Volume 327, number 3, 284–288 FEBS 12777 August 1993
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Agonist pharmacology of the neuronal α7 nicotinic receptor expressed in *Xenopus* oocytes

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Received 25 May 1993; revised version received 15 June 1993

The potencies and efficacies of seven agonists at chick α7 nicotinic receptors expressed in *Xenopus* oocytes were determined by whole cell recording. (+)-Anatoxin-a was the most potent agonist (EC_{50} = 0.58 μM) and acetylcholine was the least potent (EC_{50} = 320 μM). The rank order of agonist potencies was: (+)-anatoxin-a >> cytisine > (−)-nicotine > (+)-nicotine > DMPP > 1-acetyl-4-methylpiperazine methiodide > acetylcholine. DMPP evoked only very small currents: comparison of maximally effective agonist concentrations showed that DMPP was only one-fifth as efficacious as other agonists. Previously published IC_{50} values for rat brain [^{125}I]α-bungarotoxin sites show a similar agonist profile, and the identity of homo-oligomeric α7 receptors with native α-bungarotoxin-sensitive neuronal nicotinic receptors is discussed.

Neuronal nicotinic receptor; Nicotinic agonist; (+)-Anatoxin-a; Nicotine; α-Bungarotoxin; *Xenopus* oocyte

1. INTRODUCTION

The α7 nicotinic acetylcholine receptor (nAChR) subunit is unique among vertebrate nAChR subunits characterised so far in its ability to form robust homo-oligomeric channels when expressed in *Xenopus* oocytes [1,2]. The expressed α7 channels respond to acetylcholine (ACh) and nicotine, desensitise very rapidly and are sensitive to α-bungarotoxin (αBgt), curare and dihydro-β-erythroidine [1,3]. Antibodies raised against bacterially-expressed α7 gene product indicate that at least 90% of αBgt-binding proteins in the chick brain contain this subunit [4,5]. Until the cloning and characterisation of the α7 cDNA, the relationship between brain αBgt binding sites and nAChR was ambiguous [6]: although αBgt binding sites have a clear nicotinic profile in binding assays [7], the failure of αBgt to antagonise the majority of centrally-mediated nicotinic responses raised questions about the function of these proteins. More recent studies on autonomic neurons [8,9] have revealed that αBgt-sensitive nAChR are functional but their activity is eclipsed by the dominant nAChR subtype mediating conventional synaptic transmission. In the CNS, it is not known if αBgt-sensitive nAChR perform the same functions as in autonomic neurons. However, αBgt-sensitive channels have been demonstrated by patch-clamp analysis of cultured hippocampal neurons [10,11,12]. These channels show the rapid desensitisation characteristic of α7 nAChR.

Thus there is good evidence that α7 subunits contribute to neuronal αBgt-sensitive nAChR. What is not known is whether other subunits are present in the native protein. Protein chemistry has suggested between 1 and 4 subunits in the αBgt-binding protein [13,14] and antibody studies have indicated that some 20% of α7-containing nAChR in chick brain also contain the related α8 subunit [5]. One approach to the comparison of α7 and native αBgt binding sites is to compare their pharmacological specificities and sensitivities. Semi-rigid agonists, stereoisomers and structural analogues can be particularly discriminating. Here we have examined the effects of seven agonists on α7 nAChR expressed in *Xenopus* oocytes, for comparison with published binding data for brain membranes.

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Abbreviations: nAChR, nicotinic acetylcholine receptor; αBgt, α-bungarotoxin; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; AMP MeI, 1-acetyl-4-methylpiperazine methiodide.

2. EXPERIMENTAL

Xenopus oocytes were injected with 2 ng of α7 cDNA [1]. Electrophysiological recording was performed 2–5 days later using a conventional dual electrode voltage clamp. Cells were clamped at −70 mV and perfused (10 ml/min) with modified Barth's solution (MBS: NaCl 88 mM, KCl 1 mM, HEPES 10 mM, $MgSO_4$ 0.82 mM $Ca(NO_3)_2$ 0.33 mM, $CaCl_2$ 0.91 mM, $NaHCO_3$ 2.4 mM, pH 7.5) containing 0.5 μM atropine. Agonists were applied in perfusion as 3 s pulses. Data were stored on a digital-to-analogue DAT converter and processed using the acquisition and analysis programs AQ and PAT2L [15] and Sigmaplot version 4.1. Dose–response curves were fitted to the non-linear Hill equation: $y = 1/(1+(EC_{50}/x)^{n_H})$, where x = agonist concentration and n_H = Hill number.

