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Effects of the plant alkaloid tetrandrine on human nicotinic acetylcholine receptors

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Abstract

Functional effects of the well-characterized antagonist of L-type Ca^{2+} channels tetrandrine on recombinant human γ-aminobutyric acid type A ($GABA_A$) (α1β2γ2s) receptor or human α7, α4β2, α1β1δγ and α1β1δε nicotinic acetylcholine receptors expressed in *Xenopus* oocytes were examined using two-electrode voltage clamp. Tetrandrine inhibited the function of acetylcholine nicotinic receptors, but it had no effect on $GABA_A$ receptors. Potency of inhibition was influenced by the receptor subtype and the rank order was α4β2>α7>α1β1δγ≅α1β1δε. Functional inhibition of α4β2 and α1β1δγ receptors was noncompetitive, but only inhibition of α1β1δγ receptors was voltage-dependent. Binding of ^{125}I-α-bungarotoxin to α1β1δγ or ^{3}H-cytisine to α4β2 receptors was also inhibited by tetrandrine, but inhibition was noncompetitive and required concentrations higher than those needed to inhibit receptor function. Inhibition of both α7 receptor function and binding of ^{125}I-α-bungarotoxin to α7 receptor were mixed competitive/noncompetitive and occurred at a similar concentration range.

Keywords: Tetrandrine; Nicotinic receptor; Ca^{2+} channel ligand, L-type; *Xenopus* oocyte

1. Introduction

Tetrandrine is a bis-benzyl-isoquinoline alkaloid isolated from the tuberous roots of the Chinese herb *Stephania tetrandra* used to treat hypertension and angina (Wang and Liu, 1985). Equilibrium and kinetic binding studies with voltage-gated calcium channels have shown that tetrandrine produces the same allosteric coupling pattern as diltiazem (King et al., 1988; Felix et al., 1992). This has led to the suggestion that tetrandrine inhibits L-type Ca^{2+} channels by interacting with the benzothiazepine site of these channels and that this interaction most likely underlies the therapeutic effects of this alkaloid (King et al., 1988; Felix et al., 1992; Wang and Lemos, 1995) as it is the case for other L-type Ca^{2+} channel antagonists, such as dihydropyridines, verapamil and diltiazem. Interestingly, however, there is increasing evidence that L-type Ca^{2+} channel drugs, such as diltiazem, verapamil and the dihydropyridines, also inhibit the function of ligand-gated cation channels, including 5-hydroxytryptamine type 3 (5-HT3; Hargreaves et al., 1996) and nicotinic acetylcholine receptors (Gandia et al., 1991; Lopéz et al., 1993; Villarroya et al., 1997; Houlihan et al., 2000). These interactions occur, at least in the case of diltiazem, at concentrations similar to the therapeutic concentrations of this drug in the blood plasma (Yeung et al., 1996). Of relevance to the elucidation of the overall effects of L-type Ca^{2+} channel blockers in the treatment of cardiovascular disorders is the finding that some types of nicotinic acetylcholine receptors coexist with L-type Ca^{2+} channels in cells (e.g., chromaffin cells) (Gandia et al., 1991; Lopéz et al., 1993; Villarroya et al., 1997) involved in cardiovascular homeostasis.

Nicotinic acetylcholine receptors belong to the four-transmembrane superfamily of ligand-gated ion channels

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that also include the γ-aminobutyric acid type A ($GABA_A$), glycine and 5-HT3 receptors (Ortells and Lunt, 1995). Nicotinic acetylcholine receptors are pentamers formed from a combination of subunits that determines their structural and pharmacological properties. Muscle-type nicotinic receptors consist of 2 α1, β1 and δ subunits with either γ (foetal) or ε (adult). At least nine different α subunits (α2–α10) and three β subunits (β2–β4) have been identified in avian, rodent and or human neuronal tissue and combine in different ways to generate a large number of different subtypes of neuronal nicotinic receptors (Lukas et al., 1999; Lustig et al., 2001). Hence, three broad categories of nicotinic receptor emerge: heteromeric muscle receptors and neuronal receptors of either homomeric (α7, α8, α9) or heteromeric arrays. Recently, we have shown that both diltiazem and verapamil inhibit nicotinic acetylcholine receptors noncompetitively and that the extent of inhibition is influenced by receptor subtype (Houlihan et al., 2000). Here, we report the effects of tetrandrine on foetal (α1β1δγ) and adult (α1β1δε) muscle nicotinic acetylcholine receptors and neuronal nicotinic receptors (α4β2 and α7) expressed in *Xenopus laevis* oocytes and clonal cell lines. The results indicate that tetrandrine is an antagonist of both muscle and neuronal nicotinic acetylcholine receptors and that its potency and mode of action are determined by the receptor subtype. We also show here that tetrandrine has no effect on the function of recombinant human $GABA_A$ receptors.

2. Materials and methods

2.1. Drugs and reagents

Tetrandrine was purchased from Sigma (UK) and stock solutions were prepared in distilled water at pH 2.0 (Wang and Lemos, 1992) with dimethyl sulphoxide (<0.02%) and then neutralized to pH 7.4 with NaOH. Fresh stocks were made fortnightly and stored at −20 °C. ^{125}I-α-bungarotoxin (134–146 Ci/mmol) and [^{3}H]cytisine (35 Ci/mmol) were purchased from NEN (UK).

