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The ion channel properties of a rat recombinant neuronal nicotinic receptor are dependent on the host cell type

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1. A stable mammalian cell line (L-$\alpha 3\beta 4$) has been established which expresses the cloned rat neuronal nicotinic acetylcholine receptor (nAChR) subunits $\alpha 3$ and $\beta 4$, which are the most abundant in autonomic ganglia. Ion channel properties of nAChRs expressed in L-$\alpha 3\beta 4$ cells were investigated by single-channel and whole-cell recording techniques, and compared with both rat $\alpha 3\beta 4$ nAChRs expressed in *Xenopus* oocytes, and endogenous nicotinic receptors in rat superior cervical ganglion (SCG) neurones, using identical solutions for all cell types.
2. Acetylcholine (ACh) caused activation of single ion channel currents with a range of amplitudes. Some channels had high conductances (30–40 pS), and relatively brief lifetimes; these resembled the predominant native channel from SCG. Other channels had low conductances (20–26 pS) and long bursts of openings which were quite unlike native channels, but which were similar to channels formed by $\alpha 3\beta 4$ in oocytes. Both types often occurred in the same patch.
3. Cytisine was about 3 times more potent than ACh (low-concentration potency ratio) in L-$\alpha 3\beta 4$ cells, which is not dissimilar to the 5-fold potency ratio found in both SCG and oocytes, whereas 1,1-dimethyl-4-phenylpiperazinium (DMPP) was less potent than ACh in some cells (as in the oocyte), but more potent in others (as in SCG).
4. While the channels expressed in L-$\alpha 3\beta 4$ cells do not mimic exactly those expressed in rat SCG, they differ considerably from the same subunit combination expressed in oocytes. Larger conductance, SCG-like channels were detected frequently in L-$\alpha 3\beta 4$, but were rarely, if ever, seen in oocytes injected with $\alpha 3$ and $\beta 4$ mRNA. Our results indicate that ion channel properties such as single-channel conductance can be influenced by the choice of heterologous expression system.

Knowledge of the subunit composition of oligomeric neurotransmitter-gated ion channels is of considerable physiological and pharmacological importance. Whereas the subunit composition of the nAChR, which is expressed at the vertebrate neuromuscular junction, has been established with reasonable confidence ($\alpha_2\beta\epsilon\delta$ in the adult and $\alpha_2\beta\gamma\delta$ in fetal muscle), it is far from clear which subunits are present in the numerous nAChRs that are pharmacologically distinguishable in the central and peripheral nervous system (the 'neuronal' nAChRs). Up to now, eleven neuronal nAChR subunits ($\alpha 2$–$\alpha 9$ and $\beta 2$–$\beta 4$) have been identified by molecular cloning (Green & Millar, 1995; McGehee & Role, 1995), in addition to the five mammalian muscle nAChR subunits (α, β, γ, δ and ϵ).

One of the better characterized native neuronal nAChRs is that which mediates fast synaptic transmission in the rat superior cervical ganglion (SCG). The most abundant nAChR mRNAs present in SCG neurones encode the $\alpha 3$ and $\beta 4$ subunits, although other nAChR subunit mRNAs have been detected at lower levels (Rust, Burgunder, Lauterburg & Cachelin, 1994; Mandelzys, De Koninck & Cooper, 1995). Heterologous expression in *Xenopus* oocytes of the cloned rat $\alpha 3$ and $\beta 4$ subunits (and of other subunit combinations which might be expected to be expressed in SCG) generates channels which do not resemble the native SCG receptors in single-channel conductance or kinetics (Sivilotti, McNeil, Lewis, Nassar, Schoepfer & Colquhoun, 1997), and which also differ from SCG in relative agonist potencies (Covernton, Kojima, Sivilotti, Gibb & Colquhoun, 1994). This has led to the conclusion that either our assumptions about the subunit composition of the SCG receptor are incorrect, or that the oocyte assembles neuronal nAChRs incorrectly (Sivilotti *et al.* 1997).

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The main single-channel conductance of native rat SCG nAChRs is 35–40 pS (in 1 mM external Ca^{2+}), though the spread of conductances is unusually wide, and other levels are present (Mathie, Colquhoun & Cull-Candy, 1990; Mathie, Cull-Candy & Colquhoun, 1991; Sivilotti *et al.* 1997). In contrast, recombinant nAChRs generated by the expression in *Xenopus* oocytes of the cloned rat $\alpha3$ and $\beta4$ subunits produce channels with considerably smaller conductances (about 22 pS for $\alpha3\beta4$) (Papke, 1993; Sivilotti *et al.* 1997). Until recently, it has proved surprisingly difficult to express functional neuronal nicotinic receptors reliably in mammalian cell lines. In the present study we have examined the ion channel properties of the rat $\alpha3$ and $\beta4$ subunits expressed in a stably transfected mammalian cell line (L-$\alpha3\beta4$). Although the channel properties of nAChRs expressed in L-$\alpha3\beta4$ cells do not mimic exactly the properties of native rat SCG channels, high conductance SCG-like channels were detected frequently in L-$\alpha3\beta4$ cells, but not in *Xenopus* oocytes expressing the rat $\alpha3$ and $\beta4$ subunits.

