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Life Sciences 78 (2006) 2812 – 2819
Life Sciences
www.elsevier.com/locate/lifescie

Estimation of both the potency and efficacy of α7 nAChR agonists from single-concentration responses

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Received 17 August 2005; accepted 2 November 2005

Abstract

The assessment of functional properties is a crucial step in the screening of potential new drug candidates. The development of moderate to high throughput electrophysiological recording systems such as OpusXpress (Molecular Devices) has facilitated the process of testing new drugs to a large degree. However, while the simple screening of multiple drugs at a single concentration identifies "hits" and "misses", the generation of full concentration–response studies is still a bottleneck in drug development. The α7 nicotinic acetylcholine receptor displays a unique concentration dependence of response kinetics which permits estimates of EC_{50} and I_{max} values for experimental drugs to be generated from single-concentration responses. This method is based on the analysis of 13 different concentration–response studies utilizing either human or rat α7 nAChR. Each experimental response was first normalized to an ACh control, and then a transformation of the pooled data was generated which, based on the relationship between the net charge and peak current to their respective EC_{50} values defined the "functional concentration" (the test concentration relative to the EC_{50} for the given agonist). At low functional concentrations, net charge is large relative to peak current amplitude and at higher functional concentration this relationship reverses. For any single-concentration response, the ratio of net charge to peak current can be used to estimate functional concentration. Efficacy can then be estimated by comparing the observed (net charge) response to the expected value for a full agonist at the estimated functional concentration. This extended analysis, combined with automated recording methods, should greatly increase the efficiency with which promising new drug candidates can be characterized.

Keywords: Drug development; Alzheimer's disease; Schizophrenia; Drug screening

Introduction

The identification of new drug candidates most often begins with the screening of many experimental compounds. In this context, the concept of high throughput becomes of great importance. However, high throughput approaches often rely on either binding assays, which cannot distinguish agonists from antagonists, or fluorescence-based functional studies, which may not distinguish between full agonists and partial agonists. Therefore, for drugs that target ion channel receptors, electrophysiology, in particular voltage-clamp experiments, remains the gold standard for functional studies, and recent advances in automated recording systems have brought these methods into consideration for high throughput.

A common first pass in a screening procedure is to evaluate an array of compounds at a single test concentration. Such single-concentration testing can serve to distinguish between active and inactive compounds but provides little information about the actual potency and efficacy of the active compounds, since a potent partial agonist and a full agonist of low potency might give responses of similar amplitude. The α7-type nicotinic acetylcholine receptor (nAChR) has been acknowledged as a potential target for a number of diverse indications including Alzheimer's disease, schizophrenia, and even peripheral inflammation (Freedman et al., 2000; Kem, 2000; Wang et al., 2003). This paper describes a method for greatly increasing the efficiency of drug screening for this target by estimating both EC_{50} and efficacy values from single-concentration screens.

The α7-type nAChR exhibits a unique concentration-dependent form of desensitization such that when exposed to a high concentration of agonist, synchronous channel activa-

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doi:10.1016/j.lfs.2005.11.009

tion is maximal prior to the completion of the solution exchange. This is a consistent feature of α7-mediated responses, regardless of whether agonist concentration rises over the course of several seconds, as in an oocyte experiment (Papke and Thinschmidt, 1998), or over just a few milliseconds as in the case with rapid solution delivery to an acutely isolated neuron (Papke et al., 2000; Uteshev et al., 2002). We have suggested that this could arise from extremely rapid desensitization or deactivation of receptors fully saturated at their agonist binding sites. As a consequence of the preferential desensitization of fully liganded receptors, channels tend to be open only within a limited concentration range, corresponding to what would produce a relatively low fractional occupancy of the binding sites. The application of a relatively low concentration of agonist can sustain that condition and maintain channel activation for several seconds of agonist application, and the net charge evoked by such an agonist application can be relatively large, even though the peak current representing synchronous activation of channels is relatively small (Papke et al., 2000). These features of the α7 receptor responses account for qualitative differences in the concentration dependence for net charge and peak current responses (Papke and Papke, 2002). Of these two measurements, arguably, the net charge measure is of greater physiological significance and scientific validity (Papke and Papke, 2002). Nonetheless, peak current amplitudes, and more importantly, the relationship between net charge and peak current, can be used to define the functional concentration applied to a population of α7 receptors, functional concentration being defined as the concentration relative to the EC_{50} for the specific agonist being tested. In this paper, concentration–response data are pooled from 13 separate studies in order to generalize a method for estimating potency and efficacy based on comparisons of the net charge and peak currents of single-concentration responses.

Methods

Expression on Xenopus oocytes

The preparation of *Xenopus laevis* oocytes for RNA expression was conducted as previously described (Papke and Papke, 2002). In brief, mature (>9 cm) female *Xenopus laevis* African frogs (Nasco, Ft. Atkinson, WI) were used as a source of oocytes. Prior to surgery, the frogs were anesthetized by placing the animal in a 1.5 g/l solution of MS222 (3-aminobenzoic acid ethyl ester) for 30 min. Oocytes were removed from an incision made in the abdomen.

