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A novel peptide modulates α7 nicotinic receptor responses: implications for a possible trophic-toxic mechanism within the brain

Susan A. Greenfield,* Toni Day,* Edward O. Mann* and Isabel Bermudez†

**Department of Pharmacology, Oxford University and †Department of Molecular and Biological Sciences, Oxford Brookes University, Oxford, UK*

Abstract

The α7 nicotinic acetylcholine receptor (nAChR) plays a key role in neural development and neurodegeneration. Here, we identify a novel, modulatory receptor ligand, a 14-amino acid peptide (AEFHRWSSYMVHWK) derived from the C-terminus of acetylcholinesterase (AChE). In three different *in vitro* preparations, this 'AChE-peptide' is bioactive in a ligand-specific and concentration-dependent manner. First, it modulates acutely the effect of acetylcholine (ACh) on *Xenopus* oocytes transfected with human α7, but not α4/β2, nAChR. The action persists when intracellular calcium is chelated with BAPTA or when calcium is substituted with barium ions. This observation suggests that intracellular Ca^{2+} signals do not mediate the interaction between the peptide and nAChR, but rather that the interaction is direct: however, the intervention of other mediators cannot be excluded. Secondly, in recordings from the CA1 region in guinea-pig hippocampal slices, AChE-peptide modulates synaptic plasticity in a α-bungarotoxin (α-BgTx)-sensitive manner. Thirdly, in organotypic cultures of rat hippocampus, long-term exposure to peptide attenuates neurite outgrowth: this chronic, functional effect is selectively blocked by the α7 nAChR antagonists, α-BgTx and methyllycaconitine, but not by the α4/β2-preferring blocker dihydro-β-ethroidine. A scrambled peptide variant, and the analogous peptide from butyrylcholinesterase, are ineffective in all three paradigms. The consequences of this novel modulation of the α7 nAChR may be activation of a trophic-toxic axis, of relevance to neurodegeneration.

Keywords: α7 nicotinic receptor, acetylcholinesterase, allosteric modulation, bioactive peptide, oocytes, organotypic culture.

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It is now widely accepted that acetylcholinesterase (AChE) has non-cholinergic actions (Soreq and Seidman 2001), the most widely established of which is as a trophic agent (Layer and Willbold 1995; Holmes *et al*. 1997) that enhances calcium entry (Webb *et al*. 1996; Day and Greenfield 2002). More recently, it has been suggested that such non-classical phenomena are mediated via a peptide fragment ('AChE-peptide') at the C-terminus of AChE (Greenfield and Vaux 2002). This peptide is indeed bioactive in a manner analogous to the non-cholinergic effects of AChE: it too enhances calcium entry (Bon and Greenfield 2003), enhances neurite outgrowth (Day and Greenfield 2003) and, in higher concentrations, induces apoptotic neuronal death (Day and Greenfield 2003). However, the receptor mediating this trophic-toxic action has not as yet been identified. One clue might be that, during certain critical developmental periods, the highly Ca^{2+} permeant α7 nAChR (Seguela *et al*. 1993) is transiently expressed in rat brain in close parallel with AChE (Broide *et al*. 1996). We therefore test here the hypothesis that AChE-peptide exhibits bioactivity via the α7 nAChR, by exploring the effects on three different preparations: (i) human, α7 nAChR expressed in *Xenopus* oocytes, which would reveal direct actions of the peptide on the receptor; (ii) guinea-pig

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Address correspondence and reprint requests to Susan A. Greenfield, Department of Pharmacology, Mansfield Road, Oxford OX1 3QT, UK. E-mail: susan.greenfield@pharm.ox.ac.uk

Abbreviations used: α-BgTx, α-bungarotoxin; Ach, acetylcholine; AChE, acetylcholinesterase; ACSF, artificial cerebrospinal fluid; DAB, diaminobenzidine tetrahydrochloride; DIV, days *in vitro*; LDH, lactate dehydrogenase; LTP, long-term potentiation; nAChR, nicotinic acetylcholine receptor; PBS, phosphate-buffered saline; STP, short-term potentiation.

brain slices, which offer a reliable index of synaptic plasticity and where agents acting at α7 nAChR have already been shown to have modulatory actions (Fujii *et al*. 2000; Mann and Greenfield 2003); (iii) organotypic cultures of hippocampus, a preparation in which AChE has already been shown to have non-cholinergic actions (Day and Greenfield 2002) and which, indeed, is sensitive to α7 nAChR activation (Hory-Lee and Frank 1995; Messi *et al*. 1997).

