“Orphan” α6 Nicotinic AChR Subunit Can Form a Functional Heteromeric Acetylcholine Receptor

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SUMMARY

Previously, a rat brain cDNA was reported that was designated α6 because of its homology with nicotinic acetylcholine receptor (AChR) α subunits, being especially similar to α3, but no acetylcholine-gated cation channels were detected when it was expressed in Xenopus laevis oocytes alone or in combination with other known rat AChR subunits. We cloned chicken α6 and human β4 AChR subunits and tested for acetylcholine-gated cation channels with α6 by expression in X. laevis oocytes alone or in pairwise combination with chicken α3, β2, or β4 or with human α3, β2, or β4 AChR subunits. Chicken α6 formed detectable functional AChRs only when expressed together with the human β4 subunit. The α6/β4 AChR-mediated currents show strong inward rectification and dependence on extracellular Ca\(^{2+}\). It exhibited a distinct pharmacological profile with an EC\(_{50}\) value of 28 μM for acetylcholine, 24 nM for (+)-epibatidine, 6.6 μM cytisine, and 15 μM 1,1-dimethyl-4-phenylpiperazinium. Both cytisine and 1,1-dimethyl-4-phenylpiperazinium behaved as partial (~30%) agonists. Remarkably, nicotine (EC\(_{50}\) = 22 μM) was an even weaker partial agonist (~18%) and had a relatively long-lasting inhibitory effect. Coexpression of the previously cloned rat α6 subunit with the human the β4 subunit also resulted in functional α6/β4 AChRs with properties resembling of the chicken/human α6/β4 AChRs. Therefore, α6 can function as part of AChRs with unusual pharmacological properties.

The family of nicotinic AChRs consists of subunits termed α1–α9, β1–β4, γ, δ, and ε. All of these subunits, except the “orphans” α6 and β3, have been shown to function as components of ACh-gated cation channels either as homomers or in combination with one or more other AChR subunits (1–4). Despite the fact that the cDNA sequence of the α6 AChR subunit has been known for several years (1), little data are available regarding the properties of this subunit. These data are limited to mentions in reviews of cDNA sequences of rat and chick subunits (4, 5), a recently submitted cDNA sequence of the human subunit (6), and published abstracts concerning α6 mRNA distribution in rat brain and cochlea (7, 8). High levels of sequence homology between α6 and α3 AChR subunits (>75%) and other features common to all functional nicotinic subunits (5) indicated that α6 subunits should form functional AChRs serving as a ligand-binding subunit. However, difficulties in obtaining functional AChRs formed exclusively or partially by this subunit have suggested that α6 may serve a structural role in combination with other α and β subunits as α5 does (9, 10), that it may require the presence of another subunit yet to be identified to function as an AChR, or that α6 may function as a receptor for some other ligand yet to be identified.

We show that α6 subunits can, in fact, act as ligand-binding subunits in functional AChRs. Here, we report cloning of cDNAs encoding α6 AChR subunits from a chicken cochlea library and a β4 AChR subunit from a human neuroblastoma SH-SY5Y library. When expressed in Xenopus laevis oocytes together with the human β4 subunit, chicken or rat α6 AChR subunits form nicotinic ligand-gated cation channels with novel pharmacological properties. Identification of this new subtype of AChR may prove important for understanding the pharmacological properties of centrally acting cholinergic ligands with possible therapeutic significance.

Materials and Methods

Isolation of chicken α6 and human β4 cDNA clones. A lambda Zap II cDNA library (~9 × 10\(^6\) plaques) using chick cochlear