In order to remove the follicular cell layer, harvested oocytes were treated with 1.25 mg/ml Type 1 collagenase (Worthington Biochemical Corporation, Freehold, NJ) for 2 ho at room temperature in calcium-free Barth's solution (88 mM NaCl, 1 mM KCl, 0.33 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 10 mM HEPES (pH 7.6), 50 mg/l gentamicin sulfate). Subsequently, stage 5 oocytes were isolated and injected with 50 nl (5–20 ng) each of the appropriate subunit cRNAs. Recordings were made 5 to 15 days after injection.

Chemicals

The source of the 4OH-GTS-21 was Taiho Pharmaceuticals (Tokyo, Japan). AR-R17779 and tropisetron were synthesized and supplied by Memory Pharmaceuticals. Nornicotine was supplied by Dr. Peter Crooks (University of Kentucky, Lexington). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Electrophysiology

Experiments were conducted using OpusXpress 6000A (Axon Instruments, Union City, CA, USA). OpusXpress is an integrated system that provides automated impalement and voltage clamp of up to eight oocytes in parallel. Both the voltage and current electrodes were filled with 3 M KCl. Cells were voltage-clamped at a holding potential of −60 mV. Data were collected at 50 Hz and filtered at 20 Hz. Cells were bath-perfused with Ringer's solution, and agonist solutions were delivered from a 96-well plate via disposable tips, which eliminated any possibility of cross-contamination. Flow rates were set at 2 ml/min. Drug applications alternated between acetylcholine (ACh) controls and experimental agonists. Applications were 20 s in duration followed by 181-s washout periods.

Experimental protocols and data analysis

Responses were calculated as net charge (Papke and Papke, 2002) and peak current. Oocytes received initial control applications of 300 μM ACh, then an experimental drug application, and then a follow-up control application of 300 μM ACh, a concentration which is sufficient to evoke a maximal net charge response (Papke and Papke, 2002). Responses to experimental drug applications were calculated relative to the preceding ACh control responses in order to normalize the data, compensating for the varying levels of channel expression among the oocytes. Means and standard errors (S.E.M.) were calculated from the normalized responses of at least four oocytes for each experimental concentration. For concentration–response relations, data were plotted using Kaleidagraph 3.0.2 (Abelbeck Software; Reading, PA), and curves were generated from the Hill equation

$$\text{Response} = \frac{I_{\max}[\text{agonist}]^n}{[\text{agonist}]^n + (EC_{50})^n}$$

where I_{max} denotes the maximal response for a particular agonist, and n represents the Hill coefficient. I_{max}, n, and the EC_{50} were all unconstrained for the fitting procedures.

Results

Comparisons of two response measures

The average ACh EC_{50} value reported in papers (Papke et al., 2005b; Papke and Papke, 2002; Placzek et al., 2004; Stokes et al., 2004) from our laboratory ($n=5$) on either rat or human

α7 receptors has been 26.3 μM. In spite of the fact that these papers spanned several years and utilized many different frogs as source for oocytes, the standard deviation in our EC_{50} estimates has been only 7.6 μM. As shown in Fig. 1, responses of α7 nAChR to increasing concentrations of agonist show characteristic changes in wave form (Papke and Thinschmidt, 1998), due to the tendency for the high agonist concentrations achieved with full solution exchange to promote desensitization rather than activation (Papke et al., 2000). Responses were characterized by both the net charge and peak current amplitudes relative to 300 μM ACh control responses in the same cell. As previously reported (Papke and Papke, 2002), 300 μM evokes a saturating net charge response but is only about the EC_{50} for the evoking peak currents (Table 1). The progressive change in the relationship between net charge and peak current is clearly evident in the scaled traces to the right of Fig. 1. Compared to the 300 μM ACh response, the ratio of net charge to peak is much greater in the 10 μM ACh response and 40% less in the 1 mM ACh response.