Materials and methods

Peptide production

All peptides used were synthesised at the Dyson Perrins Laboratory for Organic Chemistry (Dr M. Pitkeathley, University of Oxford) using an Applied Biosystems 430A Automated Peptide Synthesiser, utilising the standard Fmoc protein synthesis methodology. Couplings were achieved using double couple cycles involving two 30-min incubations with a twofold excess of the HBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)-activated form of the amino acid. Any N-termini, which had not reacted and remained after the coupling step, were capped using acetic anhydride and pyridine. The progress of the peptide synthesis was monitored by UV analysis of the dibenzofulvene product released on the cleavage of the transient N-terminal Fmoc protecting group. The completed peptides were cleaved from the resin and de-protected using trifluoroacetic acid (95% TFA) and an appropriate mixture of the following scavengers (5%): water, ethandithiol, thioanisole, ethylmethyl sulfide and triisopropyl silane. After removal of the resin by filtration, the filtrate was concentrated *in vacuo* and the crude peptides were precipitated by the addition of diethyl ether.

Recordings in *Xenopus* oocytes

Xenopus laevis oocytes at developmental stages V and VI were prepared and injected with 20 nL of recombinant plasmid PcDNA3.1-human α7 (0.1 μg/μL) or combinations of PcDNA3.1-hα4 (0.1 μg/μL) + *Pc*DNA3.1-hβ2 (0.1 μg/μL) (Houlihan *et al*. 2001). Whole-cell currents were measured 3–5 days post-injection by two-electrode voltage clamp (GeneClamp 500, Axon Instruments, Union City, CA, USA) using agarose-cushioned electrodes containing 3 M KCl. Oocytes were continually supplied with fresh Ringer solution (in mM: 115 NaCl, 2.5 KCl, 10 Hepes and either 1.8 $CaCl_2$ or 1.8 $BaCl_2$, pH 7.2) in a 100-μL bath, using a gravity-driven perfusion system at a rate of 4 mL/min. ACh and peptides were diluted in the perfusion solution and then applied by gravity perfusion using a manually activated valve. ACh was applied for a period sufficient (approximately 10–15 s) to obtain a stable plateau response (at low concentrations) or the beginning of a trough after a peak (at higher concentrations). Relevant peptides were perfused for up to 5–8 min to study the effects of peptide alone.

An EC_{50} concentration of ACh (100 μM for hα7 nAChR and 30 μM for hα4β2 nAChR) was applied followed by a 3-min period of perfusion in Ringer solution to allow drug clearance and allow for recovery from receptor desensitisation (Houlihan *et al*. 2001); the same concentration of ACh was then co-applied together with the relevant peptide and data were then normalised to the preceding application of EC_{50} ACh. Because results obtained using the hα7 nAChR suggested that AChE-peptide had concentration-dependent effects, data were fitted to an equation previously used to examine the biphasic effects of cytisines (Houlihan *et al*. 2001) on human nACh receptors: $I = I_{min} + (I_{max} - I_{min})\{[1/(1 + (EC_{50}/X)^n)] - [1/(1 +)IC_{50}/X)^{(m)}]\}$, where I is the current response at a given AChE-peptide concentration, X; I_{min} is the minimal current; I_{max} is the maximal current; EC_{50} and IC_{50} are the concentrations of AChE-peptide producing half-maximal potentiation and inhibition, respectively; n and m are the Hill coefficients for potentiation and inhibition, respectively.