2.2. Receptor expression

Xenopus oocytes were prepared and injected with human α7 or combinations of α4+β2, α1+β1+δ+γ or α1+β1+δ+ε nicotinic acetylcholine receptor subunit in vitro transcribed RNAs as previously described (Houlihan et al., 2000, 2001). Human $GABA_A$ receptor subunits cDNA α1, β2 and γ2 s were coinjected nuclearly at a ratio 1:0.1:1. Injected oocytes were incubated at 20 °C for up to a week in Barth's solution containing (in mM): 88 NaCl, 1 KCl, 0.41 $CaCl_2$, 0.82 $MgSO_4$, 0.33 $Ca(NO_3)_2$, 2.5 $NaHCO_3$, 0.5 theophylline, 10 HEPES, pH 7.2, supplemented with 5% horse serum, 0.1 mg/ml gentamicin, 0.01 mg/ml streptomycin and 0.01 mg/ml penicillin.

2.3. Electrophysiology

Whole-cell currents were measured by two-electrode voltage clamp (GeneClamp 500, Axon Instruments, USA) using 0.5–1.5 MΩ agarose-cushioned electrodes containing 3 M KCl. Oocytes were continually supplied with fresh Ringer solution (in mM: 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 10 HEPES, pH 7.2) in a 100 μl bath, using a gravity-driven perfusion system at a rate of 4 ml/min. Modified Ringer solution ($CaCl_2$ replaced by $BaCl_2$) was used when recording from oocytes expressing α7 nicotinic acetylcholine receptors. Drugs were applied by gravity perfusion using a manually activated valve. Agonists were applied for a period sufficient (approximately 10–30 s) to obtain a stable plateau response (at low concentrations) or until the beginning of a sag after the peak response (at higher concentrations). Dose–response curves for agonists were constructed by normalising to the maximal response of the agonist and used to estimate EC_{50} and nHill values. To construct antagonist dose–response curves, the responses elicited by coapplication of agonist and increasing concentrations of tetrandrine were normalised to the responses elicited by EC_{50} concentrations of agonist alone. Constant responses to the agonist were obtained before the coapplication of agonist and tetrandrine. In these studies, oocytes were preincubated with tetrandrine for 3 min prior to the coapplication procedure to ensure equilibration between receptors and tetrandrine. To maintain ongoing measurements of the control response to agonist throughout the experiment, each coapplication was bracketed by an application of EC_{50} agonist alone. Between each successive drug application, the cell was perfused with Ringer (or Ba-Ringer solution as appropriate) solution for 3 min to allow drug clearance and prevent receptor desensitization.

2.4. Clonal cell lines

The SH-SY5Y-hα7 cell line, over-expressing human α7 nicotinic acetylcholine receptors, was created as previously described (Puchacz et al., 1994; Peng et al., 1999; Houlihan et al., 2000, 2001) and used to assay ^{125}I-α-bungarotoxin binding. Membrane homogenates for [^{3}H]cytisine binding studies were prepared from the SH-EP-hα4β2 clonal cell line, which express human α4β2 nicotinic receptors (Pacheco et al., 2001). Cells were maintained in Dulbecco's Modified Eagle's Medium (Gibco, UK) supplemented with 5% foetal calf serum, 10% horse serum, 2 mM L-glutamine, 10 IU/ml penicillin, 10 μg/ml streptomycin, 2.5 μg/ml amphotericin B and 0.4 mg/ml hygromycin. For SH-EP-hα4β2 culture media, the hygromycin concentration was decreased to 130 μg/ml, and zeocin (Cayla, France) was added at 250 μg/ml. Established cultures of the human rhabdomyosarcoma cell line TE671, which express human foetal (α1β1δγ) muscle nicotinic acetylcholine receptors, were kindly provided by Dr. David Beeson (Institute of Molecular Medicine, Oxford). Culture medium for TE671 cells consisted of Dulbecco's Modified Eagle's Medium supplemented with 10% foetal calf

serum, 120 μg/ml penicillin and 1 μg/ml streptomycin. All cultures were maintained at 37 °C in humidified chamber supplemented with a 5% CO_2–air mixture.

2.5. Ligand binding assays

For binding assays, confluent SH-SY5Y-hα7, SH-EP1-hα4β2 or TE cells were rinsed with ice-cold phosphate buffered saline, mechanically disaggregated and homogenised using a Polytron homogeniser for 10 s. The homogenates were centrifuged at 40,000 × *g* at 4 °C for 20 min, and the pellets resuspended in ice-cold binding saline to give a final protein concentration in the assay tubes of approximately 30–50 μg. The binding saline used in ^{125}I-α-bungarotoxin studies consisted of (in mM) 140 NaCl, 1 EDTA and 50 Tris–HCl at pH 7.4, whilst for [^{3}H]cytisine binding the saline contained (in mM) 120 NaCl, 5 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 50 Tris, pH 7.0. ^{125}I-α-bungarotoxin binding and [^{3}H]cytisine binding were carried out as described previously (Houlihan et al., 2000, 2001). Nonspecific binding was defined using 10 μM nicotine.

2.5.1. Data analyses

Dose–response data for agonists and tetrandrine were fit by nonlinear regression (Prism 3.01, GraphPad, USA) to the equations:

$$(a)\ i = i_{max}/[1 + (EC_{50}/x)^{nHill}],\ \text{or}$$

$$(b)\ i = i_{max}/[1 + (IC_{50}/x)^{nHill}],$$

where i_{max} = maximal normalised current response (in the absence of antagonist for inhibitory currents), x = agonist or antagonist concentration, EC_{50} = concentration of agonist eliciting a half-maximal response, IC_{50} = antagonist concentration eliciting half-maximal inhibition and nHill = Hill coefficient.