Our results indicate that the rat neuronal nicotinic $\alpha3$ and $\beta4$ subunits generate nAChRs which display substantially different ion channel properties according to whether they are expressed in *Xenopus* oocytes or in a cultured mammalian cell line, and that L cells produce some channels which are very similar to those in SCG.

METHODS

Plasmids and cell lines

Rat neuronal nAChR $\alpha3$ and $\beta4$ subunit cDNAs (Boulter *et al.* 1986; Duvoisin, Deneris, Patrick & Heinemann, 1989) were subcloned into the *Nhe* I and *Xho* I sites of the inducible mammalian expression vector pMSG (Pharmacia) to create plasmids pMSG-$\alpha3$ and pMSG-$\beta4$. Mouse fibroblast L929 cells (European Collection of Cell Cultures; No. 85011425) were grown in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum (Gibco). Cells were co-transfected with pMSG-$\alpha3$ and pMSG-$\beta4$ by a modified calcium phosphate co-precipitation method (Chen & Okayama, 1987). Stably transfected clonal cell lines were selected by serial dilution in 96-well plates in the presence of mycophenolic acid and aminopterin, as described by Mulligan & Berg (1981). After expansion of clonal cell populations, expression of nAChR subunit cDNAs from the mouse mammary tumour virus promoter of pMSG was induced by the addition of dexamethasone (1 μM final concentration) to the culture medium typically for 5–10 days.

Radioligand binding

Binding studies with [^{3}H]-epibatidine (DuPont NEN) were performed on cell membrane preparations as described by Lansdell, Schmitt, Betz, Sattelle & Millar (1997). Curves for equilibrium binding were fitted by weighted least squares (CVFIT program, David Colquhoun, University College London).

Intracellular calcium measurement

Fluorescence ratiometric intracellular calcium measurements were performed on populations of fura-2 AM-loaded cells (typically between 5×10^6 and 1×10^7 cells) using a Perkin-Elmer LS-50B fluorescence spectrometer fitted with a stirred cuvette holder and fast filter accessory. Details of cell loading and fluorimetry have been described previously (Cooper & Millar, 1997).

Electrophysiological recording

One of the aims of this study is to compare the properties of $\alpha3\beta4$ receptors expressed in L cells with those expressed in *Xenopus* oocytes, and with the native nicotinic receptors from rat SCG. Consequently, identical experimental methods (in particular, identical extracellular solutions) were used for all three cell types, as in our previous studies (Covernton *et al.* 1994; Sivilotti *et al.* 1997). Both whole-cell and single-channel current recordings were made at room temperature (18–20 °C) using an Axopatch 200A (Axon Instruments) amplifier, low-pass filtered at 10 kHz (4-pole Bessel, −3 dB) and stored on a digital tape recorder (DTR1204, Biologic Science Instruments). The standard bathing solution was of composition (mM): NaCl, 150; KCl, 2·8; $MgCl_2$, 2; $CaCl_2$, 1; Hepes, 10; with atropine sulphate, 0·5 μM (BDH); pH 7·2 adjusted with NaOH. Patch pipettes were pulled from thick-walled borosilicate glass (GC150F; Clark Electromedical Instruments), coated with Sylgard® (Dow Corning 184) and fire polished before use to a final resistance of 2–5 MΩ for whole-cell recordings, or 8–10 MΩ for outside-out patches.

Whole-cell currents were recorded at a holding potential of −60 mV, with a pipette solution of composition (mM): KCl, 130; MgCl, 4; Tes, 10; EGTA 10; pH 7·2 adjusted with NaOH. These currents were filtered at 1 kHz (8-pole Bessel, −3 dB) and recorded on a chart recorder. Single-channel currents were recorded at a holding potential of −100 mV with a pipette solution of composition (mM): CsCl, 80; CsF, 60; Hepes, 10; EGTA, 10; $CaCl_2$, 1; pH 7·2 adjusted with KOH.

Recordings of single-channel currents were filtered at 1–2 kHz (8-pole Bessel, −3 dB), digitized at 10–20 kHz (CED 1401plus interface; Cambridge Electronic Design) and then analysed by the method of time course fitting using the program SCAN (Colquhoun & Sigworth, 1995). Stretches of the records where more than one channel was open simultaneously were excised from the analysis. After time course fitting, a fixed time resolution was imposed on each idealized record before analysis of distributions, using as a criterion an expected false event rate of less than 10^{-8} s^{-1}. Amplitudes were obtained from fitted open periods (as defined in Sivilotti *et al.* 1997) which were longer than 3 times the filter rise time, and their distribution was fitted with Gaussian distributions by maximum likelihood estimation (Colquhoun & Sigworth, 1995). Chord conductance values assume a reversal potential of 0 mV. Distributions of apparent open periods were obtained from the idealized records after imposing a resolution of 200 μs, and the resultant distributions were fitted with mixtures of exponential densities using maximum likelihood estimation. Data records which had been heavily excised because of double openings were not analysed for open periods in order to avoid errors introduced by length-biased sampling.

Agonists were applied to whole-cell and outside-out patches with a modified U-tube (Greenfield & Macdonald, 1996), with a gravity feed. The agonists ACh chloride, (−)-cytisine (both from Sigma) and 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP; Aldrich) were stored as 10^{-2} M frozen aliquots, and dilutions were made up fresh from an aliquot each day.