Hippocampal slice recordings

Slices of guinea pig hippocampus (200–300 g) were prepared; transverse slices (400 μm) were cut and superfused (1.2 mL/min) with warm, gassed (30–32°C, 95% O_2 : 5% CO_2) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 126, $NaHCO_3$ 26, KCl 3.7, $CaCl_2$ 2.4, $MgSO_4$ 1.3, KH_2PO_4 01.3, glucose 10. Electrical stimuli (0.1 ms square pulse) were delivered through a concentric bipolar electrode (FHC Inc., Bowdoinham, ME, USA), with the stimulus strength (5–20 V) adjusted to evoke a half-maximal response. In some experiments, a short-term potentiation of the fEPSP was induced using a short burst of afferent stimulation (10 pulses, 50 Hz). For analysis of all the field recordings, the slope of the fEPSP was normalised to the long-term potentiation (LTP) induced at the end of the experiment (100 pulses, 100 Hz). Data are presented as means ± SEM. Comparisons for differences in the means were assessed by one-way ANOVA followed by post-hoc Student Newman–Keuls multiple comparison *t*-tests.

Hippocampal organotypic cultures

Hippocampal organotypic cultures were prepared according to the procedure described by Gahwiler (Gahwiler 1981, 1984). Cultures were maintained in a humidified incubator with 5% CO_2 atmosphere at 37°C and the medium was changed twice a week. Cultures were maintained for 14 days *in vitro* (14 DIV) before immunohistochemical staining for MAP-2 using the biotin-avidin-peroxidase method. Cultures were fixed in 4% paraformaldehyde and pre-incubated with phosphate-buffered saline (PBS) containing 10% goat serum. Cells were then incubated with the primary antibody (anti-MAP-2 antibody; Chemicon International Ltd, Temecula, CA, USA) for 24 h at 4°C. Following extensive rinsing with PBS, cells were incubated for 1 h with the secondary antibody (biotinylated goat-anti-mouse monoclonal antibody, Novocastra Laboratories Ltd, Newcastle–upon–Tyne, UK) and treated with 0.3% hydrogen peroxide in 10% methanol to eliminate endogenous peroxidase activity. Finally, cultures were incubated in avidin-biotin complex (ABC elite kit, Novocastra Laboratories). The peroxidase was visualised with 0.05% diaminobenzidine tetrahydrochloride (DAB) (Sigma, Poole, UK)/0.015% H_2O_2. Data are presented as mean ± SEM. Comparisons for differences in the means were assessed by one-way ANOVA followed by post-hoc Dunnett's multiple comparison *t*-tests.

Results

Recordings from oocytes

α7 or α4/β2 nAChRs were expressed in *Xenopus* oocytes and current responses were recorded of an EC_{50} concentration of

ACh (100 and 30 μM, respectively) (Houlihan *et al*. 2001) alone or when co-applied with a range of concentrations of peptides. None of the peptides tested, i.e. AChE-peptide (AEFHRWSSYMVHWK, 10 nM and 10 μM), a scrambled peptide variant (HSWRAEVFHKYWSM, 10 nM and 10 μM), nor the analogous peptide derived from butyrylcholinesterase (BuChE-peptide; AGFHRWNNYMMDWK, 10 nM and 10 μM) affected membrane conductance when applied alone (Fig. 1). In contrast, AChE-peptide did have a dose-dependent modulatory effect on EC_{50} ACh responses in α7 nAChR transfected oocytes (Figs 1a and 2a). Low concentrations (nM) of peptide potentiated the ACh response, with a maximal increase of approximately 69% ± 15 at 10 nM ($n = 10$) and an EC_{50} of 0.31 ± 0.09 nM ($n = 10$) (Fig. 2a). Higher concentrations (μM) of AChE-peptide inhibited ACh-induced currents with an IC_{50} of 274 ± 60 nM ($n = 10$) (Figs 1a and 2a). Neither scrambled peptide nor BuChE-peptide had any effect on the EC_{50} ACh responses of α7 or α4/β2 receptors (Figs 1a and b). These results show that nM concentrations of peptide potentiate ACh responses of α7 nAChR, yet higher concentrations inhibit the responses, which resembles the biphasic modulatory actions of β-amyloid (Dineley *et al*. 2001).