The binding parameters (K_d and B_{max}) of ^{125}I-α-bungarotoxin or [^{3}H]cytisine binding were determined from saturation binding isotherm data using the equation $Y = B_{max}X/K_d + X$, where B_{max} = maximal binding, K_d = apparent equilibrium dissociation binding constant, X = concentration of ligand and Y = binding.

Results are presented as means ± S.E.M. of at least four separate experiments from at least two different batches of oocytes. Where appropriate, one-way ANOVA or Student's *t*-test for unpaired data were used, and values of $P \leq 0.05$ were regarded as significant. Higher significance levels are, however, stated when relevant.

3. Results

3.1. Functional effects of tetrandrine

Application of tetrandrine did not elicit any detectable current when applied on its own or coapplied with EC_{50} concentrations of GABA (30 μM; Houlihan et al., 2000) onto oocytes expressing human α1β2γ2s $GABA_A$ receptors (Fig. 1A), even at concentrations as high as 1 mM. In contrast, tetrandrine fully inhibited the acetylcholine EC_{50} responses of α1β1δγ, α1β1δε, α7 and α4β2 nicotinic acetylcholine receptors in a dose-dependent manner (Fig. 1B, C and D). Maximal inhibition occurred at concentrations over 100 μM, but no significant inhibition was observed at concentrations lower than 0.1 μM (Fig. 2). The IC_{50} value for tetrandrine-mediated inhibition of EC_{50} ACh responses ranged from 1.1 ± 0.3 μM in α4β2 receptors (n = 5) and 2.7 ± 0.21 μM in α7 receptors (n = 7) to 5.4 ± 0.8 and 5.9 ± 0.5 μM in α1β1δγ and α1β1δε receptors, respectively. The corresponding Hill coefficients were 0.9 ± 0.08, 1.4 ± 0.05, 1.3 ± 0.04 and 1.5 ± 0.3, respectively. The mean IC_{50} and nHill values are

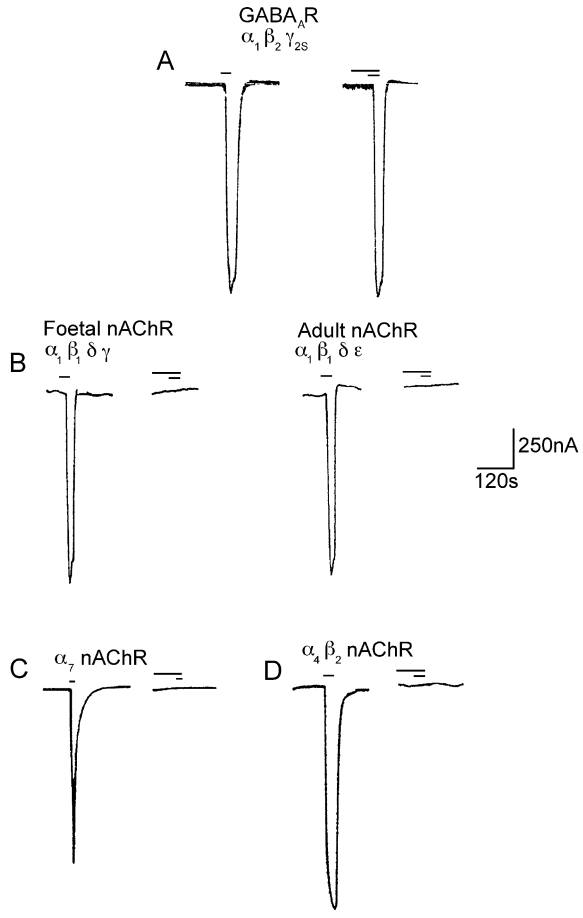

Fig. 1. Functional effects of tetrandrine on human recombinant nicotinic acetylcholine and $GABA_A$ receptors. *Xenopus* oocytes expressing either α1β2γ2S $GABA_A$ receptors (A) or α1β1δγ (foetal muscle), α1β1δε (adult muscle) (B), α7 (C) or α4β2 (D) nicotinic acetylcholine receptors were voltage-clamped at −70 mV and challenged with EC_{50} concentrations of GABA (30 μM) or acetylcholine (0.1 μM for muscle receptors, 30 μM for α4β2 receptors and 100 μM for α7 homomers) in the presence and absence of 100 μM (nicotinic acetylcholine receptors) or 1 mM ($GABA_A$ receptors) tetrandrine. Horizontal bars above each trace indicate the duration of applications. Key: nAChR, nicotinic acetylcholine receptors; $GABA_AR$, $GABA_A$ receptors.

