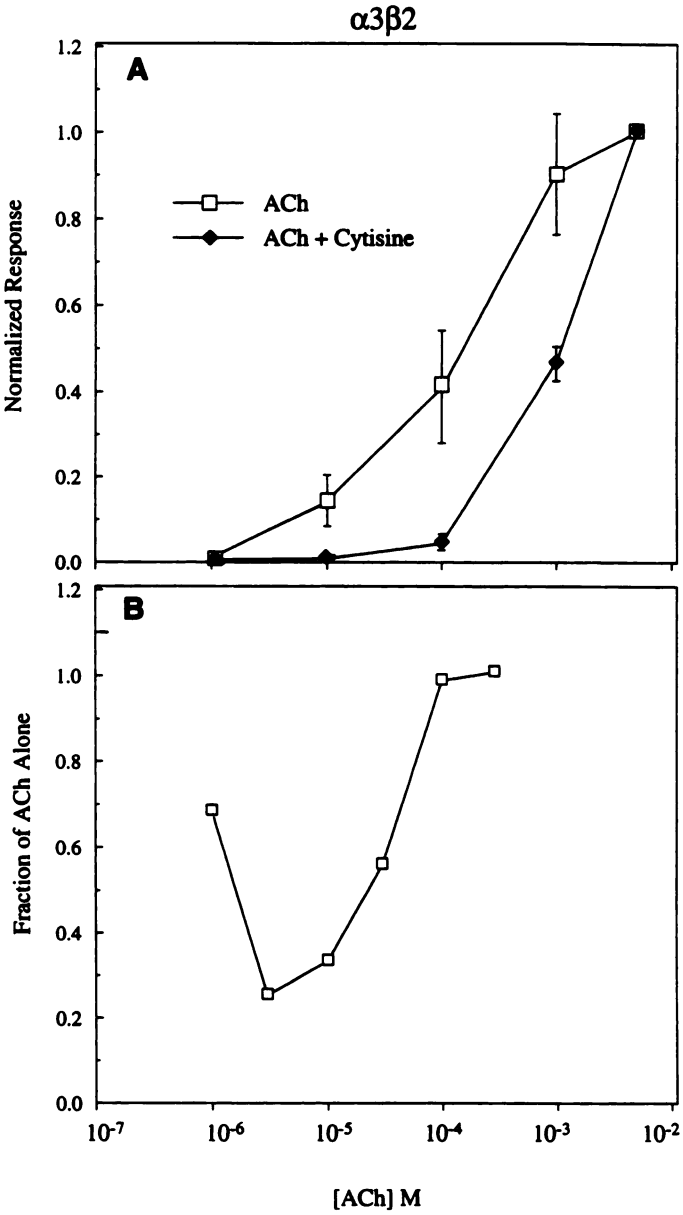

Fig. 2. A, Effect of cytisine on the ACh concentration-response relationship of $\alpha3\beta2$ receptors. The concentration-response relationships of $\alpha3\beta2$-injected oocytes for the application of ACh in the absence or presence of 30 μM cytisine are shown. Values were normalized to the response of each oocyte to 5 mM ACh. B, Effect of 3 μM cytisine on the ACh concentration-response relationship of $\alpha3\beta2$ receptors, plotted as in Ref. 8. The responses of $\alpha3\beta2$-injected oocytes to the application of ACh in the presence of 3 μM cytisine were normalized to the response of each oocyte to ACh at that concentration in the absence of cytisine.

inhibition with increasing agonist concentration (over the limited concentration range of 200 nM ACh to 10 μM ACh).

Luetje and Patrick reported that when they coapplied 200 nM ACh and 1 μM cytisine they obtained a response 120% of the size of the response to ACh alone. Their interpretation was that this was due to an absence of a blocking effect when ACh concentration was low, combined with the addition of the cytisine-induced current to the ACh-induced current. They reported their control (200 nM) ACh response as 3.4 ± 0.3 nA and their 1 μM cytisine response as 0.5 nA. Linear addition of these currents would give 111% of the control ACh response. We attempted to reproduce this observation. In our experi-