ABBREVIATIONS: AChR, acetylcholine receptor; ACh, acetylcholine; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; SDS, sodium dodecyl sulfate; SSPE, standard saline/phosphate/EDTA; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid.
mRNA, constructed by Stratagene (La Jolla, CA), was kindly provided by Dr. Paul Fuchs (Johns Hopkins University, Baltimore, MD). The chicken α6 cDNA was obtained by screening ~5 × 10^6 plaques from this library at low stringency using previously cloned chick α3, α4, α5, α7, α8, and β2, rat α2, β3, and β4, and human α1, β1, γ, and δ full-length or nearly full-length cDNA probes. The human β4 cDNA clone was obtained by screening a previously described lambda Zap II cDNA library (11) constructed using mRNA isolated from the human neuroblastoma cell line SH-SY5Y with human α3, α4, α5, α7, β2, and β4 and rat α2, β3, and α6 full-length or nearly full-length cDNA probes. All rat AChR subunit cDNAs used were kindly provided by Drs. Stephen Heinemann and Jim Boulter (Salk Institute, San Diego, CA). A human α5 cDNA clone and a partial β4 cDNA clone were kindly provided by Dr. Francesco Clementi (Università Degli Studi di Milano, Milano, Italy). The low-stringency screens were performed by hybridizing the membranes overnight at 42° in 30% formamide, 5 × SSPE (1× = 0.18 M NaCl, 0.01 M sodium phosphate, pH 7.4, 1 mM EDTA), 1% SDS, 5 × Denhardt’s solution (1 × Denhardt’s = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 150 μg/ml sonicated salmon sperm DNA. The membranes were washed successively in 5 × SSPE and 0.1% SDS, at room temperature, and in 2 × SSPE and 0.1% SDS at 42° for 30 min each. Autoradiography was performed by exposing the membranes to Kodak XAR-5 film (Eastman Kodak, Rochester, NY) for 1–2 days. Clones thus isolated were purified and subjected to dine in sequencing using the Sequenase 2 Kit (United States Biochemical, Cleveland, OH). The identity of each clone was then determined by searching for their sequences against the sequences contained in the National Center for Biotechnology Information database using the Blast suite of programs (12). Alignment of the peptide sequences was performed using MacVector (Eastman Kodak) and The Wisconsin Package (Genetics Computer Group, Madison, WI).

**Other cDNAs.** Chicken β2 cDNA was described previously (12). Rat α6 was kindly provided by Drs. Stephen Heinemann and Jim Boulter. Human α3 was cloned from a human brain library. Chicken α3, β2, and β4 cDNAs were obtained through the generosity of Dr. Marc Ballivet (Department of Biochemistry, University of Geneva, Geneva, Switzerland).

**Expression of AChR subunits in X. laevis oocytes.** Chicken and human cDNAs were cloned into a modified SP64T expression vector (14) using standard DNA cloning procedures. cRNA was synthesized in vitro using the Megascript kit (Ambion, Austin, TX). Oocytes were defolliculated and injected with either 15 or 100 ng of cRNA per oocyte. Chicken α3, β2, and β4 subunits were expressed by nuclear injections of 2 ng of genomic DNA per oocyte. The oocytes were incubated in semisterile conditions at 18° in saline solution (96 mM NaCl, 2 mM KCl, 1 mM MgCl$_2$, 1.8 mM CaCl$_2$, 5 mM HEPES, pH 7.6) containing 50% Leibovitz-15 media (GIBCO/BRL, Gaithersburg, MD) buffered to pH 7.4 with 10 mM HEPES. Oocytes were incubated at 18° for 5–6 days before use.

**Electrophysiological procedures and drug application.** Currents in oocytes were measured using a standard two-microelectrode voltage clamp amplifier (oocyte clamp OC-752; Warner Instrument, Hamden, CT). Electrodes were filled with 3 M KCl and had resistances of 0.5–1.0 MΩ for the voltage electrode and 0.4–0.6 MΩ for the current electrode. All records were digitized (MacLab/2e interface and Scope software; AD Instruments, Castle Hill, Australia), stored on a Macintosh IIE computer (Apple Computer, Cupertino, CA) and analyzed using AXOGRAPH software (Axon Instruments, Burlingame, CA).

The recording chamber was continually perfused by a flow rate of 10 ml/min with a saline solution containing 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 5 mM HEPES, pH 7.6. In most experiments, 1 μM atropine was added to the solution to suppress endogenous muscarinic responses in X. laevis oocytes. Application of the agonists was performed as described in detail previously (15). All agonists were applied by means of a set of 2-mm glass tubes directed on the animal pole of the oocyte. Application was achieved by manual unclamping and clamping of a flexible tube connected to the syringe with the test solution. Typical delay between beginning of the application and first detection of the induced current was approximately 0.3 sec.