Low-dose concentration–response curves were obtained from whole-cell recordings with two or three points for each agonist. By using low doses to determine the limit of the potency ratios, the effects of desensitization are minimized. The potency ratios were defined as the ratio of the concentration of ACh to the equi-effective concentration of the agonist being tested. The horizontal distance between the concentration–response curves was determined by least-squares fitting of power functions (straight lines in log–log co-

ordinates) constrained to be parallel. Separate, unconstrained fits were also done to judge the degree of parallelism. The standard deviations of the mean for the potency ratios were estimated using Fieller's theorem (see Colquhoun, 1971).

RESULTS

Construction of stable cell lines

The rat neuronal nAChR $\alpha3$ and $\beta4$ subunit cDNAs (Boulter *et al.* 1986; Duvoisin *et al.* 1989) were subcloned into the mammalian expression vector pMSG to create plasmids pMSG-$\alpha3$ and pMSG-$\beta4$. Mouse fibroblast L929 cells were co-transfected with pMSG-$\alpha3$ and pMSG-$\beta4$, and stably transfected clonal cell lines isolated by serial dilution in selection medium. A clonal cell line (L-$\alpha3\beta4$-12) which showed specific binding of nicotinic radioligands was then recloned by serial dilution. After expansion, the resulting clonal cell lines were screened for expression of functional nAChRs by fluorescent ratiometric measurement of agonist-induced elevations in intracellular calcium, as has been described previously (Cooper & Millar, 1997). A clonal isolate (L-$\alpha3\beta4$-12/36) which expressed functional nAChR channels and moderate levels of nicotinic radioligand binding (B_{max}, $0{\cdot}1 \pm 0{\cdot}05$ pmol mg^{-1}) was selected for detailed electrophysiological characterization.

As might have been expected from the reported failure of either the $\alpha3$ or $\beta4$ subunits to generate functional channels when expressed alone in *Xenopus* oocytes (Boulter, Connolly, Deneris, Goldman, Heinemann & Patrick, 1987; Duvoisin *et al.* 1989), we were unable to detect specific binding of nicotinic radioligands or functional nAChRs when the $\alpha3$ or