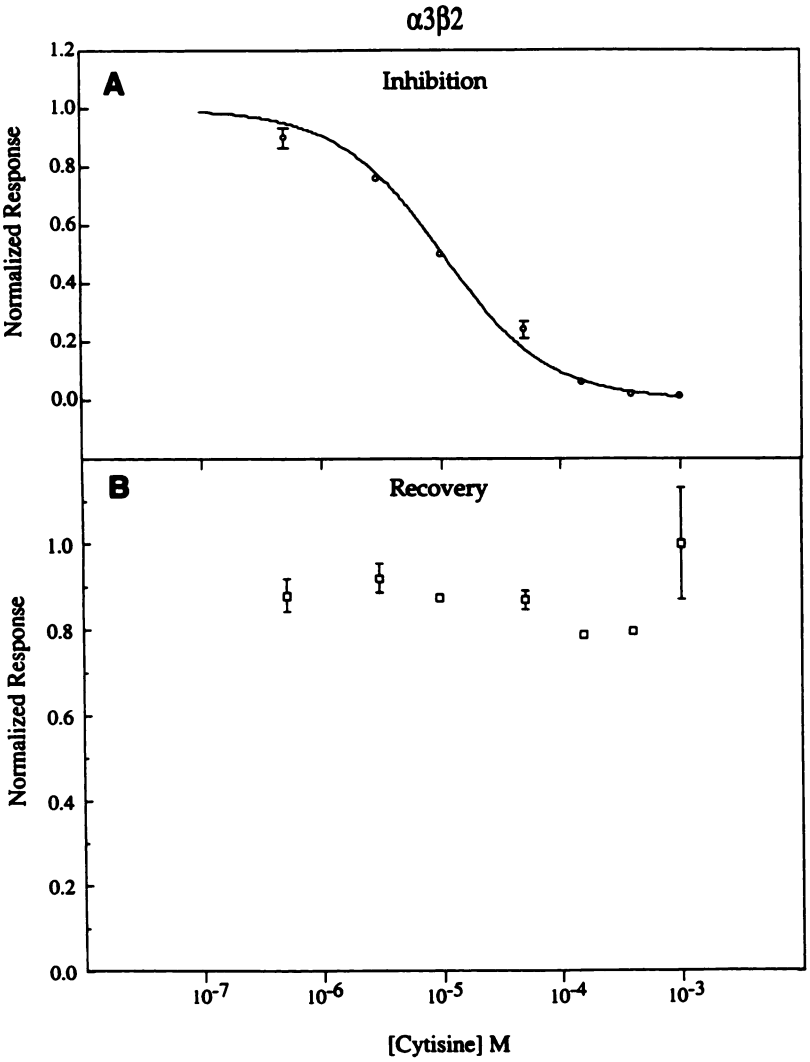

Fig. 3. Inhibition by cytisine of the ACh response of $\alpha 3\beta 2$ receptors. A, Inhibition of the response of $\alpha 3\beta 2$-injected oocytes to ACh by increasing concentrations of cytisine. The inhibition curve was generated by Sigmaplot curve-fitting routines. B, Recovery of $\alpha 3\beta 2$-injected oocytes after inhibition of the ACh response by cytisine. In both A and B, values were normalized to the initial response of each oocyte to 30 μM ACh.

ments the response to 200 nM ACh was 14.4 ± 2.6 nA (n = 5) and the response to 1 μM cytisine was 6.2 ± 0.7 nA (n = 5), a response to cytisine that was nearly half the size of the response to the lower concentration of ACh. However, in our experiments there was no additive effect with coapplication; the response to 200 nM ACh plus 1 μM cytisine was only 10.7 ± 2 nA (n = 5), which represents a 27% inhibition of the 200 nM ACh response by 1 μM cytisine. Our experiments therefore support the hypothesis that there are competitive interactions between ACh and cytisine throughout the concentration range.

Cytisine inhibition of $\alpha 4\beta 2$ responses. Fig. 4 shows the concentration-response relationships for the responses of $\alpha 4\beta 2$-injected oocytes to ACh in the presence or absence of 3 μM cytisine. The coapplication of 3 μM cytisine and ACh clearly shifted the ACh concentration-response relationship to the right, resulting in a change in the EC_{50} from 2.5 μM in the absence of cytisine to 140 μM in the presence of 3 μM cytisine.

As noted above, the application of cytisine alone to $\alpha 4\beta 2$-injected oocytes stimulated a significant response, nearly 10 times greater than the relative responses of $\alpha 3\beta 2$-injected oocytes to cytisine. For this reason, cytisine must be interpreted as a partial agonist for $\alpha 4\beta 2$ receptors. Fig. 5A shows the response of $\alpha 4\beta 2$-injected oocytes to the application of 1 μM ACh and increasing amounts of cytisine. There was significant inhibition of the ACh response with the coapplication of only 10 nM cytisine, and the inhibition was maximal with the coapplication of 300 nM cytisine. With the coapplication of 1 μM ACh and concentrations of cytisine greater than 300 nM there was a decrease in the apparent inhibition due to the additional current evoked by cytisine. The inhibitory effects of cytisine were reversible when the cells were tested with a second application of 1 μM ACh alone, 5 min after the ACh/cytisine coapplication (data not shown).