The Hill equation was fitted to the concentration-response relationships using a nonlinear least-squares error curve fit method (KaleidaGraph software; Abbebeck/Synergy, Reading, PA): $I(x) = I_{\text{max}} [x^n / (x^n + EC_{50}^n)]$, where $I(x)$ is current measured at the agonist concentration $x$, $I_{\text{max}}$ is the maximal current response at the saturating agonist concentration, $EC_{50}$ is the agonist concentration required for the half-maximal response, and $n$ is the Hill coefficient.

**Drugs used.** Epibatidine (oxalate salt) was synthesized at Merck Sharp & Dohme Research Laboratories (Essex, UK) and was a gift from Stephen Fletcher (16). (-)Nicotine tartrate, cytisine, DMPP, and ACh chloride were obtained from Sigma (St. Louis, IL).

### Results

**Isolation and primary structure of the chick α6 AChR subunit.** A chicken cochlea cDNA library was screened at low-stringency hybridization conditions using a cocktail of AChR subunit cDNA probes. In addition to identifying cDNAs for α4, α7, β2, and β4 subunits, one 2233-bp cDNA was identified that closely resembled the previously cloned rat α6 AChR subunit (Genbank accession number L08277) as shown in Fig. 1A. The cDNA encodes a predicted mature protein of 464 amino acid residues, preceded by a leader peptide of 30 residues. The sequence contains a cysteine pair homologous to α1 128 and 142 and a second cysteine pair homologous to α1 192 and 193, which identify it as an α subunit. The sequence also contains the four putative transmembrane sequences typical of all AChR subunits. The predicted amino-acid sequence of mature chicken α6 is 86%, identical to that of rat α6 and 88% identical to human α6 (Fig. 1). Most sequence differences occur in the putative large cytoplasmic loop between transmembrane domains three and four, which is the region that typically shows the most variation between species of AChR subunits. Chicken and rat α6 subunits are identical both in parts of the sequence believed to contribute to the ACh binding site (e.g., amino acids of the mature protein 180–200) and in the lining of the cation channel (i.e., amino acids 200–250); therefore, α6 subunits from the two species would be expected to have both similar ligand binding and cation channel characteristics.

**Isolation and primary structure of the human β4 AChR subunit.** The deduced amino acid sequence of the human β4 AChR subunit obtained by low-stringency screening of a SH-SY5Y cDNA library is compared with the rat and chicken β4 subunits in Fig. 1B. Protein encoded by the human β4 cDNA has substantial identity with sequences of the chick (75%) and rat (85%) β4 AChRs. A leader peptide, four hydrophobic putative transmembrane domains, and two highly conserved cysteine residues at positions 153 and 167 are characteristic of all β-type subunits. Designation of this clone as a β subunit was confirmed by functional tests in which it was shown to form functional AChRs when expressed in combination with human α3 subunits (Fig. 2). An incomplete, nonfunctional human β4 AChR cDNA was published earlier (17).

**Functional expression of the chicken α6 AChR subunit.** Multiple attempts to detect functional nicotinic AChRs in X. laevis oocytes injected with in vitro synthesized chicken α6 transcripts either alone or after prior nuclear injection of α6β4 AChRs
chick $\beta_2$, $\beta_3$, or $\beta_4$ cRNAs were unsuccessful (Fig. 2). Parallel control experiments with pair-wise coexpression of $\beta_2$ or $\beta_4$ together with $\alpha_3$ confirmed the functionality of these cDNAs. Additionally, no functional AChRs were detected when oocytes were injected with mixtures containing 15–100 ng per oocyte of both in vitro synthesized $\alpha_6$ and $\beta_4$ transcripts (these were obtained by linearizing the chicken $\alpha_3$ cDNA recloned into the NotI site of the pBS SK(−) vector). Functionality of $\beta_4$ cRNA was confirmed by successful coexpression with the chicken $\alpha_4$AChR subunit. In general, maximal currents resulting from expression of the chicken $\alpha_3\beta_2$ (Fig. 3), $\alpha_3\beta_4$, and $\alpha_4\beta_4$ subunit combinations in oocytes clamped at $-70$ mV did not exceed 500 nA.