Table 1
Cruve fit values for human (A) and rat (B) α7

Agonist	Net charge α7			Peak α7		
	EC_{50} (μM)	n	I_{max}	EC_{50} (μM)	n	I_{max}
(A) Human α7						
ACh	32±4.4	1.6±0.3	1.0*	74±9.1	1.5±0.6	1.4±0.1
Choline	300±20	2.1±0.3	0.88±0.02	977±65	2.5±0.5	1.3±0.12
Cytisine	11.7±0.6	1.9±0.2	0.85±0.01	65±3	1.5±0.8	1.2±0.03
4OH-GTS-21	4.7±0.5	1.9±0.3	0.43±0.01	15.0±3.9	1.2±0.3	0.72±0.06
TMA	29±2.4	1.7±0.2	1.07±0.03	195±10	1.3±0.1	2.9±0.1
AR-R17779	4.0±0.4	1.2±0.2	1.1±0.2	36.4±2.9	1.3±0.6	1.9±0.24
Tropisetron	0.59±0.03	2.0±0.1	0.26±0.03	10.0±2.4	4.0±2.0	0.65±0.06
Tropane	103±0.13	3.2±0.01	0.28±0.03	392.1±13	2.1±0.1	0.61±0.1
Tropinone	230±3.3	1.9±0.1	0.64±0.01	515±19	2.7±0.2	0.9±0.02
(B) Rat α7						
ACh	28±7.4	1.1±0.3	1.0*	530±84	0.9±0.1	2.7±0.2
Nor-nicotine	17±5	2.7±1.4	0.52±0.05	588±140	1.0±0.1	2.2±0.2
Cytisine	12.8±0.6	1.8±0.3	0.65±0.02	141±3	1.2±0.8	2.1±0.3
4OH-GTS-21	2.5±0.6	3.9±2.0	0.47±0.03	60±36	0.8±0.2	1.6±0.3

* The efficacy of ACh in the net charge analyses was defined as 1, and was the basis for the comparison of all other efficacy measures.

Evaluation of agonists and partial agonists

Although experimental drugs targeting α7 vary greatly in potency and efficacy, concentration-dependent desensitization is a consistent feature. For example, the full agonist ACh and the α7 partial agonists tropisetron, tropane, and tropinone (Papke et al., 2005a), all show qualitatively similar differences in waveform between responses evoked by concentrations near their respective net charge EC_{50} values and concentrations that produce saturated responses (Fig. 2A). This consistent relationship is further supported by comparisons of the concentration–response relationships for net charge and peak current or these agonists (Fig. 2B). As previously reported (Papke et al., 2005a), the net charge EC_{50} for tropisetron is 600 nM, while that for tropinone is 230 μM. The I_{max} value for tropisetron is approximately 25% that of ACh and tropinone, although less potent, is significantly more efficacious than tropisetron. Although these agents differ by large factors in potency and efficacy, they all show (Fig. 2B) qualitatively similar relationships between the two concentration–response curves (Papke and Papke, 2002): compared to the net charge responses, the curves for peak currents are shifted to the right and show higher maximum values (expressed relative to the respective measures of ACh control responses).

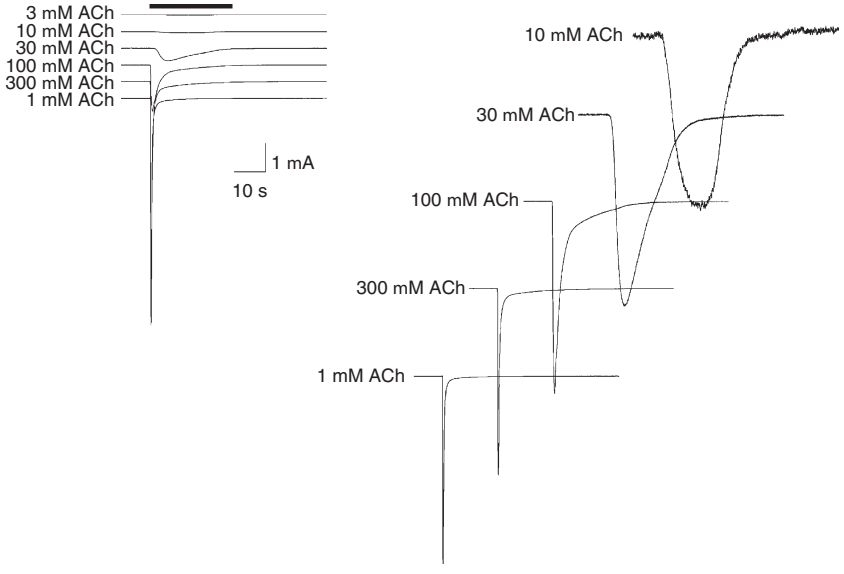

Fig. 1. Concentration-dependent changes in the receptor-mediated responses of human α7 nAChR expressed in *Xenopus* oocytes. The application of ACh at increasing concentrations differentially evokes increases in the net charge and peak currents of the receptor mediated responses. Progressive increases in net charge are seen only up to concentrations of about 100 μM while peak current amplitude increases throughout the entire concentration range tested. A series of responses from a single oocyte are shown on the upper left, and the same responses are scaled on the right to have matching amplitudes in order to illustrate how the ratio of net charge to peak current decreases with increasing agonist concentrations.