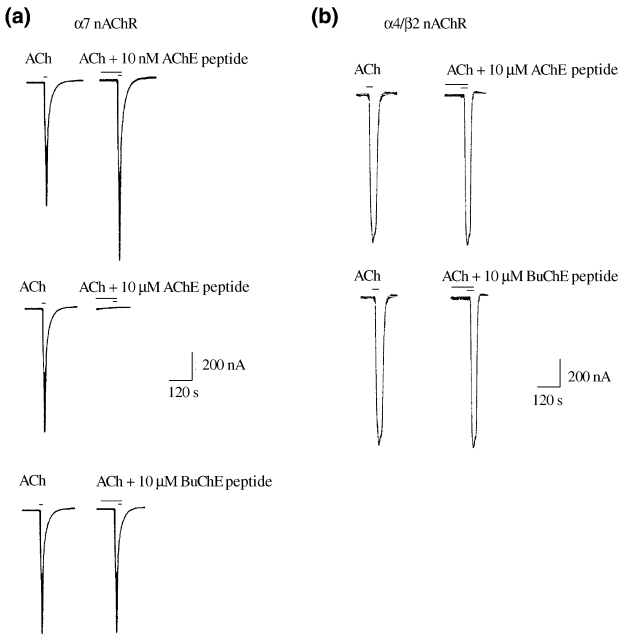

Fig. 1 The effects of AChE-peptide on the function of human α7 and α4/β2 nAChRs in *Xenopus* oocytes. (a) Current responses of a human α7 nAChR-expressing oocyte to 100 μM ACh (EC_{50} concentration) before and during co-application of 10 nM (top trace), 10 μM (middle trace) AChE-peptide or 10 μM BuChE-peptide (bottom trace). Note application of AChE-peptide alone (longer bar) has no effect. (b) Current responses of α4/β2 nAChR-expressing oocytes to 30 μM ACh (EC_{50} concentration) before and during application of 10 μM AChE-peptide (top trace) or 10 μM peptide (bottom trace).

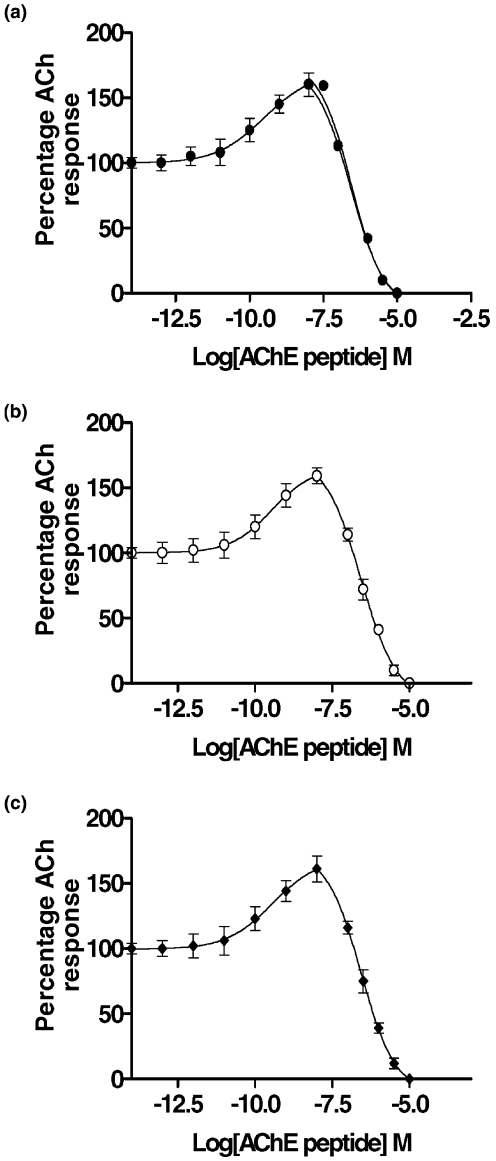

Fig. 2 The effects of AChE-peptide on the concentration-response curve of ACh at human α7 nAChRs expressed in *Xenopus* oocytes. (a) The effects of AChE-peptide on EC_{50} ACh-induced current responses in human α7 nAChR-expressing oocytes were plotted as a percentage of the response to ACh alone (mean ± SEM, 10 oocytes). One group of oocytes was incubated in 10 μM BAPTA/AM (b) for 1 h prior to recordings. In (c) oocytes were continuously superfused with Ba^{2+}-Ringer solution. Data were fitted as indicated in Materials and methods.