We continued to search for $\alpha_6$ function by coexpressing this subunit with human $\alpha_3$, $\beta_2$, or $\beta_4$ subunits (Fig. 2). Only oocytes injected with both $\alpha_6$ and $\beta_4$ subunits produced detectable responses to ACh. These responses could be detected only more than 72 hr after cRNA injection and only in about 50% of the injected oocytes. Expression typically reached a plateau on day 5 or 6 after cRNA injection. Peak amplitudes of the currents in oocytes clamped at $-100$ mV ranged from 5 to 250 nA; most responses were lower than 100 nA. By contrast, currents mediated by human $\alpha_3\beta_2$ and $\alpha_3\beta_4$ AChRs were much larger (3–5 $\mu$A). $\alpha_6\beta_4$ AChR currents usually did not show significant rundown, even after 2 hr of recording.

Oocytes expressing $\alpha_6\beta_4$ AChRs responded to ACh in a concentration-dependent manner (Fig. 3) with an $EC_{50}$ value of 28 $\mu$M and a Hill coefficient greater than 1. Maximal responses were obtained using 300 $\mu$M ACh. Further increase of the concentration resulted in decreased peak amplitude. Presence of “rebound” currents after termination of the application of high ACh concentrations (>100 $\mu$M) indicated a possible channel block effect of this agonist. At all concentrations, responses exhibited relatively slow activation and desensitization kinetics.

$\alpha_6\beta_4$ AChR-mediated responses exhibited a nonlinear voltage dependence typical of neuronal nicotinic AChRs. Currents reversed at $-17 \pm 3$ mV ($n = 5$). Strong inward rectification was observed not only at positive potentials but also at negative potentials at which the current/voltage dependence significantly deviated from linearity (Fig. 3). “Rebound” current upon agonist removal (Fig. 3) was attributed to recovery from agonist-mediated channel blockage. It correlated with holding potential, being more prominent at more negative potentials.

Amplitude of the $\alpha_6\beta_4$-mediated currents was dramatically attenuated [to $33 \pm 5\%$ ($n = 5$)] upon removal of Ca$^{2+}$ ions from the external solution (Fig. 3). Voltage dependence of the resulting responses showed less inward rectification at both positive and negative potentials (Fig. 3). Reversal potential in low Ca$^{2+}$ had a tendency to shift to the more positive potentials. More precise estimation of this shift was...
not result in detectable responses. However, chicken AChRs:

observed after 1-hr incubation in 200 nM
responses every 2 min. No attenuation of the responses was
recovered fully only after 8–10 min of washing with test ACh

blockage by classical nicotinic antagonists. Curare at 20
mM behaved as a very poor partial agonist with maximal cur-
tion of curare with ACh was approximately 2 sec (Fig. 4). Inhibition by curare showed relatively fast on and off
times. Half-time for inhibition of the response by coappli-
cation (Fig. 4). Inhibition by curare was revealed also as an independent measure of the quality of the
expression insufficient for functional detection. We have no
to compare the properties of these cDNAs ex-
pression with which to compare the properties of these cDNAs ex-
pression insufficient for functional detection. We have no

tFORMANCE OF THE RAT α6 ACHR SUBUNIT.

Coexpression of the rat α6 subunit, along with the human β4 subunit, resulted in appearance of ACh-induced inward cur-
rents that resembled those observed for chick α6 human β4

fundingsubunits were almost two orders of magnitude
higher compared with the α6β4 combination. This might indicate that α6 and α3 subunits differ in assembly affinity
for the β4 subunit and/or, possibly, additional subunits are
required for more effective functional expression of the α6
subunit. On the other hand, the relatively small amplitudes of the α6β4-mediated currents and failure to detect function
on coexpression with the β2 subunit could reflect levels of α6
expression insufficient for functional detection. We have no
independent measure of the quality of the α6 cRNA or the
amount of α6 protein produced. The low overall levels of α6β4
AChR function detected also may reflect inefficient process-
ing or assembly of α6 in X. laevis oocytes compared with the
neurons in which α6 might normally be found. There is, as
yet, no characterization of α6 protein or function in neurons
with which to compare the properties of these cDNAs ex-
pressed in X. laevis oocytes.