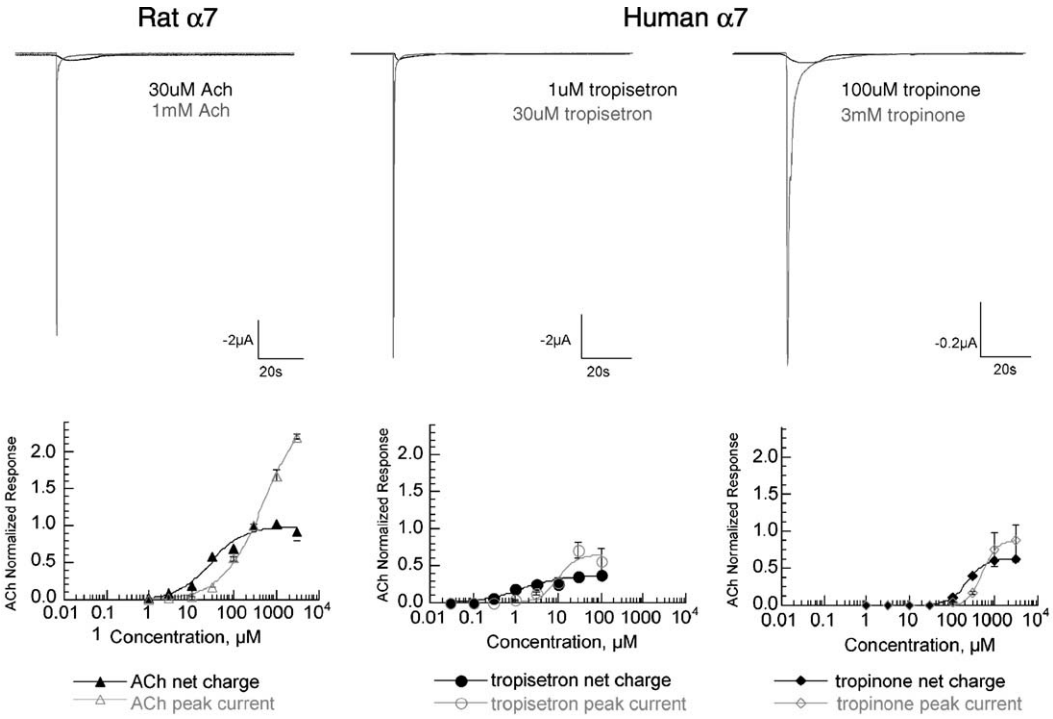

Fig. 2. Both full and partial agonists show similar differences between responses to low and high functional concentrations of agonist. At the top of the figure, pairs of responses of rat α7 receptors to ACh and human α7 receptors to the high potency, low efficacy, partial agonist tropisetron and the low potency, higher efficacy, partial agonist tropinone. Contrasted are responses to each drug at concentrations near the EC_{50} values for net charge (Table 1) and responses to 30-fold higher concentrations. The complete peak and net charge concentration–response curves for these drugs are shown at the bottom of the figure. Data were normalized to the net charge or peak currents of control 300 μM ACh responses obtained 3 min before the experimental agonist-evoked responses. Each point represents the average ± S.E.M. of the normalized responses of at least 4 oocytes. The data on net charge were previously published (Papke et al., 2005a).

Transformation of concentration–response data based on functional concentrations

Concentration–response data for net charge and peak currents were compiled for nine agonists with human α7 (ACh, tropisetron, tropane, tropinone, 4OH-GTS-21, choline, cytisine, AR-R17779, and tetramethylammonium) and four agonists for rat α7 (ACh, 4OH-GTS-21, cytisine, and nornicotine). Responses were initially measured relative to the net charge and peak currents of 300 μM ACh-evoked responses in the same oocytes. The wide range of efficacies and potencies shown by these various agents results in a chaotic display of the combined data (Fig. 3A). However, these data can be restructured through a two-step transformation process. The first step in the transformation involves converting actual concentration values to "functional concentrations"; that is, the concentrations relative to the EC_{50} concentration for each particular drug. The EC_{50} estimates from the net charge data were used for this step in the transformation. Specifically, for each of the 13 pairs of concentration–response data, the concentration values were divided by the EC_{50} fit to the corresponding net charge data. For example, the concentrations used in the tropisetron data were divided by 0.6, while the concentrations used for tropinone data were divided by 230. This has the effect of aligning all of the net charge data so that the EC_{50} values equal to 1, with the peak current data making a parallel shift.

The second step in the transformation process involves correction for the partial efficacy of some agonists. To do this, all response values were divided by the empirically determined maximum net charge response (relative to ACh). This had the effect of equalizing the I_{max} of the net charge data for all of the agonists in the compiled data set, with again, parallel shifts in the peak current data. A plot of the transformed data is shown in Fig. 3B with separate *Y*-axes for the net charge and peak current data. As expected, the transform of the pooled net charge data was well fit with a Hill curve with an EC_{50} and I_{max} both equal to 1. The transformed data for peak currents were fit with an I_{max} of 1.8 ± 0.1 and an EC_{50} of 5.1 ± 0.7, relative to the net charge data. The Hill slopes for the transformed net charge and peak current data were similar (1.5 ± 0.1 and 1.4 ± 0.2, respectively).