To separate the inhibitory and excitatory effects of cytisine, the concentration-response relationship for cytisine was examined, and the results are presented in Fig. 5B. The response of each oocyte to 1 μM ACh was first measured, and the cytisine responses were normalized to these values. As illustrated in Fig. 5B, the concentration-response relationship for cytisine was complex. There were measurable responses to even low nanomolar concentrations of cytisine, a plateau in the response relationship at concentrations of approximately 100 nM, and then a second phase of increasing response in the low micromolar concentration range, reaching a second plateau with concentrations greater than 30 μM. The inhibitory effects of cytisine on the ACh-induced response, independently of the current stimulated by cytisine, were then extrapolated by subtracting the cytisine response in Fig. 5B from the coapplication re-

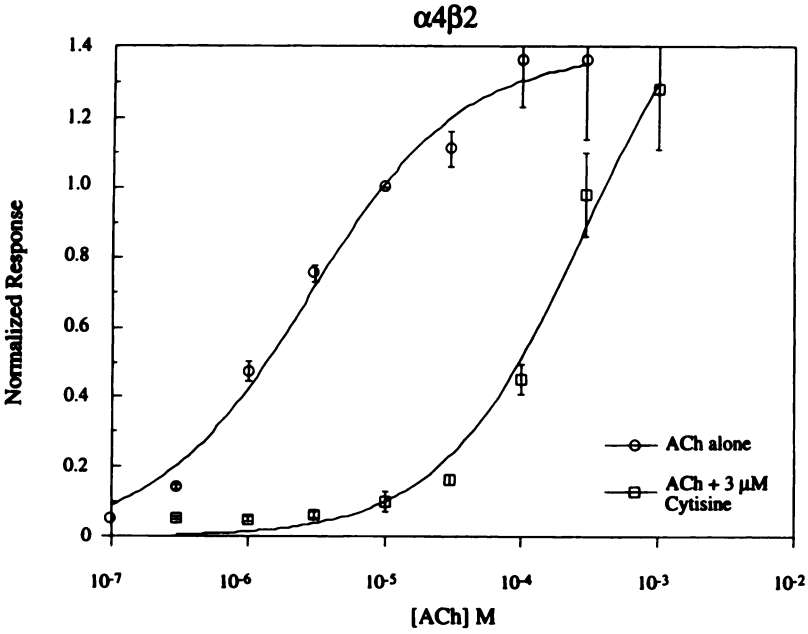

Fig. 4. Effect of cytisine on the ACh concentration-response relationship of $\alpha4\beta2$ receptors. The concentration-response relationships of $\alpha4\beta2$-injected oocytes for the application of ACh in the absence or presence of 3 μM cytisine are shown. Values were normalized to the initial response of each oocyte to 10 μM ACh.

sponses in Fig. 5A. The results, representing the inhibition of the ACh response by cytisine, are shown in Fig. 5C. Cytisine appears to be effective at inhibiting the ACh response in the concentration range where it stimulates very little current.

Even when the cytisine concentration was taken to very high values, the peak currents never approached those stimulated by ACh. The concentration-response relationship for the peak currents evoked by cytisine is compared with the ACh curve in Fig. 6. The maximum values for cytisine were only 10–20% of the maximum currents evoked by ACh.

From this analysis we estimate an IC_{50} of 20 nM for cytisine inhibition of the responses of $\alpha4\beta2$-injected oocytes to 1 μM ACh. This value is reasonable in light of the binding data available for this ligand (16–18).