To test whether the effects of AChE-peptide at the α7 nACh receptors were direct and independent of intermediary intracellular cascades that may be activated by α7 nAChR-dependent Ca^{2+} entry, we examined the effects of the peptide in oocytes incubated for 1 h with 10 μM BAPTA/AM (Fig. 2b). In these cells, the EC_{50} was 0.34 ± 0.08 nM ($n = 4$) with a maximal potentiation of 66 ± 9% ($n = 4$).

The IC_{50} value in cells treated with BAPTA/AM was 280 ± 40 nM ($n = 4$) which is not significantly different from that seen in standard conditions 274 ± 60 nM. Similar results were obtained when Ca^{2+} ions in Ringer solution were substituted for an equimolar concentration (1.8 mM) of Ba^{2+} ions. In Ba^{2+}-Ringer (Fig. 2c), the EC_{50} was 0.36 ± 0.05 nM, with a respective maximal potentiation of 70 ± 28% ($n = 4$), compared with 69 ± 15% and an EC_{50} of 0.31 ± 0.09 nM with normal Ca^{2+} levels. The IC_{50} in Ba^{2+}-containing medium was 270 ± 35 nM ($n = 4$) compared with 274 ± 60 nM in control conditions These data strongly suggest that the actions of AChE-peptide on α7 nAChR mediated calcium influx is a direct one that does not involve any intermediary, intracellular reactions.

Hippocampal slice recordings

To explore further whether lower concentrations of AChE-peptide could have physiological effects at endogenously expressed α7 nAChR, electrophysiological experiments were continued in an acute slice preparation. Previous work has demonstrated that nicotine significantly lowers the threshold for synaptic plasticity in the hippocampal CA1, through the inactivation of α7 nAChR (Fujii and Sumikawa 2000; Mann and Greenfield 2003). Here, application of 1 μM AChE-peptide mimicked the effect of nicotine: a long-lasting potentiation could be induced by a tetanus (10 pulses, 50 Hz) that would normally elicit only a short-term potentiation (STP) (42.6 ± 3.7% of LTP, $p < 0.01$) (Fig. 3a). Analysis of the potentiation produced by the pre-drug STP stimulation showed that this effect could not be attributed to differences in the inherent plasticity of the slices used for different drug groups ($F_{2,6} = 0.14$, $p = 0.87$). Furthermore, the effect was selective for AChE-peptide, as it was not mimicked by 1 μM BuChE-peptide ($p < 0.01$). We have already reported a similar effect on STP for nicotine, where we concluded that the agonist desensitised the α7 nAChR: as endogenous activation of the receptors would normally inhibit the effects of STP stimulation, the net effect of nicotine, and here of AChE-peptide, would be one of potentiations (Mann and Greenfield 2003).

However, α7 nAChR antagonists can mimic the net effects of α7 nAChR desensitisation and lower the threshold for synaptic plasticity (Fujii and Sumikawa 2000; Mann and Greenfield 2003). Therefore, it was not possible to test directly whether the observed effect of AChE-peptide was mediated by α7 nAChR and the pharmacology of AChE-peptide effects were tested in a different paradigm. We have recently demonstrated that, following blockade of NMDA receptors, α7 nAChR activation, rather than desensitisation, can produce a long-lasting potentiation of the CA1 fEPSP in the absence of high-frequency stimulation (Mann and Greenfield 2003). It was found here that AChE-peptide could also mimic this effect: following NMDA receptor blockade (50 μM D-AP5), application of 1 μM AChE-peptide