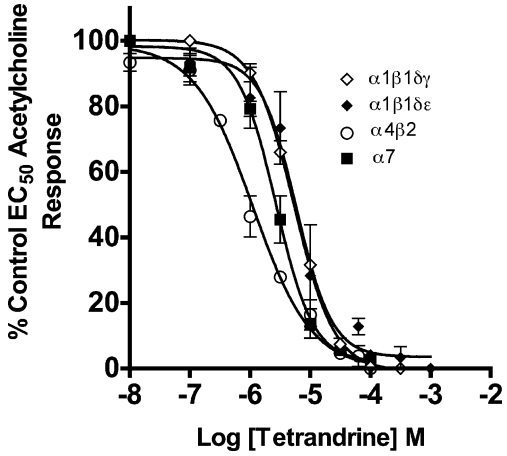

Fig. 2. Concentration–response curve for tetrandrine inhibition of human nicotinic receptors. Oocytes expressing either α1β1δγ, α1β1δε, α4β2 or α7 nicotinic acetylcholine receptors were held at −60 mV and tetrandrine was applied at a concentration range from 10 nM to 1 mM in the presence of EC_{50} acetylcholine (0.1 μM for α1β1δγ, α1β1δε receptors, 30 μM for α4β2 receptors and 100 μM for α7 receptors). Data points are the means ± S.E.M. of three to five experiments from different oocyte batches.

summarised in Table 1. IC_{50} values for the inhibition of α7 and α4β2 receptors were significantly different from each other, as well as from those for α1β1δγ, α1β1δε receptors. In contrast, the IC_{50} values for the latter receptors were not significantly different from each other. The rank order of functional inhibition of nicotinic acetylcholine receptors by tetrandrine is, therefore: α4β2>α7>α1β1δγ ≅ α1β1δε.

To determine how tetrandrine inhibits the function of nicotinic acetylcholine receptors, we investigated the effect of 10 μM tetrandrine (approximate IC_{70-80} concentration) on the acetylcholine concentration–response curves for α1β1δγ, α4β2 and α7 receptors. As shown in Fig. 3, tetrandrine significantly reduced the maximal acetylcholine response of α1β1δγ, α7 and α4β2 receptors to 67 ± 4% (n = 3), 49 ± 3% (n = 3) and 59 ± 1% (n = 3), respectively (P < 0.001). No significant changes in the EC_{50} values or Hill coefficients for the acetylcholine concentration–response curves were observed with the addition of tetrandrine in the case of α1β1δγ or α4β2 receptors, but in the case of α7 receptors, the presence of tetrandrine caused a significant shift of the EC_{50} value from 106 ± 8 to 650 ± 10 μM. These results are consistent with tetrandrine exerting

Table 1
Summary of the effects of tetrandrine on human nicotinic acetylcholine receptors

Nicotinic acetylcholine receptor subtype	Functional IC_{50} (μM)	Radioligand binding competition IC_{50} (μM)
α1β1δγ	5.4 ± 0.8	43 ± 0.3
α1β1δε	5.9 ± 0.5	ND
α7	2.7 ± 0.2	4 ± 1
α4β2	1.1 ± 0.3	43 ± 3

Data represent the means ± S.E.M. of three to five experiments. Key: ND, not determined.

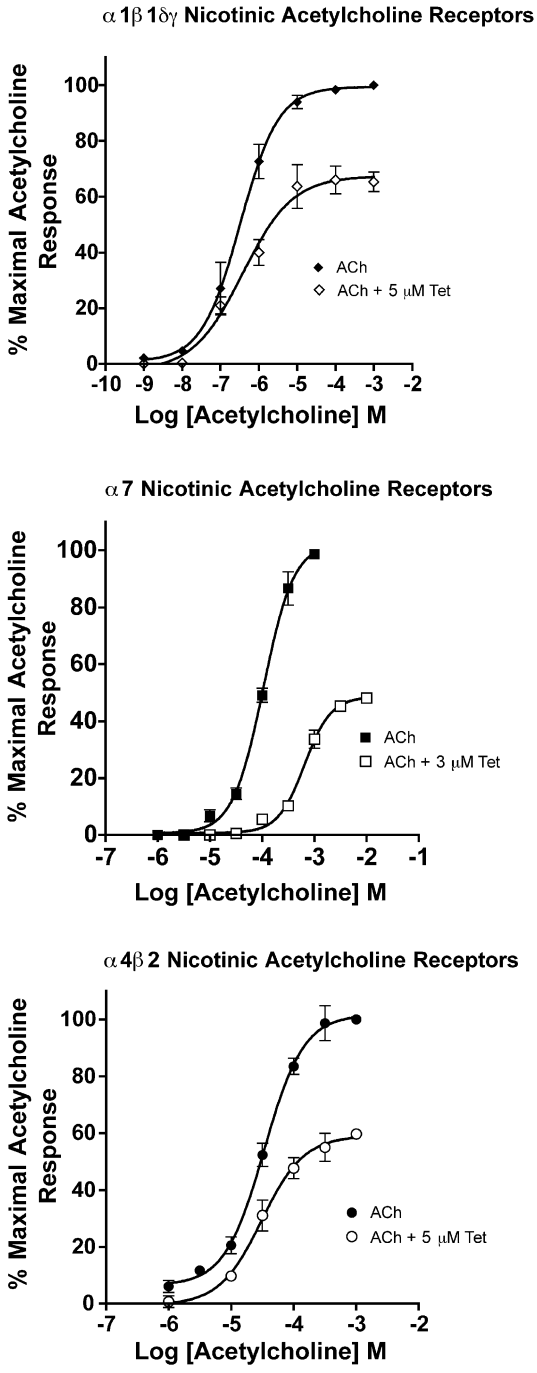

Fig. 3. The effect of tetrandrine on the acetylcholine concentration–response curve for α1β1δγ, α7 and α4β2 nicotinic acetylcholine receptors. Oocytes were exposed to increasing concentrations of acetylcholine in the presence and absence of 10 μM tetrandrine. Data points were normalized to the control response elicited by the maximal acetylcholine concentration (1 mM) and are the means ± S.E.M. of at least three experiments. Key: ACh, acetylcholine; tet, tetrandrine.

noncompetitive block at α1β1δγ, α4β2 and a mixed competitive/noncompetitive blockade at α7 receptors.