Discussion

Our results support the hypothesis that cytisine inhibits the response of $\beta2$-containing receptors to ACh by competing with ACh for the agonist binding site, because the inhibition may be overcome by the coapplication of higher concentrations of ACh. Cytisine therefore appears to be a ligand for the agonist binding site of both $\beta2$-containing and $\beta4$-containing receptors, but for receptors containing the $\beta2$ subunit the binding of cytisine is relatively ineffective at activating the channel. The previous study by Luetje and Patrick (8) reported that cytisine was a selective agonist for $\beta4$-containing receptors and also noted that cytisine inhibited the response of $\alpha3\beta2$ receptors to applied ACh. Those authors, however, suggested that cytisine inhibited $\alpha3\beta2$ receptors by behaving as a channel blocker. They asserted that a biphasic relationship between percent inhibition and ACh concentration argued against a competitive mechanism of inhibition. However, their analysis focused on very low concentrations of ACh and did not factor out the current that would have been evoked by cytisine alone in this low ACh concentration range. As we have shown in our analysis, if cytisine-induced current is corrected for then their data are consistent with cytisine behaving as a partial agonist. Moreover, Luetje and Patrick demonstrated that inhibition by cytisine was not voltage dependent, which also argues against inhibition through a mechanism of channel block.

A basic nitrogen and a hydrogen bond acceptor group separated by 5.9 Å are essential elements for a nicotinic agonist (22). Presumably, the receptor molecule has counterparts to these elements in the agonist binding sites, i.e., a negative subsite and groups that serve as hydrogen bond donors. It has long been known that at least part of the agonist binding site is on the α subunits, and there is substantial evidence that part of the agonist binding site is located near the adjacent cysteine residues at positions analogous to amino acids 192 and 193 of the *Torpedo* sequence (for review, see Ref. 23). Recently, mutagenesis experiments have suggested that the tyrosine at position 190 may function as the hydrogen bond donor in the agonist binding reaction (24). It is still unclear, however, what residues provide the negative subsite and how it is that agonist binding is coupled to the conformational change that gates the channel. Recently it has been proposed that the agonist may bind in a pocket formed by two subunits.

Based on studies of the binding of curare (25, 26) and photoaffinity labeling with nicotine (27), it has been suggested that the agonist binding sites of the muscle receptor are located at the interfaces between the α subunits and the γ and δ subunits. By analogy, that would imply that the agonist binding sites of neuronal receptors contain elements from both the α and β subunits. It may be that it is the binding of the same molecule of agonist to subsites on both the α and β subunits that is an essential factor for the translation of binding energy to the conformational change associated with channel gating. The functional discrimination between $\beta2$-containing and $\beta4$-containing receptors by cytisine may have to do with specific differences in the agonist binding site on the β subunit and whether changes in conformational states are stimulated by the binding of cytisine. There are several possible models explaining how cytisine may discriminate between $\beta2$- and $\beta4$-containing receptors. 1) The channel opening rate of $\beta2$-containing receptors may be extremely low when cytisine is bound. 2) The open state activated by cytisine may be unstable (i.e., the channel closing rate may be faster than that for a full agonist),