Method for the estimation of potency

An important extension from the transformed data set is an analysis of the ratio between the net charge and peak current values as a function of functional concentration. This is shown in Fig. 4. These data for the human and rat experiments were fit with a form of the Hill equation having a negative Hill coefficient and an offset factor reflecting the ratio (*R*) between saturating net charge and peak current responses (see below).

$$R = \frac{\text{net charge relative to ACh control}}{\text{peak current relative to ACh control}}$$

The curves fit separately for the rat and human data set were not significantly different in any of the fit parameters (Fig. 4A)

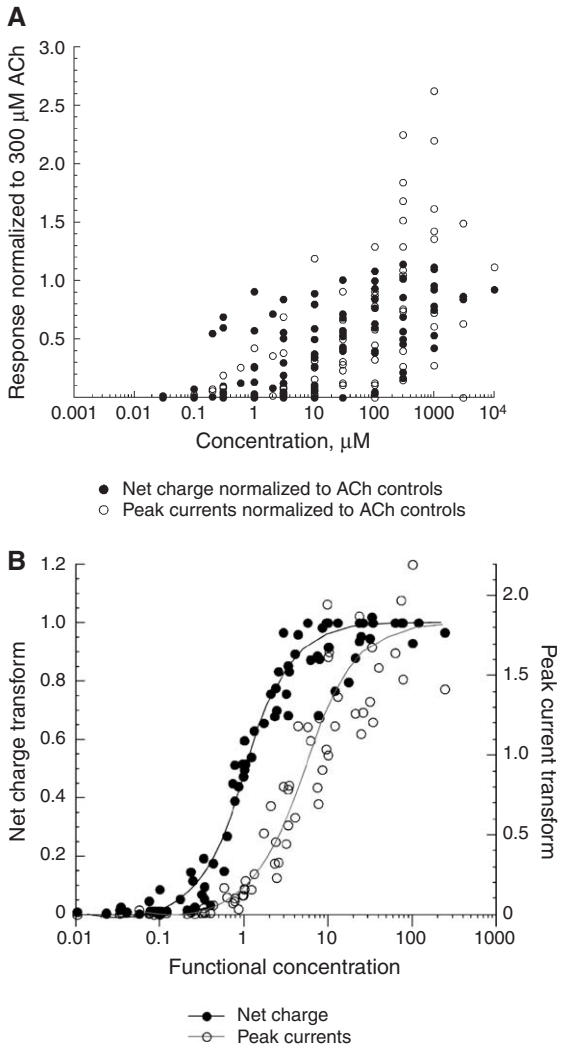

Fig. 3. Transformation of α7 concentration–response curves. (A) The upper plot shows all of the net charge and peak current data for 13 concentration response studies of human or rat α7 receptors expressed in *Xenopus* oocytes. All values plotted were calculated relative to the net charge and peak currents of 300 μM ACh responses in the same oocytes. The scatter in the data reflects the fact that the agonists tested varied greatly in both potency and efficacy. (B) The same data shown in panel A after two transform functions were performed on each pair of concentration–response data sets. The first transform function involved expressing concentration relative to the EC_{50} for net charge. The second transform set the I_{max} for net charge equal to 1 (see text for more information). Note the different *Y*-axes for peak currents and net charge.

and, therefore, the data sets were pooled to generate a function that could be used to estimate potencies based on the net charge-to-peak current ratios of single-concentration responses. The curve shown in Fig. 4B is described by Eq. (1) below:

$$R = \left(\frac{5.2[\text{ Functional concentration}]^{-1}}{[\text{ Functional concentration}]^{-1} + 0.67} \right) + 0.26 \qquad (1)$$

This equation can be used directly to derive an estimate for functional concentration from a calculated ratio of net charge to peak (Eq. (2) below).

$$\text{Functional concentration} = \frac{R - 5.46}{0.174 - 0.67R} \qquad (2)$$

EC_{50} is then estimated as follows (Eq. (3));

$$EC_{50} = \frac{\text{test concentration}}{\text{Functional concentration}} \qquad (3)$$

When the functional concentration is equal to 1, i.e. when a drug is used at its EC_{50}, the ratio (R) of net charge to peak current (measured relative to the ACh controls) should be around 3.4. A drug used at one-fifth its EC_{50} should produce responses with a ratio value of about 4.8 and a drug used at 5 times its EC_{50} should produce responses with a ratio of about 1.5.

An alternative approach for rapid estimation is by graphical extrapolation using the plot in Fig. 4B.