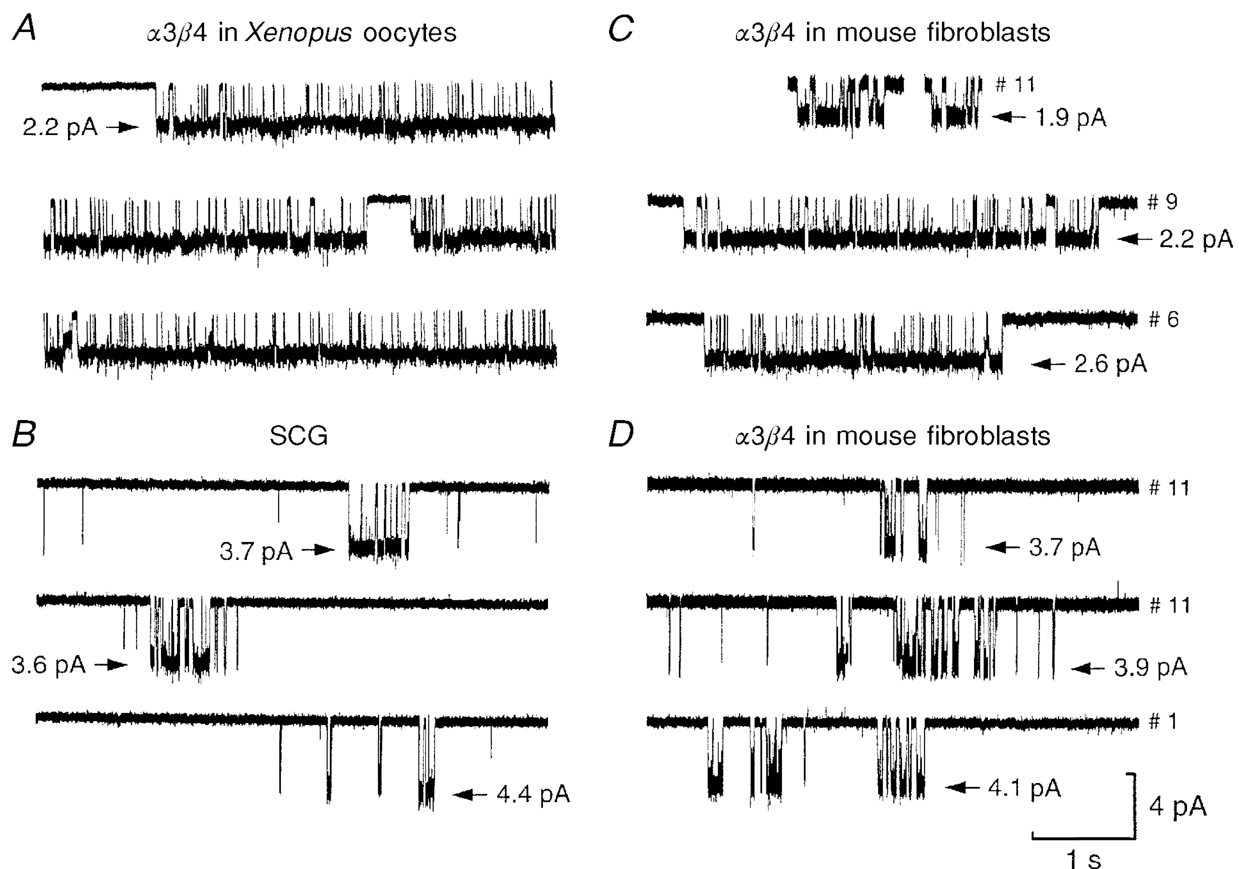

Figure 1. Single-channel activity of native and recombinant nicotinic receptors

Single-channel activity of $\alpha3\beta4$ recombinant nicotinic receptors expressed in *Xenopus* oocytes (*A*) and mouse fibroblasts (*C* and *D*). Similar recordings obtained from native nicotinic receptors of adult SCG are displayed for comparison in *B*. Recordings were obtained in the outside-out patch configuration (holding potential, −100 mV) from *Xenopus* oocytes (*A*) and from mouse fibroblasts stably expressing $\alpha3\beta4$ nicotinic subunits (*C* and *D*). The oocyte trace in *A* shows a continuous stretch of record. The channels from SCG in *B* show three separate segments of record from one patch. Both *A* and *B* show only the most common amplitudes, for comparison. The records from L-$\alpha3\beta4$ cells in *C* and *D* show a more representative selection of amplitudes. Each trace is labelled with (a) the number of the patch (#) from which they came (numbering as in Fig. 2*B*) and (b) the approximate amplitude of the channels that are shown. The amplitude distributions for patches 1, 9 and 11 are shown in Fig. 3. The variability of amplitudes is illustrated by the fact that the individual channels shown in the upper two traces of *D* (patch 11) have amplitudes of about 3·6 and 3·8 pA, though the mean large conductance in Fig. 3*C* was 3·45 pA.

$\beta 4$ subunits were expressed individually in various eukaryotic cell lines (data not shown). The ability of the $\alpha 3\beta 4$ nAChR expressed in L-$\alpha 3\beta 4$ cells to bind nicotinic radioligands indicates that the formation of an agonist binding site requires the co-assembly of the $\alpha 3$ and $\beta 4$ subunits.

Single-channel properties of nAChRs in L-$\alpha 3\beta 4$ cells

The level of expression of $\alpha 3\beta 4$ nAChRs in L cells was assessed by the whole-cell current elicited from application of 1 mM ACh. Of eighteen cells tested with this concentration, fifteen responded with currents ranging from 10 to 180 pA, with a mean ($\pm$ S.D.) of 58 ± 47 pA. Cells were used from 5 days after induction with dexamethasone, and recordings of nicotinic currents could be obtained up to 21 days after induction.

Single-channel recordings were made from outside-out patches in which activity could be identified unequivocally as resulting from nAChRs after application of ACh (1–10 μM). A variety of single-channel amplitudes were observed in all patches, as described previously for both native SCG nAChRs and $\alpha 3\beta 4$ expressed in oocytes (Sivilotti *et al.* 1997). Examples of single-channel events from L-$\alpha 3\beta 4$ cells are shown in Fig. 1*C* and *D*, alongside previously recorded examples of the main conductance displayed by $\alpha 3\beta 4$ in *Xenopus* oocytes (Fig. 1*A*) and by native receptors from SCG (Fig. 1*B*).

Patches from L-$\alpha 3\beta 4$ cells may contain low conductance levels with openings clustered in long bursts as in oocyte-expressed $\alpha 3\beta 4$, and/or higher conductance levels active in short bursts as in SCG channels. Figure 2*A* shows examples