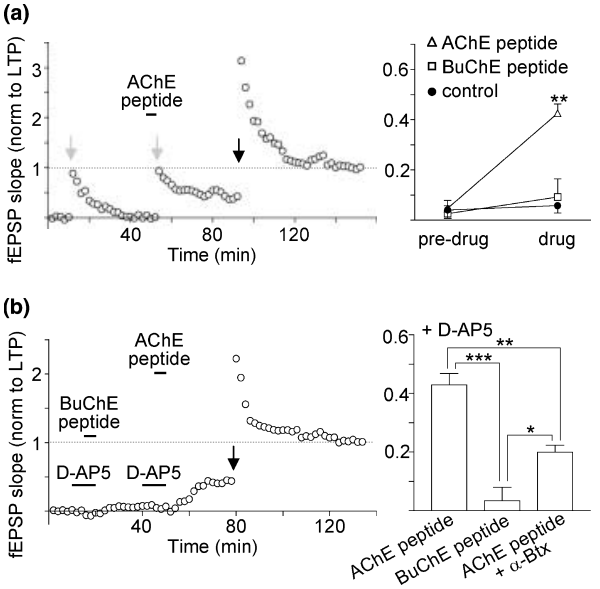

Fig. 3 The effects of AChE-peptide on electrophysiological responses in the hippocampal slice *in vitro*. (a) Application of 1 μM AChE-peptide had no effect on the baseline fEPSP recorded in the CA1, but augmented the response to STP stimulation (grey arrow, 10 pulses, 50 Hz) into a long-lasting potentiation. The degree of potentiation was measured relative to LTP induced at the end of the experiment by 100 Hz tetanic stimulation (black arrow, 100 pulses). The effect of AChE-peptide was not mimicked by the application of 1 μM BuChE-peptide; $n = 3$. (b) AChE-peptide (1 μM) produced a selective potentiation of the fEPSP in the presence of 50 μM D-AP5, which was inhibited by 100 nM α-BgTx. The degree of potentiation was measured relative to LTP induced at the end of the experiment by 100 Hz tetanic stimulation (black arrow, 100 pulses). Application of 1 μM BuChE-peptide had no effect; $n = 3$; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

produced a long-lasting potentiation of the fEPSP (Fig. 3b), an effect that was not produced by 1 μM BuChE-peptide (43.0 ± 3.9 vs. 3.4 ± 4.6% of LTP, respectively, $p < 0.001$). This effect of AChE-peptide was sensitive to 100 nM α-BgTx ($p < 0.01$) and therefore, at least partially, mediated via α7 nAChR.

Effects on neuronal growth and survival in organotypic cultures

In order to see whether this selective, modulatory effect of α7 nAChR by AChE-peptide had any functional consequences over a longer time-frame, an organotypic culture preparation was used (Gahwiler 1981; Pugh and Berg 1994; Day and Greenfield 2002). Control cultures of hippocampus maintained in serum-free media had a mean neurite length of 150 ± 10.8 μm after 14 days *in vitro* ($n = 158$). Application of the scrambled peptide or the BuChE-peptide analogue for the same time period had no effect on neurite outgrowth at any concentration tested (data not shown). However, addition of AChE-peptide for the cultivation period of

14 days significantly decreased neurite outgrowth at 1 nM (70.3 ± 4.0 μm, $p < 0.01$, $n = 151$) and 1 mM (37.6 ± 3.7 μm, $p < 0.01$, $n = 151$) (Figs 4a and b). When co-applied with 1 nM AChE-peptide, the α7 nAChR antagonist α-BgTx, in a range of concentrations (0.1–10 μM) significantly attenuated the effect of peptide (Fig. 4c), whilst having no effect alone at even the highest dose. Co-application of 1 nM AChE-peptide and methyllycaconitine (0.1–10 μM), another potent α7 nAChR antagonist, also inhibited the effect of 1 nM AChE-peptide, whereas dihydro-β-ethroidine (10–100 nM), an α4/β2 nAChR-preferring antagonist, was ineffective (Table 1). Exposure over 14 days to 1 mM ACh peptide also decreased neurite outgrowth, but unlike lower doses of AChE-peptide also increased lactate dehydrogenase (LDH) release, an index of membrane disruption (control = 0.7 ± 0.1 U/mL, $n = 151$; 1 mM AChE-peptide = 7.2 ± 0.1 U/mL, $n = 154$; $p < 0.01$). The effects of these very high concentrations of AChE-peptide were not blocked by α-BgTx (0.1–10 μM; Fig. 4d), methyllycaconitine (0.1–10 μM) nor by dihydro-β-ethroidine (10–100 nM) (Table 1).