Noncompetitive inhibition of ligand-gated ion channels may result from open-channel blockade by ligands that are charged at physiological pH. Because tetrandrine is charged

at physiological pH, we tested whether the noncompetitive effects of tetrandrine are due to open-channel blockade. Thus, we analysed the acetylcholine current–voltage relationship in oocytes expressing either α1β1δγ, α7 or α4β2 nicotinic acetylcholine receptors in the presence and absence of 3 μM tetrandrine. As shown in Fig. 4, the

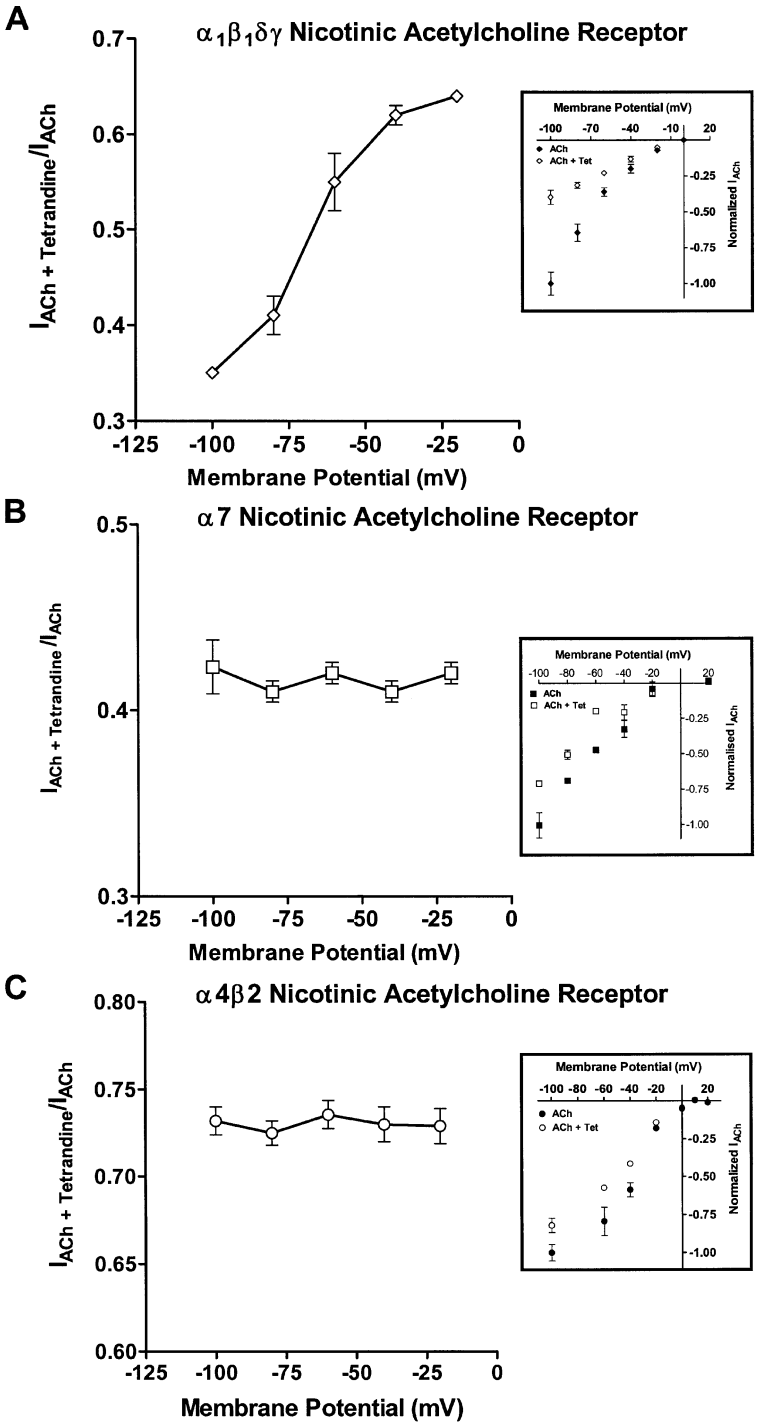

Fig. 4. Effect of tetrandrine on the current–voltage relationship of α1β1δγ, α7 and α4β2 nicotinic acetylcholine receptors. Plots show the inhibition of α1β1δγ (A), α7 (B) and α4β2 (C) nicotinic acetylcholine receptors by tetrandrine at a range of membrane potentials. Acetylcholine responses elicited with EC_{50} acetylcholine concentrations (0.1 μM for α1β1δγ, α1β1δε receptors, 30 μM for α4β2 receptors and 100 μM for α7 receptors) were recorded in the absence and presence of 3 μM tetrandrine. Inhibition was equivalent at all potentials at which acetylcholine responses could be elicited in α7 or α4β2 receptors, but it was voltage-dependent in α1β1δγ receptors (ANOVA test). Insets show the corresponding current–voltage relationship in the absence and presence of 3 μM tetrandrine. Voltage steps were applied from −100 to +20 mV and the response to applications of EC_{50} acetylcholine alone and then acetylcholine plus tetrandrine. Responses were normalised to the acetylcholine responses obtained at −120 mV. Values represent the means ± S.E.M. of three to five experiments. Key: ACh, acetylcholine; tet, tetrandrine.