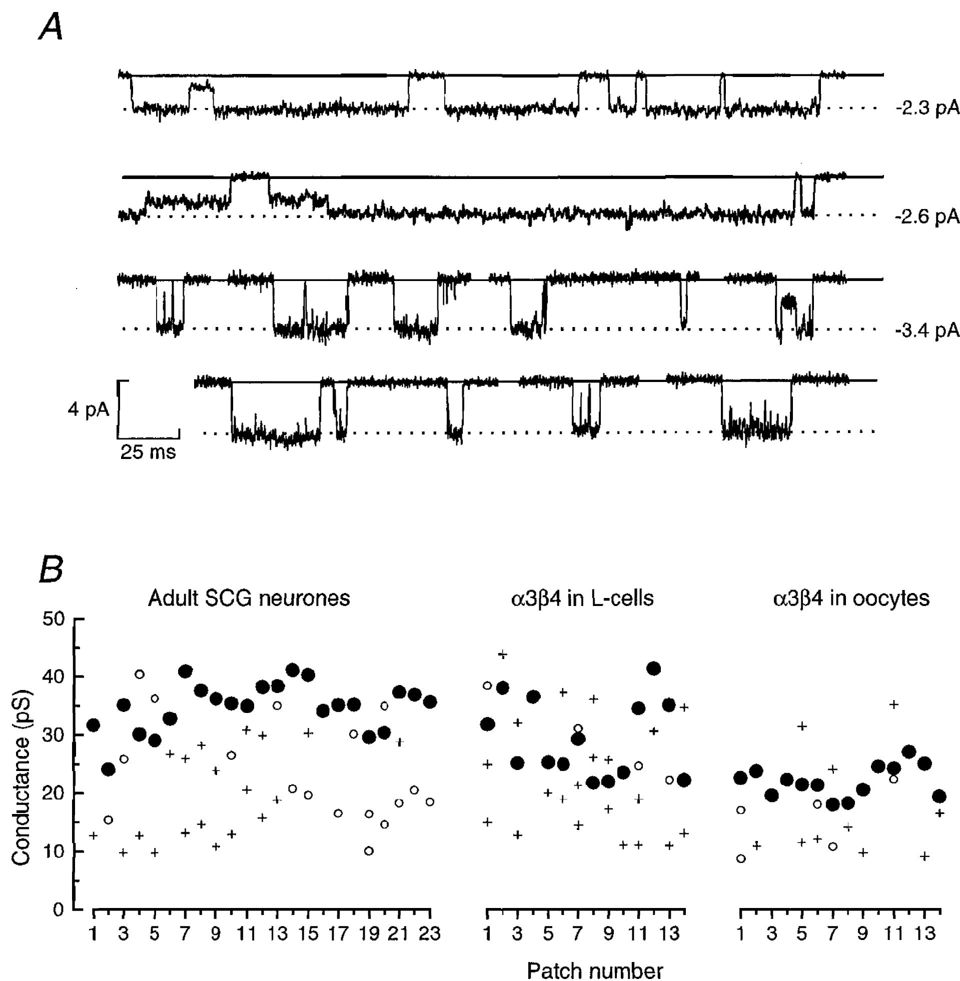

Figure 2. Properties of recombinant $\alpha 3\beta 4$ single-channels expressed in mouse fibroblasts

A, examples of current recordings showing the pattern of $\alpha 3\beta 4$ single-channel activity. Note that the higher conductance states (bottom) have much shorter apparent open times and more infrequent openings to sublevel states compared with the lower conductance states (top). *B*, summary of chord conductances elicited by nicotinic agonists in outside-out patches from adult superior cervical ganglia and from mouse fibroblasts and *Xenopus* oocytes expressing $\alpha 3\beta 4$ nicotinic subunits. Filled circles represent the most common conductance in each patch, while open circles and crosses show secondary conductance levels (more than 20% of the total number of fitted amplitude values or less than 20%, respectively). Results for SCG and oocytes are adapted from Sivilotti *et al.* (1997).

of both classes of channels from L-$\alpha3\beta4$ cells at a higher sweep speed, and Fig. 2*B* shows a summary of all the chord conductances from L-$\alpha3\beta4$ cells (centre), compared with those from SCG (left) and oocytes (right). It can be seen from this summary that, in six of fourteen L-$\alpha3\beta4$ cell patches, the main Gaussian component was a high conductance similar to that of SCG channels (30–40 pS; see also Fig. 3*A* and *C*). The majority of all channel openings were above 30 pS in four of fourteen patches (as in Fig. 3*A*); indeed three of fourteen patches displayed only conductances above 30 pS. Another three patches displayed only small conductances (less than 30 pS; see also Fig. 3*B*). In the remaining eight patches, conductances below 30 pS represented an average of 67·7 ± 9·7 % of all fitted openings (see Fig. 3*C*).

The complexity of the single-channel amplitudes is better illustrated by the amplitude distributions shown in Fig. 3, which shows examples of a predominantly 'SCG-like' patch (*A*), a predominantly 'oocyte-like' patch (*B*) and a patch containing a mixture of both (*C*).

A characteristic of nAChRs recorded in outside-out patches from oocytes or native SCG is the rapid 'run-down' of activity after excision: this phenomenon is independent of the presence of agonist and appears to be faster in oocytes than in SCG. Run-down was observed also in outside-out patches from L-$\alpha3\beta4$ cells, though it was not as fast as previously seen in oocytes. There appeared to be a differential run-down of the small and large conductance events with the large conductance events persisting for longer (as in SCG) than the smaller oocyte-like channels.

The kinetics of channel activations were hard to examine in detail because of run-down and because of channel heterogeneity. Nevertheless it was obvious that the smaller conductance events tended to occur in long bursts much like those previously described for $\alpha3\beta4$ in oocytes, and the larger conductance events tended to occur in short bursts, like the SCG receptors. These differences were reflected also in differences in the mean apparent open period. In patches where there were predominantly small conductance (22–25 pS) events, fitted open-period distributions were