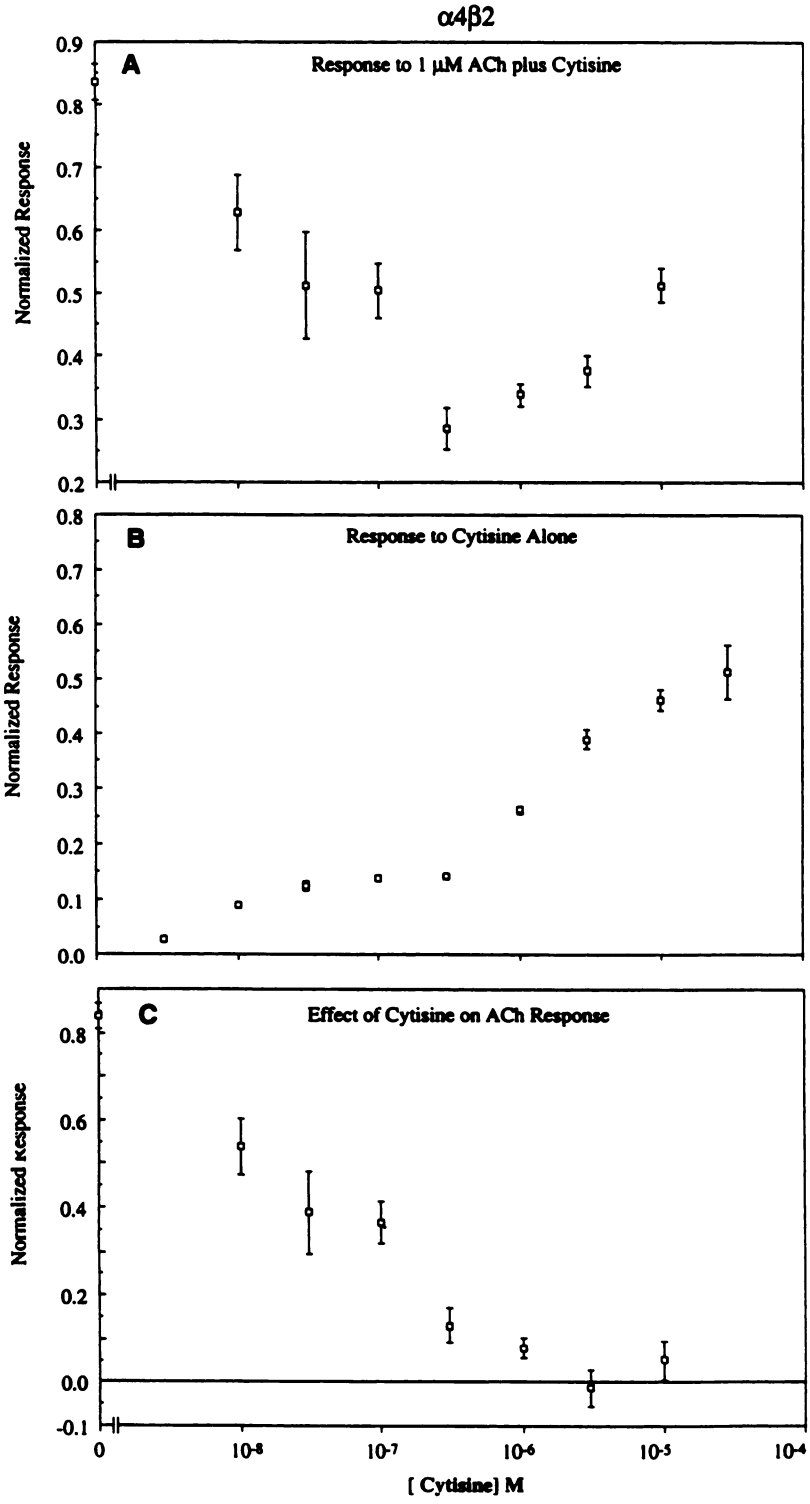

Fig. 5. Inhibition by cytisine of the ACh response of $\alpha4\beta2$ receptors. A, Response of $\alpha4\beta2$-injected oocytes to coapplication of 1 μM ACh and increasing concentrations of cytisine. B, Response of $\alpha4\beta2$-injected oocytes to application of cytisine alone. C, Inhibitory effect of increasing concentrations of cytisine on the response of $\alpha4\beta2$-injected oocytes to 1 μM ACh, calculated as the difference between the values in A (the response to coapplication of ACh and cytisine) and the values in B (the current stimulated by cytisine alone). All values were normalized to the initial response of each oocyte to 1 μM ACh.

or it may be a subconductance state. 3) Cytisine may induce very fast desensitization. 4) Cytisine may have high affinity for the closed state of the receptor and lower affinity for the binding site when the channel opens.

It has been shown that, in general, the presence of agonist favors a shift in the equilibrium of conformational states toward desensitized forms of the receptor, which have higher affinity for agonists such as ACh or nicotine than does the resting state of the receptor. Because binding experiments require prolonged exposure to agonist and this stabilizes desensitized states, binding experiments typically estimate K_d values that are orders of magnitude lower than the EC_{50} values for channel activation derived from physiology experiments. For example, ACh displaces [^{3}H]ACh from receptors in brain membranes with a K_i of 7.6 nM (16), whereas our estimate for the EC_{50} for $\alpha4\beta2$ channel activation by ACh is 2.5 μM (see Fig. 4).

The analysis of single-channel bursts suggests that agonist dissociation rates are faster for nondesensitized closed channels than for channels in the open state. That is, channels remain in the (agonist-bound) open state for longer periods of time