current–voltage relationship for acetylcholine currents mediated by either α7 or α4β2 receptors had a similar inward rectification in the presence or absence of tetrandrine, and the percentage of inhibition was equivalent at all negative holding potentials. These results are consistent with non-voltage-dependent inhibition. In contrast, the extent of tetrandrine inhibition of α1β1δγ receptors increased with membrane hyperpolarisation, which indicates

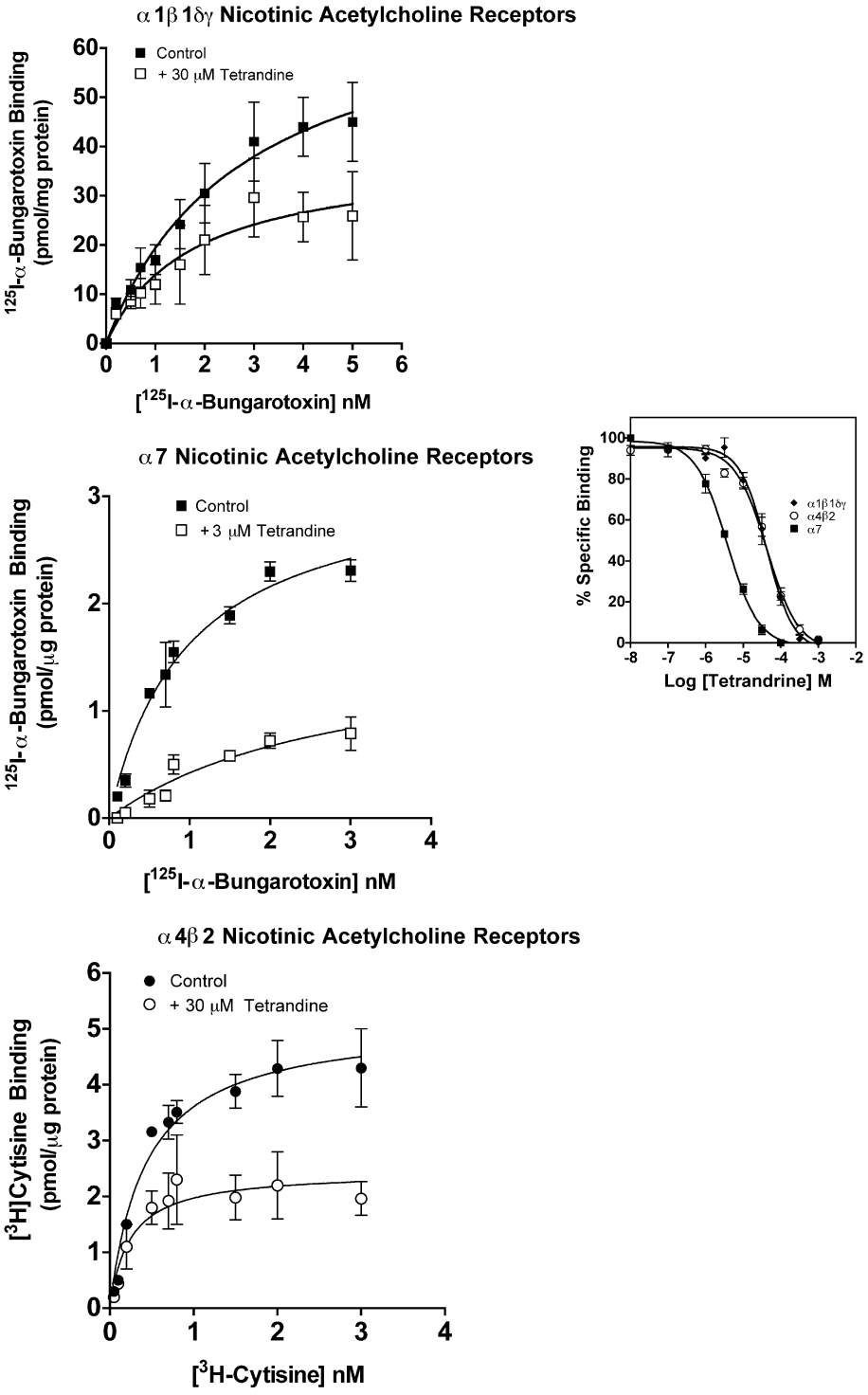

Fig. 5. Saturation analysis of specific binding of ^{125}I-α-bungarotoxin to either α7 or α1β1δγ and ^{3}H-cytisine to α4β2 cells in the absence and presence of tetrandrine. Data are the means ± S.E.M. of three experiments, each with triplicate samples. Tetrandrine reduced significantly the value of B_{max} in each receptor type studied. Insert shows the effect of increasing concentrations of tetrandrine on the specific binding of 1 nM ^{125}I-α-bungarotoxin to either α7 or α1β1δγ and [^{3}H]cytisine to α4β2 nicotinic acetylcholine receptors. Data points represent the means ± S.E.M. of three to five experiments, each carried out in triplicate.

that the effect of tetrandrine on α1β1δγ receptors was voltage-dependent.