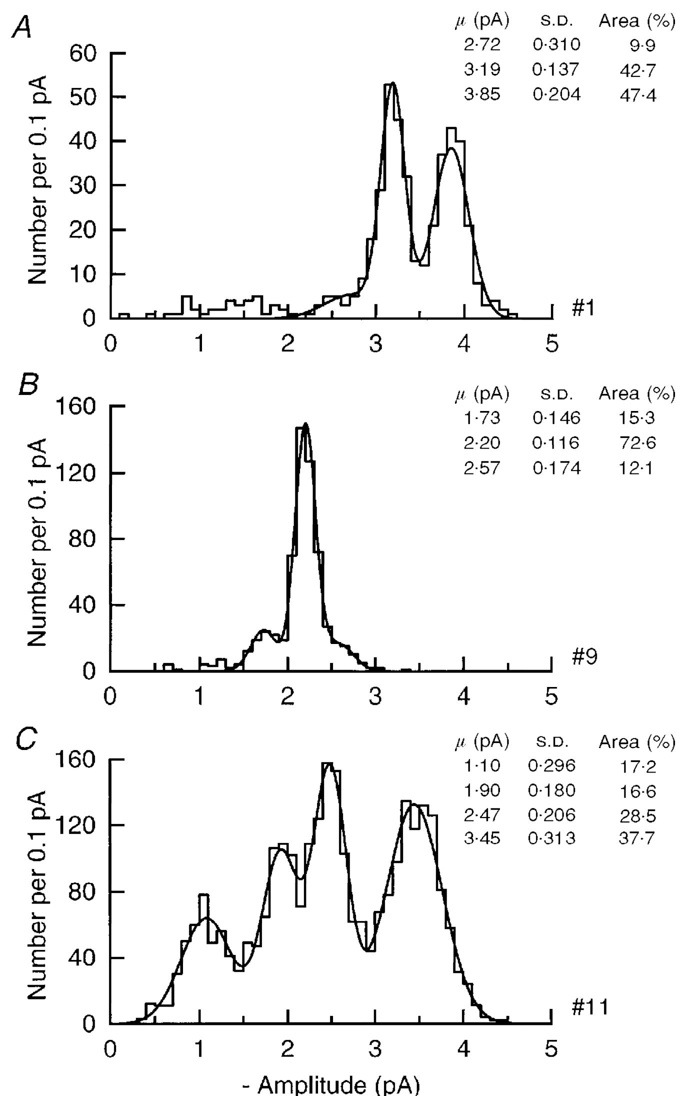

Figure 3. Examples of single-channel current amplitude distributions from mouse fibroblasts expressing $\alpha3\beta4$ nicotinic subunits

Examples were chosen to show a patch with predominantly high conductances (patch 1 in Fig. 2*B*) (*A*); a patch with predominantly low conductances (patch 9 in Fig. 2*B*) (*B*); and a patch which displayed the whole range of conductances (patch 11 in Fig. 2*B*) (*C*). Note also the difference between patches in the means and standard deviations of the Gaussian components fitted to the distributions (even for similar conductance levels).

dominated by a single exponential component with a mean open time of 48·6 ± 3·2 ms ($n = 6$). This may be compared with a predominant component of 47·8 ms found for oocyte-expressed $\alpha3\beta4$ (Sivilotti *et al.* 1997). Patches with predominantly large events usually required two exponentials with components 0·43 ± 0·14 ms (32%) and 14·7 ± 0·7 ms (68%) ($n = 4$), with an overall mean of 10·2 ± 0·9 ms. This is comparable to values found for SCG channels of 1·1 ms (44%) and 20·2 ms (56%), with an overall mean of 11·8 ms (Sivilotti *et al.* 1997). For these analyses a resolution of 200 μs was imposed on both open and shut times throughout, even when the record had better resolution. This is essential in order to ensure comparability with results in other cells, some of which could not be analysed at better resolution. With the optimum resolution the open periods were a good deal shorter, because fewer brief shuttings were missed (for example in one experiment the slower time constant was 15·0 ms at 200 μs resolution, but 4·6 ms at 50 μs resolution). Generally, burst analysis of the small conductance events was not possible because of their long open periods and the short recordings due to run-down.

Agonist potency ratios

Partial concentration–response curves were determined for the nicotinic agonists ACh, DMPP and cytisine. Examples of these experiments are shown in Fig. 4*A* and *B*.

In most of the cells tested (6 of 8, see Fig. 4*A*), DMPP was less potent than ACh. The limiting low-concentration potency ratios for these agonists, relative to ACh, were 0·60 ± 0·03 ($n = 6$) for DMPP, and 3·0 ± 0·26 ($n = 7$) for cytisine. These values are rather closer to those found in oocytes (DMPP, 0·43; cytisine, 5·1), than to those in SCG neurones (DMPP, 2·5; cytisine, 4·8) (Covernton *et al.* 1994). However, in two of eight cells, DMPP was slightly (about 1·2-fold) *more* potent than ACh (cf. 2·5-fold in SCG), though the relative potency of cytisine was similar to that in the other cells (as illustrated in Fig. 4*B*). This could result from heterogeneity of the predominant channel type; it is clear that there are at least two populations of receptors in L-$\alpha3\beta4$ cells, as judged by their conductance and kinetic properties, and the whole-cell response will be the sum of their separate responses.

DISCUSSION

Molecular cloning has revealed a baffling diversity of subunits for oligomeric ion channels that mediate fast synaptic transmission in the brain and periphery (Green & Millar, 1995; McGehee & Role, 1995). It is, therefore, important to know which of the enormous number of possible subunit combinations occur in real synapses. One of the most important tools for investigation of this problem is heterologous expression of combinations of cloned ion channel subunits and comparison of these recombinant channels with native channels. Progress in this field is critically dependent on the assumption that a given subunit composition will give rise to the same channel behaviour whether it is expressed in a heterologous system or in its