Method for the estimation of efficacy

Once an estimate of functional concentration is generated, as described above, that value can be used to make a prediction

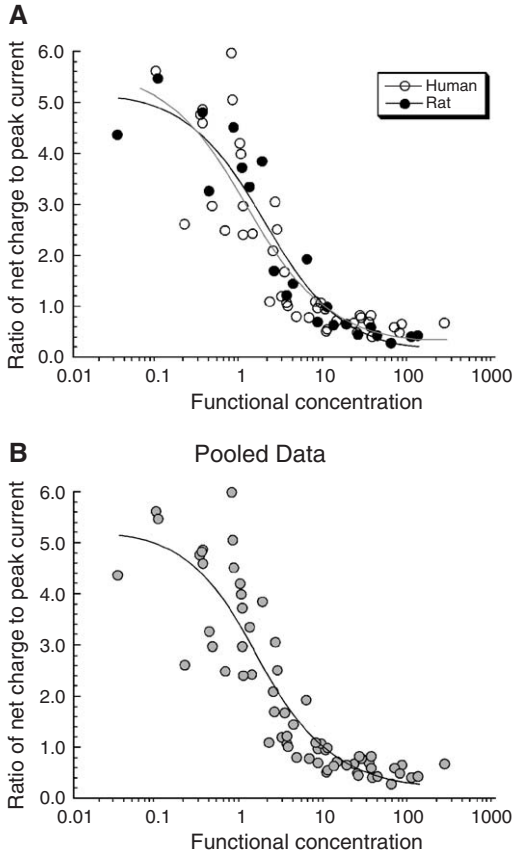

Fig. 4. Relationship between functional concentration and the ratio of net charge to peak current. After the first transformation described above, which converted the response data to reflect functional concentration rather than concentration applied, ratios of the ACh-normalized net charge and peak currents were calculated for each of the data pairs, reflecting the 13 different agonists at varying functional concentrations. (A) Data for rat and human α7 receptors are distinguished by plot symbols, as indicated. The curves fit to the rat and human data were not significantly different in any of the fit parameters and, therefore, the data were pooled (B) and used to generate a curve, the formula for which (Eq. (1)) can be used to estimate functional concentration (Eq. (2)) from the ratio of net charge to peak current for any test response of either rat or human α7.

of what the net charge response should have been for a full agonist. For a full agonist, the net charge response (relative to the ACh control), as a function of functional concentration can be predicted by Eq. (4) below, which is the equation for the pooled net charge curve in Fig. 4B.

$$\text{Predicted response} = \frac{[\text{Functional concentration}]^{1.5}}{[\text{Functional concentration}]^{1.5}+1} \quad (4)$$

By definition, for any given functional concentration, partial agonists will have net charge responses less than those predicted for full agonists at that functional concentration. The fractional efficacy of a partial agonist can therefore be predicted from the ratio of the observed to the predicted responses (Eq. (5)).

$$\text{Fractional efficacy} = \frac{\text{Observed response}}{\text{Predicted response}} \quad (5)$$

Sample analyses

Numerous complex quinuclidine derivatives, including AR-R17779, PSAB-OFP and PNU-282987, have been identified as α7-selective agonists (Broad et al., 2002; Hajos et al., 2005; Mullen et al., 2000), suggesting that quinuclidine itself and related simple compounds may also have agonist activity. This hypothesis was tested with single-concentration screens. Sample responses of oocytes expressing rat α7 receptors to the application of either quinuclidine or quinuclidinone at 100 μM are shown in Fig. 5. The average responses to quinuclidine had net charge values 91% those of the ACh controls and peak currents 71% those of the ACh controls, giving a ratio of 1.25. Using Eq. (2) gives a functional concentration of 6.4 times the EC_{50}. With 100 μM estimated to be 6.4 times the EC_{50}, the predicted EC_{50} for quinuclidine would then be 16 μM. The average responses to quinuclidinone had net charge values 52% those of the ACh controls and peak currents only 16% those of the ACh controls, giving a ratio of 3.25. Using Eq. (2) gives a functional concentration of 1.1 times the EC_{50}. With 100 μM estimated to be 1.1 times the EC_{50}, the predicted EC_{50} for quinuclidinone then would be 90 μM. Graphical extrapolation of these estimates is illustrated in Fig. 6.

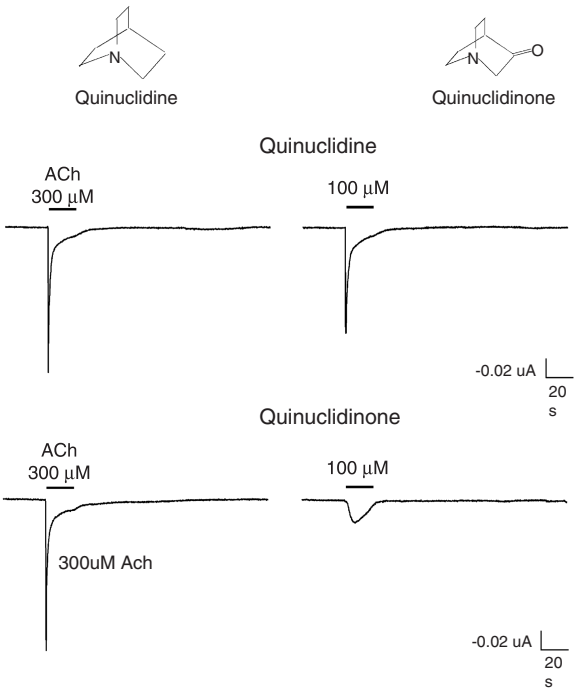

Fig. 5. Sample single-concentration quinuclidine responses. Shown are representative responses of oocytes expressing rat α7 receptors to 100 μM applications of two previously uncharacterized compounds, quinuclidine and quinuclidinone.