3.2. Ligand binding assays

Clonal cell lines expressing α4β2, α7 or α1β1δγ nicotinic acetylcholine receptors (SH-EP-hα4β2, SH-SY5Y-hα7 and TE671 clonal cells, respectively) were used to investigate the effect of tetrandrine on the agonist site of these receptors. Tetrandrine fully displaced the binding of ^{125}I-α-bungarotoxin to α7 or α1β1δγ receptors and [^{3}H]cytisine to α4β2 receptors. Inhibition in all cases was dose-dependent with IC_{50} values of 43 ± 0.3 μM in α1β1δγ receptors, 4 ± 1 μM in α7 receptors and 43 ± 3 μM in α4β2 receptors (Fig. 5, inset; Table 1). Saturation binding curves were constructed in the presence and absence of approximate IC_{50} concentrations of tetrandrine (Fig. 5). Tetrandrine had no effect on the apparent K_D of ^{125}I-α-bungarotoxin binding to α1β1δγ receptors (2.1 ± 0.5 nM, $n=3$; 1.8 ± 0.5 nM, $n=3$, in the absence and presence of 30 μM tetrandrine, respectively) or [^{3}H]cytisine binding to α4β2 receptors (0.43 ± 0.08 nM, $n=3$; 0.28 ± 0.05 nM, $n=5$, in the absence and presence of 30 μM tetrandrine, respectively; $P<0.05$), but significantly reduced B_{max} from 73 ± 5 to 38 ± 6 fmol/mg protein and from 5.1 ± 0.6 to 2.4 ± 0.3 pmol/μg protein, respectively. These data are consistent with noncompetitive inhibition of radioligand binding by tetrandrine for α1β1δγ or α4β2 receptors and with noncompetitive functional effects of tetrandrine on these receptors. In contrast, ^{125}I-α-bungarotoxin binding to α7 receptors significantly increased the apparent K_D from 0.92 ± 0.015 to 1.6 ± 0.1 nM ($n=5$; $P<0.05$) and reduced B_{Max} from 3.3 ± 0.2 to 1.5 ± 0.2 pmol/μg protein ($n=5$; $P<0.05$). This mixed competitive/noncompetitive block of radioligand binding is comparable to the functional effects of tetrandrine on α7 receptors.

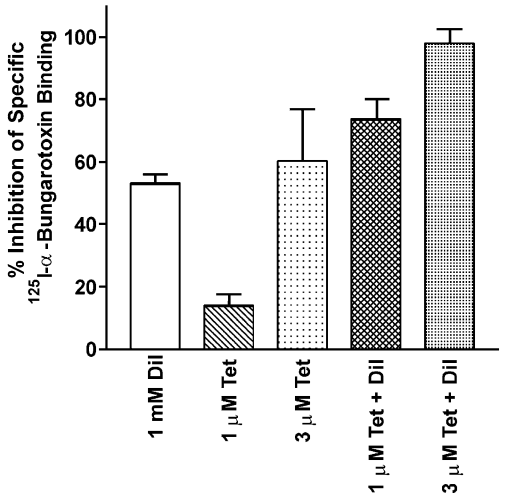

Fig. 6. The additive effects of tetrandrine and diltiazem on ^{125}I-α-bungarotoxin binding to SH−SY5Y−hα7 cells. These studies used the maximal inhibitory concentration (1 mM) of diltiazem (Dil) and 1 μM or 3 μM tetrandrine (Tet). Data are expressed as percentage of inhibition of control values (specific ^{125}I-α-bungarotoxin binding with no test alkaloid) and represent the mean of 4 experiments, each carried out in triplicate.

Tetrandrine and the L-type Ca^{2+} channel drug diltiazem interact with identical sites in L-type Ca^{2+} channels (King et al., 1988; Felix et al., 1992). To test whether tetrandrine and the L-type Ca^{2+} channel blocker, diltiazem, interact with the same locus in human α7 nicotinic acetylcholine receptors, we examined the effect of tetrandrine on maximal diltiazem inhibition of ^{125}I-α-bungarotoxin binding to SH-SY5Y-hα7 cells. In agreement with previous reports (Houlihan et al., 2000), diltiazem alone at a concentration of 1 mM produced a maximal inhibition of 53 ± 3% of ^{125}I-α-bungarotoxin binding, whereas upon simultaneous application with tetrandrine, there was an increase in the level of inhibition that was greater than the effects of the two drugs added on their own (Fig. 6). Furthermore, the combined effects of diltiazem and tetrandrine were similar to the level expected by adding together the two independent effects.

4. Discussion

The results presented here show that tetrandrine caused reversible inhibition of the acetylcholine-mediated current in both muscle (α1β1δγ and α1β1δε) and neuronal (α4β2 and α7) human nicotinic acetylcholine receptors in a concentration-dependent and receptor subtype-specific manner. The plant alkaloid tetrandrine appears to have a remarkably broad spectrum of action. It blocks Ca^{2+} currents through not only L-type channels (King et al., 1988; Felix et al., 1992; Bickmeyer and Weigand, 1993; Liu et al., 1991, 1995), but also through N- and T-type channels (Liu et al., 1992; Weinsberg et al., 1994). In addition, tetrandrine also inhibits Ca^{2+}-dependent K^{+} channels (Wang and Lemos, 1992, 1995), α_1-adrenoceptor cells (Kwan et al., 1996) and suppresses T-cell proliferation by inhibiting a protein kinase C-dependent mechanism (Ho et al., 1999). To add to this profile, the results presented here establish that tetrandrine interacts with a site on nicotinic acetylcholine receptors. The inhibitory effect of tetrandrine on four-transmembrane receptors may be restricted to cationic channels though, as $GABA_A$ receptors were found to be insensitive to tetrandrine at concentrations up to and including 1 mM (to date, there appears to be no published study detailing the effects of tetrandrine on either 5-HT_3 or glycine receptors).