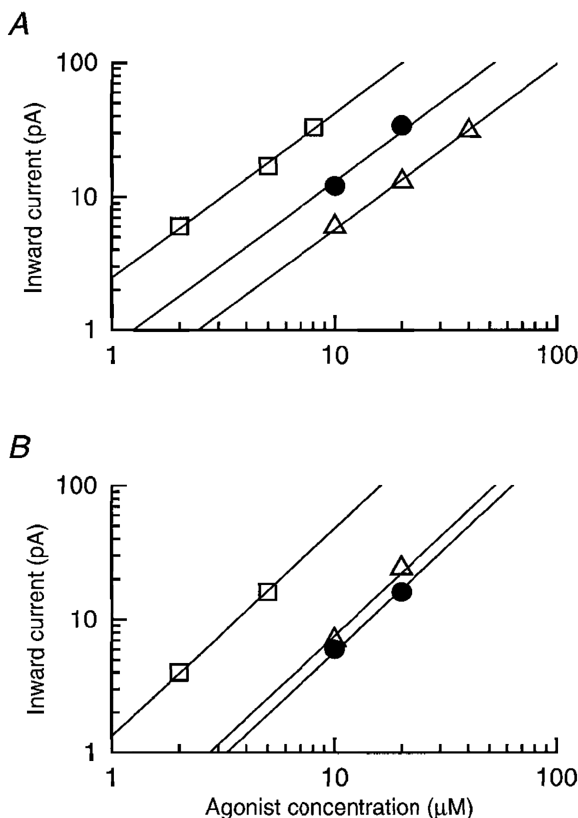

Figure 4. Fitted dose–response curves for whole-cell responses to nicotinic agonists in mouse fibroblasts stably expressing $\alpha3\beta4$ receptors

The curves were fitted with power functions (straight lines in log–log coordinates) constrained to be parallel for all the agonists applied to a cell, and potency ratios were found from the horizontal separation of the fitted curves. *A* shows an example of the most common type of response (DMPP less potent than ACh; $n = 6$ of 8 cells tested). In some cells (2 of 8 tested) DMPP was more potent than ACh (*B*). Symbols: ●, ACh; △, DMPP; □, cytisine.

native environment. We have found previously that recombinant nicotinic acetylcholine receptors (nAChRs), expressed in the widely used *Xenopus* oocyte system, do not resemble native channels expressed in mammalian sympathetic ganglia (Sivilotti *et al.* 1997). Now we find that the single-channel properties of neuronal nAChRs generated by the heterologous co-expression of the $\alpha 3$ and $\beta 4$ subunits in a mammalian cell line differ considerably from those measured under identical conditions in oocytes, and that a subclass of these channels mimics more closely the endogenous channels in autonomic ganglia than do those expressed by the oocyte.

Brief reports of the expression of $\alpha 3\beta 4$ in mammalian cell lines have appeared, but give little detail (Wong, Holstad, Mennerick, Hong, Zorumski & Isenberg, 1995; Stetzer *et al.* 1996); the latter reported a slope conductance of 29 pS (in 2 mM external Ca^{2+}), but showed no distributions. A recent study has investigated the single-channel properties of $\alpha 3\beta 4$ expressed transiently in the human BOSC 23 cell line (Ragozzino *et al.* 1997). They found, as we do, that both large and small conductance channels were produced by transfection of the rat $\alpha 3$ and $\beta 4$ subunits. They found large (average 34 pS) channels in nine of eleven patches and small (21 pS) channels in two patches. Beyond this, direct comparison with our results is difficult, because their results were fitted by a threshold-crossing method which is not well suited to records that contain many different amplitudes, such as we find in both native (SCG) and recombinant (oocyte and L cell) recordings. In addition, their solutions differ somewhat from ours; they used (mM): NaCl, 116·4; KCl, 5·4; $CaCl_2$, 1·8; $MgSO_4$, 0·8; $NaHCO_3$, 26·2; NaH_2PO_4, 1; Hepes, 25, for single-channel recording, whereas we used the same extracellular solution for all three cell types ((mM): NaCl, 150; KCl, 2·8; $MgCl_2$, 2; $CaCl_2$, 1; Hepes, 10) to ensure comparability of conductance values for native and recombinant receptors. This is necessary because single-channel conductances depend (at least) on the external sodium and calcium concentrations. Nevertheless their larger conductance channel, like ours, shows similarities to the native channel found in SCG. They give no details concerning sublevel transitions, or of their lower conductance channel, but the conductance of the latter seems to be similar to the channel which we describe here as 'oocyte-like'. Their results seem to be compatible with the inference from ours, that mammalian cell lines can express $\alpha 3\beta 4$ channels which resemble closely the native channels found in SCG, but do not always do so, and they may also produce 'incorrect' channels which, from our results, appear to be similar (in respect of their low conductance and long bursts of openings) to those usually produced by expression in oocytes. Admittedly, we cannot be sure that all (or indeed any) of the channels in the heterogeneous population of SCG receptors are made up only of $\alpha 3$ and $\beta 4$ subunits (for a discussion of the possible role of other subunits, see McGehee & Role, 1995).

In summary, it is clear that there are at least two populations of receptors in L-$\alpha 3\beta 4$ cells, as judged by their conductance and kinetic properties. Exactly what these two putative populations are, and how they come about, is still unknown. They could result from variations in folding or assembly of the subunits, from variable stoichiometry or variable post-translational modification. Whatever the reason may be, the practical consequences are that neither oocytes nor L cells can be relied on to produce a homogeneous population of channels when transfected with two subunit types; that these two expression systems produce different mixtures of channels, and that the mammalian cell line produces far more channels that resemble native neuronal nicotinic receptors of SCG. Further work will clearly be needed to find an expression system which reproduces faithfully the properties of neuronal nicotinic receptors in their native environment. This aim is undoubtedly hindered by the fact that native channels, as well as those expressed in oocytes and L-$\alpha 3\beta 4$ cells, are not homogeneous.