Despite the very high level of homology between α6 and α3
AChR subunits, they exhibit significant differences in their
functional properties. As discussed above, in addition to dif-
frences in expression levels and coexpression with β2 and β4
subunits, α6β4 AChRs exhibit significantly different phar-
macological properties compared with either chicken or human recombinant $\alpha_3\beta_4$ AChRs (Table 1) (16, 18). Therefore, both chicken $\alpha_6$ human $\beta_4$ AChRs and rat $\alpha_6$ human $\beta_4$ AChRs retain a unique agonist profile with nicotine, cytisine, and DMPP as poor partial agonists. In contrast, nicotine was shown to be a full agonist for chicken, rat, and human $\alpha_3\beta_4$ AChRs (Table 1) (16, 18, 19). Nicotine behaves virtually as an antagonist on $\alpha_6\beta_4$ AChRs. The time course of the nicotine-induced current does not indicate either accelerated desensitization or channel block of the AChRs. Inhibition of $\alpha_6\beta_4$ AChRs by nicotine strongly resembles the action of nicotine previously described for chicken $\alpha_3\beta_2$ AChRs but not for $\alpha_3\beta_4$ AChRs (18). After extensive studies of this phenomenon, these authors concluded that nicotine behaves as a competitive antagonist at low concentrations, but as a partial agonist at higher concentrations, and that its inhibitory action is at least in part contributed by the $\beta_2$ subunit. Nicotine also behaves as an antagonist for rat homomeric $\alpha_9$ AChRs (20). Epibatidine exhibits extremely high potency for $\alpha_6\beta_4$ AChRs. High potency of this alkaloid also was des-

Fig. 3. Chicken $\alpha_6$ human $\beta_4$ AChRs, activation by acetylcholine and channel properties. Top left, typical currents induced by application of increasing concentrations of ACh on oocytes co-injected with chick $\alpha_6$ and human $\beta_4$ cRNAs. Top right, a plot of the dose-response curve for ACh obtained from five oocytes held at $-100$ mV. Currents were normalized to the maximal current (at 300 $\mu$M ACh). Further increase of the ACh concentration induced currents with lower amplitude, suggesting the occurrence of channel blockage by the ACh. Recordings were performed 5 days after injection on an oocyte voltage clamped at $-100$ mV. Data (mean ± standard error) were fitted with the Hill equation. Middle, potential dependence of chick $\alpha_6$ human $\beta_4$ AChRs; left, the family of currents induced by application of 300 $\mu$M ACh to oocytes expressing $\alpha_6\beta_4$ held at different potentials from $-90$ to $+30$ mV is shown at 20-mV increments; right, a plot of the peak current versus holding potential. The current reverses at $-18$ mV. Bottom, dependence of the $\alpha_6\beta_4$ AChR-mediated current on extracellular Ca$^{2+}$ concentration; left, currents induced at normal Ca$^{2+}$ concentration (1.8 mM) and after removal of the Ca$^{2+}$ ions from the perfusion solution (5 mM EGTA was added to chelate possible Ca$^{2+}$ contamination); right, voltage dependence of the $\alpha_6\beta_4$ AChR-mediated current in control and in the "0" Ca$^{2+}$ solution. Voltage ramps were obtained by continuously changing the holding potential from $-100$ to $+50$ mV more than 2 sec. Traces are the result of the subtraction of the currents obtained in the absence of the drug from currents during application of 30 $\mu$M ACh.

Depletion of Ca$^{2+}$ ions from the extracellular solution resulted in a dramatic decrease of $\alpha_6\beta_4$ AChR-mediated currents. Similar phenomena were characterized originally for oocyte-expressed and native rat $\alpha_9$ AChRs (21, 22). It was concluded that physiological concentrations of extracellular Ca$^{2+}$ ions enhance neuronal AChR-mediated currents by direct binding on the extracellular side of these AChRs. Alternatively, decrease of the $\alpha_6\beta_4$ AChR-mediated currents in low Ca$^{2+}$ could be the result of the prevention of activation of a secondary endogenous Ca$^{2+}$-dependent Cl$^{-}$ current. This current is known to accompany currents mediated by recombinant AChRs or N-methyl-D-aspartate receptors with rela-
tively high Ca\(^{2+}\) permeability expressed in *X. laevis* oocytes (11, 15, 22). However, the time course of the currents mediated through the oocyte-expressed \(\alpha_6\beta_4\) AChRs suggests that the contribution of the Ca\(^{2+}\)-dependent Cl\(^{-}\) current is minimal or nonexistent. ACa\(^{2+}\)-dependent Cl\(^{-}\) current usually is observed as a peak current with a relatively fast inactivation at the beginning of the agonist application and sometimes is misinterpreted as a fast component of desensitization (15, 22, 23).