Materials. (−)-Nicotine base, ACh-HCl and cytisine were from Sigma Chemical Co., Poole Dorset, UK, DMPP I was from Aldrich, Gillingham, Dorset, and (+)-nicotine hydrogen tartrate was from BDH,

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Poole, Dorset. (+)-Anatoxin-a was provided by Dr. E.X. Albuquerque and AMP MeI was provided by Dr. I. Stolerman. Stock solutions (10 mM) of agonists, except AMP MeI, were made up in MBS and adjusted to pH 7.5 if necessary. Because of the low solubility of AMP MeI, a stock solution (50 mM) was prepared in DMSO. Aliquots were stored at −20°C, thawed on the day of use and diluted in MBS containing 0.5 μM atropine. Control samples of diluted DMSO (no agonist present) were used to confirm that DMSO itself had no effect on oocytes expressing α7.

3. RESULTS

Nuclear injection of α7 cDNA into *Xenopus* oocytes resulted in responses to nicotinic agonists when tested 2–5 days later. Large currents, typically 0.2–2 μA, were recorded (Fig. 1), and these displayed characteristic fast onset and rapid desensitisation at higher agonist concentrations [1]. Currents evoked by (−)-nicotine were completely blocked by 10 nM α-cobratoxin with no recovery following 30 min of washing, whereas 10 nM methyllycaconitine produced a complete blockade that was slowly reversible. Several agonists were investigated for their abilities to activate nicotinic currents (Fig. 1): a range of agonist concentrations was tested on a single oocyte and dose–response curves of the peak currents averaged from several such experiments. (+)-Anatoxin-a was clearly the most potent agonist, with an EC_{50} value of 0.58 μM, whereas ACh (EC_{50} = 320 μM) was the least potent (Table I). Intermediate potencies were observed for cytisine (5.6 μM), (−)-nicotine (24 μM), (+)-nicotine (45 μM), and DMPP (64 μM). Hill slopes were greater than one (Table I), consistent with the binding of more than one agonist molecule for activation; (+)-anatoxin-a and (+)-nicotine produced steeper curves than the other agonists.

When agonist dose–response data were normalised with respect to (−)-nicotine (Fig. 1), it was clear that DMPP elicited much smaller responses than (−)-nicotine despite having comparable potency. A similar effect on α7 nAChR was noted by Bertrand et al. [3]. To explore this further, we examined the structurally related compound 1-acetyl-4-methylpiperazine methiodide (AMP MeI) [16]. The dose–response curve for AMP MeI (Fig. 1) gave an EC_{50} of 170 μM (Table I) and the maximum response was 70% of the maximum