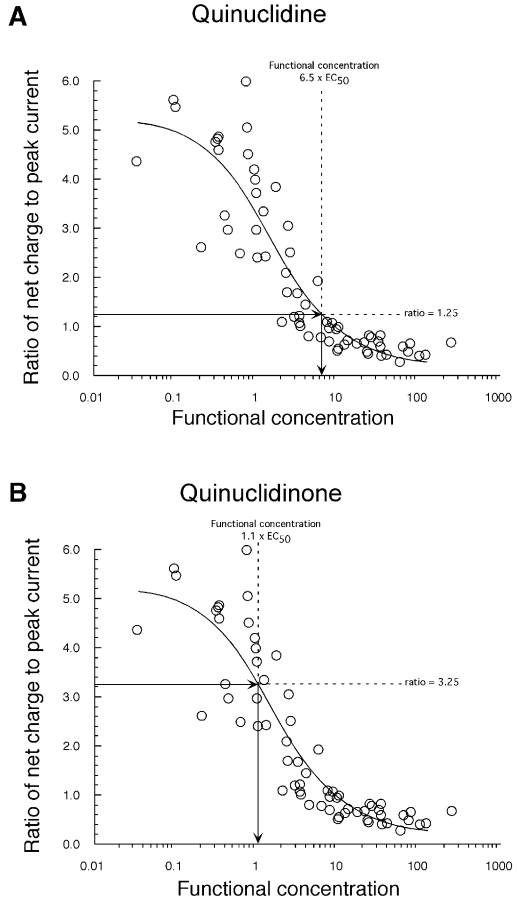

Fig. 6. Graphic extrapolation of EC_{50} estimates. Rather than calculating functional concentration from Eq. (2), an alternative approach is to extrapolate from the curve shown in Fig. 4.

Using Eq. (4), we could predict that, if quinuclidine is a full agonist, at a functional concentration of 6.4 times the EC_{50}, it would produce a net charge response of 95% the ACh control. The observed value of 91% is sufficiently close to predict that quinuclidine is a full agonist. For quinuclidinone, used at a concentration 1.1 times the EC_{50}, the expected net charge response for a full agonist would be 52% so that the observed response of 52% would also be consistent with a full agonist.

Quinuclidine and quinuclidinone were subsequently subjected to full concentration–response studies using our typical procedure of applying the drugs at approximately half log concentration increments from 30 nM to 1 mM. These eight point concentration–response studies are shown in Fig. 7 and

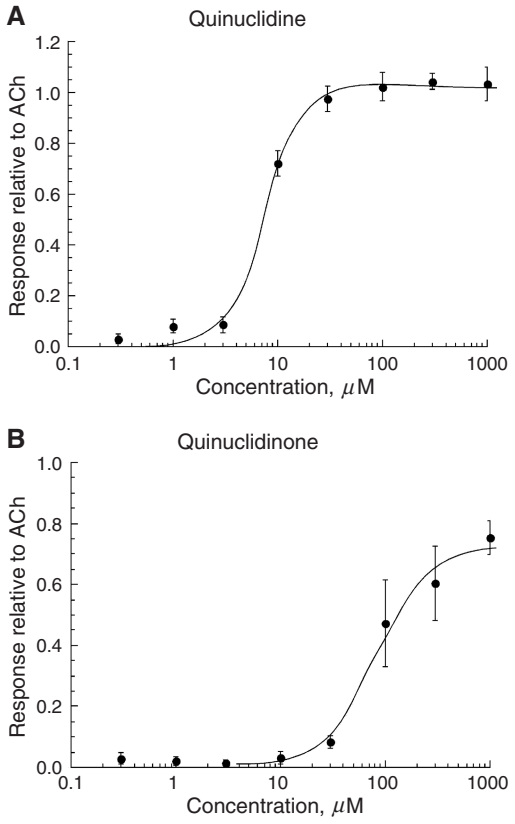

Fig. 7. Full concentration response curves for the net charge responses to quinuclidine agonists. Data were normalized to the net charge of control 300 μM ACh responses obtained 3 min before the experimental agonist-evoked responses. Each point represents the average ± S.E.M. of the normalized responses of at least 4 oocytes.

are in generally good agreement with the single-point estimates. For quinuclidine, the I_{max}, Hill Coefficient, and EC_{50} values from the curve fit were 1.03 ± 0.02, 2.5 ± 0.4, and 7.2 ± 0.6 μM, respectively. For quinuclidinone, the I_{max}, Hill Coefficient and EC_{50} values from the curve fit were 0.73 ± 0.04, 1.7 ± 0.4, and 78 ± 11 μM, respectively.