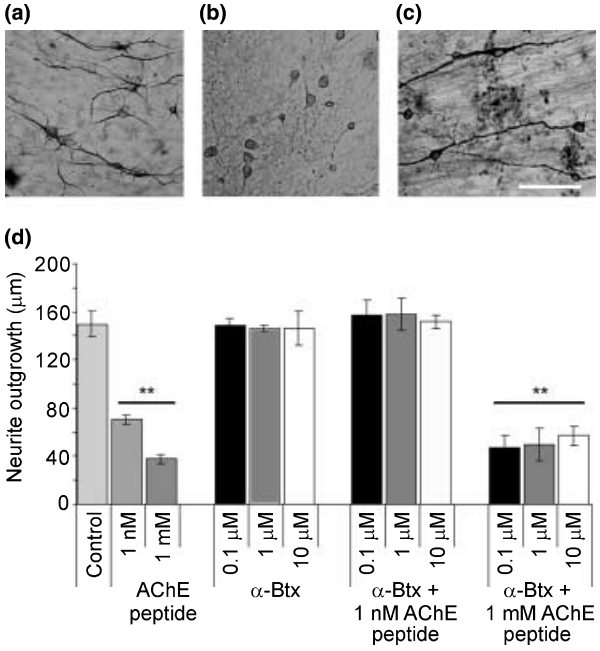

Fig. 4 The effect of nicotinic ACh receptor antagonism on *in vitro* AChE-peptide induced toxicity in rat hippocampal organotypic cultures. Cultures were maintained in serum-free medium for 14 days and then processed for MAP-2 immunochemistry. Representative photomicrographs of organotypic hippocampal cultures: (a) control, (b) treated with 1 nM AChE-peptide for 14 days and (c) treated with 1 nM AChE-peptide and 10 μM α-BgTx for 14 days (scale bar = 50 μm). (d) Quantification of effects of AChE-peptide and antagonism by α-BgTx (10 μM). Neurite outgrowth was measured by selecting cells in a non-biased manner and using camera Lucida drawings. Experiments were repeated a minimum of three times with separate culture groups; $n = 131–134$; $^{**}p < 0.01$.

Table 1 The effect of the AChE-peptide and of nAChR antagonists, alone and in combination, on neurite outgrowth (μm) in hippocampal organotypic cultures

AChE-peptide	0	1 nM	1 mM
	150 ± 10.80	70.3 ± 4.0**	37.6 ± 3.7**
+ Methyllycaconitine			
1 nM	152 ± 11.3	147.8 ± 13.4	35.6 ± 12.4**
10 nM	149.8 ± 13.3	151.2 ± 12.4	43.7 ± 9.9**
+ Dihydro-β-erythroidine			
10 nM	150 ± 12.3	76.3 ± 14.9**	49.6 ± 13.3**
100 nM	159.8 ± 3.3	70.3 ± 4.0**	52.3 ± 3.4**

**= $p < 0.01$.

Discussion

The value of comparing the three somewhat disparate preparations used in this study, is that each reveals the action of AChE-peptide over a different time/space scale. The electrophysiological studies in oocytes and brain slices monitor, respectively, single cells through to large neuronal populations over seconds through to minutes, whilst the culture preparation demonstrates the longer term consequences on simplified neuronal matrices, from hours to days. Similarly, each of the three preparations compensates for the other in the pay-off between demonstration of a direct action, as seen in oocytes, against the more physiological yet highly complex circuitry that characterises the mammalian brain slice. The organotypic slice preparation lies somewhere between and can be used to show that the net effect of peptide is a function of dose and duration of application (Day and Greenfield 2003). The efficacy of even the lowest dose of the two different α7 nAChR blockers compared with the insensitivity of peptide to the α4β2 nAChR-preferring blocker, suggests that AChE-peptide is dependent selectively on α7 nAChR.