Boulter, J., Connolly, J., Deneris, E., Goldman, D., Heinemann, S. & Patrick, J. (1987). Functional expression of two neuronal nicotinic acetylcholine receptors from cDNA clones identifies a gene family. *Proceedings of the National Academy of Sciences of the USA* **84**, 7763–7767.

Boulter, J., Evans, K., Goldman, D., Martin, G., Treco, D., Heinemann, S. & Patrick, J. (1986). Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor α-subunit. *Nature* **319**, 368–374.

Chen, C. & Okayama, H. (1987). High-efficiency transformation of mammalian cells by plasmid DNA. *Molecular and Cellular Biology* **7**, 2745–2752.

Colquhoun, D. (1971). *Lectures on Biostatistics.* Clarendon Press, Oxford.

Colquhoun, D. & Sigworth, F. J. (1995). Fitting and statistical analysis of single-channel records. In *Single-Channel Recording*, ed. Sakmann, B. & Neher, E., pp. 483–587. Plenum Press, New York.

Cooper, S. T. & Millar, N. S. (1997). Host cell-specific folding and assembly of the neuronal nicotinic acetylcholine receptor $\alpha 7$ subunit. *Journal of Neurochemistry* **68**, 2140–2151.

Covernton, P. J. O., Kojima, H., Sivilotti, L. G., Gibb, A. J. & Colquhoun, D. (1994). Comparison of neuronal nicotinic receptors in rat sympathetic neurones with subunit pairs expressed in *Xenopus* oocytes. *Journal of Physiology* **481**, 27–34.

Duvoisin, R. M., Deneris, E. S., Patrick, J. & Heinemann, S. (1989). The functional diversity of the neuronal nicotinic acetylcholine receptors is increased by a novel subunit: $\beta 4$. *Neuron* **3**, 487–496.

Green, W. N. & Millar, N. S. (1995). Ion-channel assembly. *Trends in Neurosciences* **18**, 280–287.

Greenfield, L. J. Jr & Macdonald, R. L. (1996) Whole-cell and single-channel $\alpha 1$ $\beta 1$ $\gamma 2$S $GABA_A$ receptor currents elicited by a 'multipuffer' drug application device. *Pflügers Archiv* **432**, 1080–1090

Lansdell, S. J., Schmitt, B., Betz, H., Sattelle, D. B. & Millar, N. S. (1997). Temperature-sensitive expression of *Drosophila* neuronal nicotinic acetylcholine receptors. *Journal of Neurochemistry* **68**, 1812–1819.

McGehee, D. S. & Role, L. W. (1995). Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annual Review of Physiology* **57**, 521–546.

Mandelzys, A., De Koninck, P. & Cooper, E. (1995). Agonist and toxin sensitivities of ACh-evoked currents on neurons expressing multiple nicotinic ACh receptor subunits. *Journal of Neurophysiology* **74**, 1212–1221.

Mathie, A., Colquhoun, D. & Cull-Candy, S. G. (1990). Rectification of currents activated by nicotinic acetylcholine receptors in rat sympathetic ganglion neurones. *Journal of Physiology* **427**, 625–655.

Mathie, A., Cull-Candy, S. G. & Colquhoun, D. (1991). Conductance and kinetic properties of single nicotinic acetylcholine receptor channels in rat sympathetic neurones. *Journal of Physiology* **439**, 717–750.

Mulligan, R. C. & Berg, P. (1981). Selection for animal cells that express the *Escherichia coli* gene coding for xanthine–guanine phosphoribosyltransferase. *Proceedings of the National Academy of Sciences of the USA* **78**, 2072–2076.

Papke, R. L. (1993). The kinetic properties of neuronal nicotinic acetylcholine receptors: genetic basis of functional diversity. *Progress in Neurobiology* **41**, 509–531.

Ragozzino, D., Fucile, S., Giovannelli, A., Grassi, F., Mileo, A. M., Ballivet, M., Alemá, S. & Eusebi, F. (1997). Functional properties of neuronal nicotinic acetylcholine receptor channels expressed in transfected human cells. *European Journal of Neuroscience* **9**, 480–488.

Rust, G., Burgunder, J.-M., Lauterburg, T. E. & Cachelin, A. B. (1994). Expression of neuronal nicotinic acetylcholine receptor subunit genes in the rat autonomic nervous system. *European Journal of Neuroscience* **6**, 478–485.

Sivilotti, L. G., McNeil, D. K., Lewis, T. M., Nassar, M. A., Schoepfer, R. & Colquhoun, D. (1997). Recombinant nicotinic receptors, expressed in *Xenopus* oocytes, do not resemble native rat sympathetic ganglion receptors in single-channel behaviour. *Journal of Physiology* **500**, 123–138.

Stetzer, E., Ebbinghaus, U., Storch, A., Poteur, L., Schrattenholz, A., Kramer, G., Methfessel, C. & Maelicke, A. (1996). Stable expression in HEK-293 cells of the rat $\alpha3/\beta4$ subtype of neuronal nicotinic acetylcholine receptor. *FEBS Letters* **397**, 39–44.

Wong, E. T., Holstad, S. G., Mennerick, S. J., Hong, S. E., Zorumski, C. F. & Isenberg, K. E. (1995) Pharmacological and physiological properties of a putative ganglionic nicotinic receptor, $\alpha3\ \beta4$, expressed in transfected eucaryotic cells. *Molecular Brain Research* **28**, 101–109.

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