While the single-concentration efficacy estimate for quinuclidine was correct, the EC_{50} estimate was somewhat higher than that derived from the full study. For quinuclidinone, the EC_{50} estimates are essentially the same for both analyses and while the concentration–response curve fit suggests a slightly lower efficacy than the single-concentration estimate, it is unclear whether the concentration–response curve in fact provides a very good estimate of efficacy, since the data do not show a clearly defined maximal response.

Discussion

The unique concentration-dependent desensitization of α7 nAChR permits every drug application to cells expressing this receptor to generate a dynamic analysis through the course of solution application and inherent concentration change. The fact that this quality of the α7 response is seen for all agonists, be they high potency or low, full agonist or partial, has allowed for rules to be defined to extend the analysis of single-concentration responses. We show that for two previously uncharacterized agonists the single-point analysis is almost as good (or in one case perhaps even better) than a full concentration–response study which required almost an order of magnitude more time and effort. The utility of this extended analysis for drug screening is clear.

While single-concentration analysis provides fast and reasonably good estimates of potency and efficacy, admittedly the precision of estimated values will not be so great as those usually derived from full studies. Although presumably the binding stoichiometry is the same for all α7 agonists, as shown in Table 1, Hill slopes derived from full concentration–response studies of the different agonists vary significantly. This variation may be due to any number of factors, including differences in desensitization rates or the potency of channel block by the various agonists. Variability in Hill slopes is probably the limiting factor for the accuracy of the single-concentration analyses.

Typically, when a full CRC on a novel compound is generated, a wide range of concentrations are used to be sure to bracket the active concentration range. When precise estimates of potency are required, single-concentration analysis can be used a guide to focus and increase the efficiency of the follow-up studies. Note that, for the two quinuclidine studies shown, if the single response estimates had been used, the active concentration range for each drug might have been very effectively bracketed with relatively few concentrations. Virtually no information was obtained by testing quinuclidine at concentrations higher than 100 μM or quinuclidinone at concentrations lower than 10 μM. That is, for the quinuclidine data set, removal of the points at concentration >100 μM does not give a different curve fit. Likewise, for the quinuclidinone data set, you get the same curve fit whether or not the data for concentrations <10 μM are used. Based on the single point estimates fewer concentrations might have been used for more effectively in the follow-up experiments and importantly the quinuclidinone single-concentration analysis would have suggested that the drug be tested at higher concentrations.

The range for good single-concentration estimates probably falls from a factor of 10 below the EC_{50} to 100 fold above the EC_{50} and this concept is good to keep in mind when choosing the single concentration to be used for the test analysis. However, it is probably best to err on the side of higher test concentrations, since with too low a concentration there may be problems not only with limits of detection but also with signal-to-noise in making good estimates of both net charge and peak currents. For the most potent drugs, it may be sufficient to estimate their relative efficacy and provide an upper limit on their EC_{50} values (e.g. EC_{50} values no greater than one-hundredth the test concentration).

It seems possible that this analytical approach might be applied to other receptors. However, to do so would require as full a characterization of concentration-dependent aspects of the receptor mediated response as we provide here for rat and human α7 nAChR. We have tested a number of agonists on α7 receptors cloned from Rhesus monkey and all the agonists tested, including ACh, were less potent for monkey α7 than for

the human and rat α7 receptors (Papke et al., 2005b). Because the ACh controls were at a different functional concentration, single-concentration analysis of monkey α7 receptors would use different curves and formulae (not shown), but would nonetheless be easily done. On the other hand, the response waveforms of beta subunit-containing nAChR, such as α4β2 receptors, are rather variable in our experience and although there are some concentration-dependent features, it is unclear whether they could be easily separated from cell-to-cell variations.

In conclusion, while it may remain a challenge for others to test and validate this approach for other receptors, extended analysis of single-concentration responses of α7 nAChR may be a great aid in expediting the drug development process for this potentially important therapeutic target.

Acknowledgements

This work was supported by NIH grants GM57481-01A2 and PO1 AG10485. The author thanks Drs. Alan Finkel and Cathy Smith-Maxwell of Molecular Devices for comments and support and Taiho Pharmaceuticals (Tokyo, Japan), Memory Pharmaceuticals and Dr. Peter Crooks for experimental agonists. Technical support was provided by Brian A. Jack, Clare Stokes and Joshua D. Buhr. Thanks to Maryanne Rose Papke for fuzzy lemons and careful algebra.

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