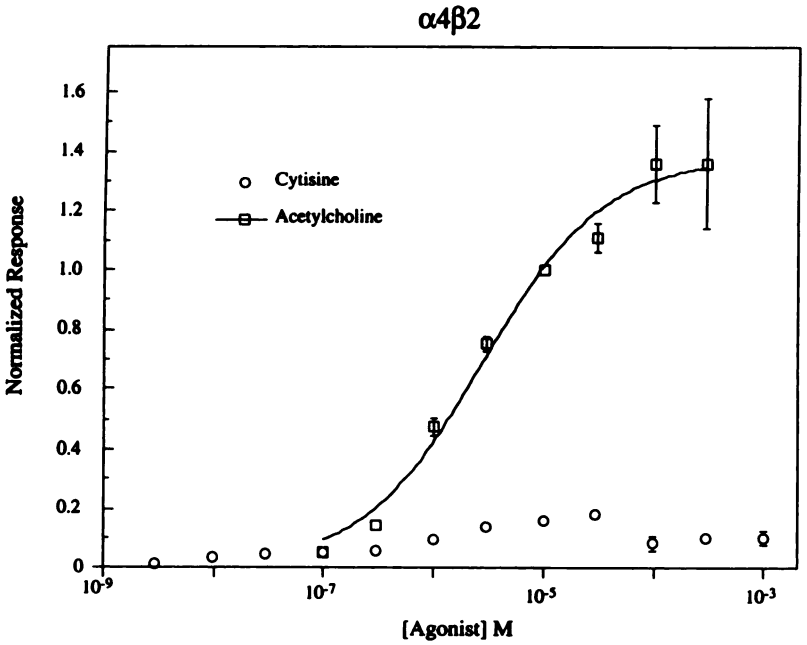

Fig. 6. Concentration-response relationships of $\alpha4\beta2$ receptors for cytisine and ACh. Responses to ACh were normalized to the response of each cell to 10 μM ACh. Responses to cytisine were first normalized to the response of each cell to 1 μM ACh and were then scaled to the ratio of the 1–10 μM responses in the ACh concentration-response relationship.

than agonist tends to remain bound to nondesensitized closed states (21). This means that the conformational change associated with channel gating also changes the agonist binding site in such a way that, by virtue of a decreased off-rate, open channels have a high affinity for full agonists that is similar (if not identical) to the affinity of desensitized receptors for agonist. This may not be true for cytisine.

As noted in Results, increasing the concentrations of ACh above 1 mM may lead to submaximal responses, possibly due to rapid desensitization or channel block by ACh. It is interesting to speculate whether specific predictions may be made for the effects of the partial agonist cytisine on the decrease in responses in this high ACh concentration range. Any predictions that could be made would depend, however, on knowing whether the partial agonist itself also induced desensitization (perhaps inducing the channel to bypass open states). If cytisine supports transitions to desensitized states and the high ACh concentration effects are due to desensitization, then cytisine might possibly alter the time course but not the extent of high concentration inhibition. If cytisine does not induce desensitization, then it might shift the concentration dependence of ACh desensitization to the right. If decreased responses at high ACh concentration are due to channel block by ACh, then the effects of cytisine would be somewhat difficult to predict. The channel-blocking effects of ACh may require the sequential acts of the channels first being open and then becoming blocked, and both of these events are dependent on agonist concentration. If cytisine shifts the agonist dose-response curve for channel opening but does not directly interfere with channel block by ACh, then cytisine might cause an apparent decrease in E_{max}, as well as causing a shift to the right for inhibitory effects; however, it would be difficult to predict the magnitude of such an effect.

Cytisine has an extremely high affinity for nicotinic receptors in brain membranes, displacing [^{3}H]ACh with a K_i of 1.3 nM (16). In our experiments the IC_{50} we estimated for the inhibition of $\alpha4\beta2$ responses to ACh was 20 nM and the apparent K_d derived from the partial agonist analysis was 12 nM. These values estimated from the physiological effects (10–20 nM) are closer to those obtained with binding experiments (1.3 nM) than is usually the case for full agonists. This suggests the possibility that cytisine may have an affinity for the resting closed states of $\alpha4\beta2$ receptors that is similar to that of full agonists for the desensitized and open states. In our experiments, effective inhibition by cytisine was followed by full recovery to the control levels of ACh response within 5 min, suggesting that cytisine did not inhibit ACh responses through a mechanism that resulted in prolonged desensitization.

Cytisine and nicotine share with ACh the same two-dimensional orientation of the essential elements for nicotinic agonists (22). These alkaloids, however, are more rigid than ACh and when their crystal structures are compared, with the aromatic rings aligned, there is a difference of 1.35 Å in displacement between the reactive groups (28). The fact that nicotine is a full agonist for $\alpha4\beta2$ receptors, equipotent with ACh (8), whereas cytisine is a partial agonist may be due to this difference in structure and to how the β subunits configure a site of agonist interaction in the different conformational states of the receptor. Studies of chimeric β subunits place a region that determines the efficacy of cytisine, relative to the two neuronal β subunits, in the amino-terminal portion of the β subunit extracellular domains (29, 30).

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