Functional inhibition of α1β1δγ nicotinic receptors was noncompetitive and dependent on membrane potential, whereas inhibition of α4β2 receptors was noncompetitive but voltage-independent. Moreover, inhibition of α7 receptors was voltage-independent and occurred by a mixed competitive/noncompetitive manner. These results show that the effects of tetrandrine on human nicotinic acetylcholine receptors are influenced by receptor subtype. This finding is not unique to tetrandrine; indeed, there is ample evidence that subunit composition plays a crucial role in determining the pharmacological properties of nicotinic

acetylcholine receptors. For example, the affinity of diltiazem at rat (Herrero et al., 1999) and human (Houlihan et al., 2000) nicotinic acetylcholine receptors is influenced by receptor subtype; both α and β subunits define the efficacy and affinity of the alkaloid cytisine at nicotinic acetylcholine receptors (Houlihan et al., 2001) such that cytisine consistently display higher affinity at α4β4 than at α4β2 or α7 receptors and is significantly more efficacious at α4β4 than at α4β2 receptors.

The effects of tetrandrine on the K_D and B_{max} values of ^{125}I-α-bungarotoxin binding to α1β1δγ or α7 receptors or [^{3}H]cytisine binding to α4β2 receptors are consistent with the mechanism underlying the functional effects of tetrandrine on these receptors. The finding that tetrandrine inhibited the function of α7, α4β2 or α1β1δγ nicotinic acetylcholine receptors at concentrations lower than those needed to displace binding of radioligand to these receptors further demonstrates that the fundamental nature of the effect of tetrandrine on nicotinic acetylcholine receptors is noncompetitive antagonistic. Interestingly, the difference between the concentration range of tetrandrine required to inhibit receptor function and radioligand binding to the receptors was less marked on α7 receptors than on α4β2 or α1β1δγ receptors, which further shows that receptor subtype influences the cholinergic activity of tetrandrine.

Tetrandrine appears to be more selective for neuronal nicotinic receptors than muscle receptors as the rank order of potency for functional inhibition was α4β2>α7>α1β1δγ≅α1β1δε. However, tetrandrine does not display high selectivity for a specific nicotinic receptor type as the IC_{50} values for functional inhibition of all receptors tested were within a narrow micromolar range (1–7 μM). This pattern of inhibition is very similar to that observed for the L-type Ca^{2+} channel blocker diltiazem, where IC_{50} values were within a similar range (1–5 μM) and human neuronal α7 and α4β2 receptors were more sensitive to diltiazem than were muscle types (Houlihan et al., 2000). Although tetrandrine is not structurally related to diltiazem, there is evidence that it interacts at the diltiazem site in L-type Ca^{2+} channels (King et al., 1988; Felix et al., 1992). In the case of nicotinic acetylcholine receptors, our findings suggest that tetrandrine is unlikely to interact with the site that diltiazem binds to exert its inhibitory effects in these receptors. The strict additivity that was observed between the effects of diltiazem and tetrandrine displacement of ^{125}I-α-bungarotoxin binding suggests that these substances interact at different loci in the human α7 receptor.

Tetrandrine exerts noncompetitive block at α4β2, α1β1δγ human nicotinic acetylcholine receptors, which suggest a mechanism of action that involves channel blockade. This does seem to be the case for foetal muscle acetylcholine nicotinic receptors evaluated in this study, as tetrandrine-mediated inhibition was dependent on membrane voltage. However, tetrandrine did not appear to be a channel blocker of the α4β2 or α7 receptor subtypes as the observed inhibition was not dependent on membrane voltage. This suggests that tetrandrine interacts with the open conformation of foetal muscle receptor channels whilst inhibition of neuronal receptors occurs by a mechanism that does not require interaction with the active form of the receptors. This observation is not unique to tetrandrine inhibition of nicotinic receptors. A recent study demonstrated that inhibition of the acetylcholine response by strychnine is voltage-dependent for rat neuronal nicotinic acetylcholine receptors yet voltage-independent for mouse muscle nicotinic acetylcholine receptors (García-Colunga and Miledi, 1999). Although the subunit composition of the receptors is probably a key determinant in the effects of strychnine on nicotinic receptors, it should be noted that the findings of García-Colunga and Miledi (1999) might reflect species-specific differences rather than structural differences between the receptors. The effects of tetrandrine on nicotinic receptors are, however, clearly influenced by the subunit composition of these receptors.

Finally, the inhibition of both muscle and neuronal nicotinic receptors by tetrandrine demonstrates that caution should be applied when studying the effects of this drug on systems that contain both nicotinic acetylcholine receptors and L-type Ca^{2+} channels, as previously noted by Gandia et al. (1991) in relation to the blockade of bovine chromaffin cell nicotinic receptors by dihydropyridines. This is particularly important as the half-maximal concentration of tetrandrine reported here for inhibition of nicotinic receptors is within the range (i.e., 2–10 μM) reported for blockade of Ca^{2+} channels from a diverse set of tissue preparations (King et al., 1988; Wiegand et al., 1990; Liu et al., 1991; Kwan et al., 1996; Wang and Lemos, 1995). This raises the possibility that the previously unrecognized nicotinic effects of tetrandrine may also contribute to its well-known medicinal effects, such as antihypertension. For example, because α7 nicotinic acetylcholine receptors contribute to the entry of Ca^{2+} into chromaffin cells and the ensuing catecholamine release from these cells (Lopéz et al., 1993), their inhibition by tetrandrine could contribute to the overall antihypertension effects of tetrandrine, particularly during stressful conditions when the release of catecholamines increases markedly due to higher sympathetic activity.

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