We have already demonstrated participation of α7 receptors in tissue cultures in mediating the non-cholinergic action of AChE (Day and Greenfield 2002), but the question remains regarding the source of the primary ligand. One possible mechanism, beyond the participation of cholinergic interneurons, would be a basal release of choline, which will also act as a primary ligand at α7 nAChR (Uteshev *et al.* 2003). However, if indeed endogenous choline is acting tonically upon the receptor, it is puzzling that α-BgTx failed to have any blocking effect when administered alone. Nonetheless, neurite extension is regulated by complex processes and there is no reason to assume a simple linear correlation between α7 nAChR activation and neurite length. Rather, the most likely explanation for these and previous (Day and Greenfield 2002) observations is that levels of

endogenous, *tonic* activation of the receptor in the culture preparation have subliminal effects on neurite structure. Alternatively, *phasic* modulation of an allosteric site could produce dramatic effects, that were therefore modifiable significantly by antagonist, as observed.

In general, it is most likely that the inhibitory effects of higher doses of AChE-peptide block α7 nAChR responses through channel inactivation (Standen 1981) and/or desensitization (Quick and Lester 2002). Therefore, the dose–response (activating or desensitizing) curve for peptide will depend on the kinetics of the channel in each system, the activation state of the channel when the peptide is applied and the temporal profile of the concentrations of peptide delivery in the region of the receptors. These parameters may render quantitative comparisons of specific doses misleading, across the different species/preparations.

Nonetheless, these data, taken together, present a consistent, albeit more qualitative, scenario whereby AChE-peptide acts as an allosteric modulator of the α7 nAChR in the nanomolar to micromolar concentration range. In lower doses and/or for short periods of application, the peptide has an excitatory/trophic action that becomes functionally inhibitory as dose/time increases and toxic when time or dose would be high enough to result in sustained influxes of calcium into the cell. Once in the millimolar range, AChE-peptide bioactivity shifts to non-specific, as shown by the loss of sensitivity to α7 nAChR antagonists. Non-specific effects are unsurprising at such non-physiological concentrations and could be attributed to gross damage of the neuronal membrane, perhaps caused by peptide aggregation (Cottingham *et al*. 2002). This clear difference between the nanomolar/micromolar doses of peptide, compared with those in the millimolar range, highlight the truly physiological action of the lower doses and clarify the actual concentration range within which peptide can act as a selective allosteric modulator.

AChE-peptide forms part of a helix on the surface of the protein, remote from the opening of the gorge that contains the active site (Greenfield and Vaux 2002): its functional association with the α 7nAChR reported here may be of fundamental relevance to neurodegeneration. The α7 nAChR has already been implicated in the neuropathological mechanism(s) underlying Alzheimer's disease (AD), in conjunction with β-amyloid (Dineley *et al*. 2001; Nagele *et al*. 2002). However, the most established function of the α7 nAChR is as a powerful calcium ionophore (Seguela *et al.* 1993), which contributes to the survival and naturally occurring death of neurons (Hory-Lee and Frank 1995; Messi *et al*. 1997). Yet the same concentrations of Ca^{2+} that are beneficial in development can lead to excitotoxicity in mature systems (Eimerl and Schramm 1994). Hence, neurodegeneration may represent an aberrant reactivation of developmental mechanisms (Woolf and Butcher 1991; Greenfield and Vaux 2002), with the α7 nAChR as a key intermediary in the neurodegenerative process. Interestingly enough, AChE acting in a non-cholinergic capacity, and indeed AChE-peptide, have also been implicated in this trophic-toxic axis by means of a calcium-dependent, excitotoxic action (Greenfield and Vaux 2002). The data presented here, in identifying AChE-peptide as a novel modulatory ligand for the α7 nAChR, may offer further insight into this potentially pivotal trophic-toxic mechanism and hence be of potential pharmaceutical relevance for combating neurodegeneration.

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