Preliminary *in situ* hybridization studies of mRNA expression for the \(\alpha_6\) AChR subunit revealed the pattern of distribution of this subunit in developing rat brain (8). Message for the \(\alpha_6\) subunit was localized within the medial habenula, locus ceruleus, ventral tegmental area, and substantia nigra compacta. This restricted pattern distribution of \(\alpha_6\) message in brain contrasts with the more diverse and diffuse distribution of the \(\alpha_4\) (see Refs. 1 and 2 for review) and \(\alpha_7\) (24, 25) subunits and rather parallels the distribution of the \(\alpha_3\) AChR subunit (1, 26). Now that we have demonstrated that \(\alpha_6\) can participate in functional AChRs in combination with \(\beta_4\), the significance of \(\alpha_6\) localization in brain will have more effect on our understanding the central effects of ACh, nicotine, and nicotinic drugs. Message for \(\beta_4\) subunit is colocalized in, but not limited to, the brain areas that contain \(\alpha_6\) mRNA (27). Message for \(\alpha_6\) RNA is localized in parts of the brain traditionally believed to participate in the rewarding properties of drugs of abuse, with one of the putative mechanisms of addiction involving dopamine release in the neurons of these areas (28). Functional, unidentified, neuronal AChRs were shown to be present in these areas (29–31). The substantia

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**TABLE 1**

<table>
<thead>
<tr>
<th>Chik (\alpha_6) Human (\beta_4)*</th>
<th>Chik (\alpha_3\beta_4)*</th>
<th>Human (\alpha_3\beta_4)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>28 ± 4</td>
<td>100%</td>
</tr>
<tr>
<td>Nicotine</td>
<td>22 ± 3</td>
<td>18% (c)</td>
</tr>
<tr>
<td>Cytisine</td>
<td>6.6 ± 0.6</td>
<td>36% (c)</td>
</tr>
<tr>
<td>DMPP</td>
<td>15 ± 1</td>
<td>27% (c)</td>
</tr>
<tr>
<td>Epibatidine</td>
<td>0.024 ± 0.002</td>
<td>100%</td>
</tr>
</tbody>
</table>

\(a\) Data from Fig. 4.

\(b\) Data from Ref. 15.

\(c\) Partial agonist.

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**Fig. 4.** Pharmacological properties of chick \(\alpha_6\) human \(\beta_4\) AChRs. Top, inhibition of \(\alpha_6\beta_4\)-mediated currents by \(\beta\)-tubocurarine (left) and mecamylamine (right). Curare was coapplied for 6 sec after 4 sec of perfusion of 30 \(\mu\)M ACh. Currents induced by 30 \(\mu\)M ACh before, after 2-min perfusion with 10 \(\mu\)M mecamylamine, and after 10-min washout. Middle left, currents induced by 300 \(\mu\)M ACh before and after 4-sec application of 100 \(\mu\)M nicotine (middle trace) to illustrate the long-lasting antagonism produced by exposure to nicotine. Middle right, currents induced by application of increasing concentrations of \((+)-epibatidine are compared with the control current induced by 300 \(\mu\)M ACh. Bottom, a family of concentration-response curves obtained for ACh, nicotine, cytisine, DMPP, and \((+)-epibatidine. Averaged data from three to five experiments are presented.
nigra, which degenerates in Parkinson's disease, expresses α6 (8). Nicotinic AChRs are lost in Parkinson's disease (32, 33), and smoking seems to be protective in this disease (34); therefore, AChR subtypes with a limited distribution, including this nucleus, might be useful drug targets for subtype-specific AChR agonists intended for therapy of Parkinson's disease.

With our initial demonstration that α6 can function as part of AChRs formed from subunit cDNAs expressed in X. laevis oocytes, α6 leaves the ranks of orphan subunits and joins the company of numerous potential AChR subtypes that are much better characterized as expressed cDNAs in oocytes than they are in any native neurons. Now the challenge is to detect α6 AChR proteins in neurons, determine their subunit composition, and relate their functional properties to those we have observed in oocytes.

Acknowledgments

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