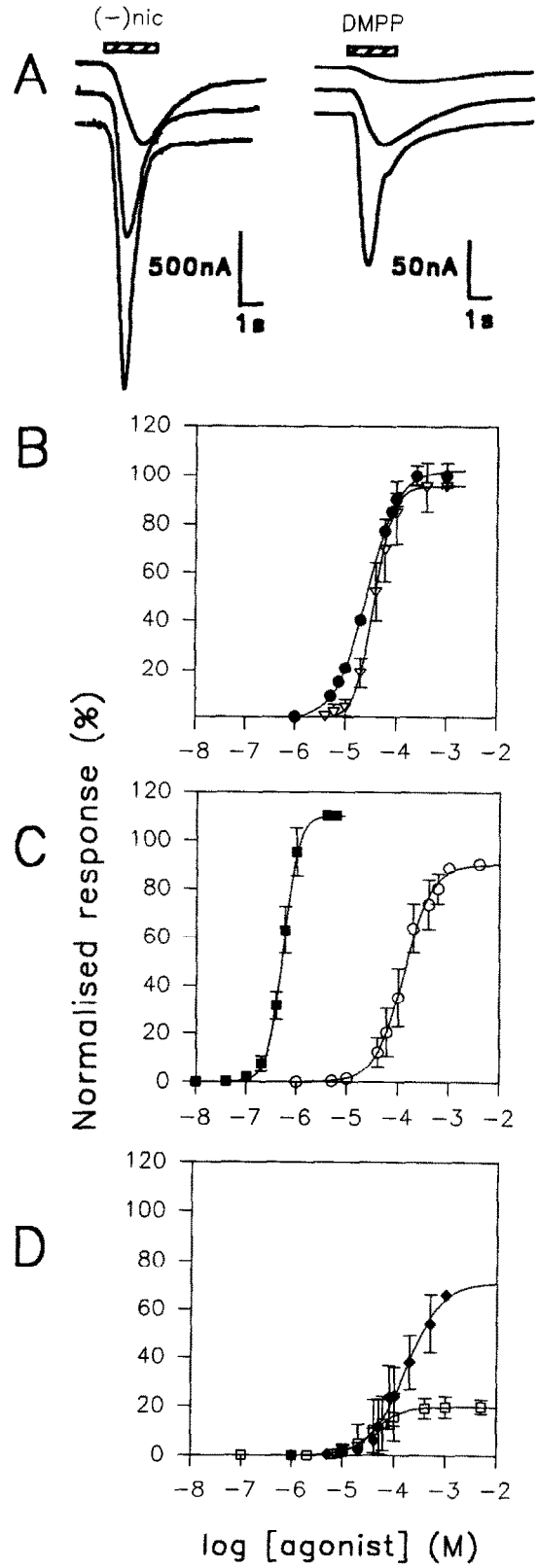

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Fig. 1. Dose–response data for agonists activating α7 nAChR. (A) Superimposed inward currents recorded from two oocytes, each exposed to 3 sec pulses of increasing concentrations of agonist. Ten minute intervals were given between each agonist pulse to allow full recovery from desensitisation, V_H = −70 mV. Left: (−)-nicotine 5×10^{-6}, 5×10^{-5}, 2×10^{-4} M; Right: DMPP 1×10^{-6}, 3×10^{-5}, 1×10^{-3}M. (B,C,D) Dose–response curves compiled from data from several oocytes, and normalised against (−)-nicotine (2.5×10^{-4} M) applied to the same oocyte. Symbols represent the mean data points with S.E.M. indicated by the vertical bars. Lines represent the theoretical dose–response curve fitted to the data points using the non-linear Hill equation (see section 2). (B) ●-●, (−)-nicotine; ▽-▽, (+)-nicotine; (C) ■-■, (+)-anatoxin-a; ○-○, ACh; (D) ◆-◆, AMP MeI; □-□, DMPP.

Table I

Potencies of nicotinic agonists at reconstituted chick α7 nAChR; comparison with ligand binding data for rat brain

Agonist	EC_{50} (M) α7	n_H	Rel. potency (EC_{50})	K_i (M) αBgt binding to rat brain	Rel. potency (K_i)	EC_{50}/K_i
(+)-Anatoxin-a	$5.8 \pm 0.9 \times 10^{-7}$ (5)	2.6 ± 0.3	41	9.1×10^{-8} [a]	97	6.4
Cytisine	$5.6 \pm 1.3 \times 10^{-6}$ (7)	1.9 ± 0.2	23	1.1×10^{-6} [b]	8	5.1
(−)-Nicotine	$2.4 \pm 0.7 \times 10^{-5}$ (4)	1.4 ± 0.1	1	8.9×10^{-6} [c]	1	2.7
(+)-Nicotine	$4.5 \pm 1.0 \times 10^{-5}$ (3)	2.5 ± 0.7	0.53	5.2×10^{-5} [c]	0.17	0.8
DMPP	$6.4 \pm 2.7 \times 10^{-5}$ (7)	2.0 ± 0.2	0.37	7.6×10^{-6} [d]	1.2	8.4
AMP MeI	$1.7 \pm 0.3 \times 10^{-4}$ (5)	1.3 ± 0.1	0.14	3.7×10^{-5} [d]	0.24	4.6
ACh	$3.2 \pm 1.5 \times 10^{-4}$ (5)	1.8 ± 0.3	0.07	1.1×10^{-5} [c]	0.8	29.1

Data from: [a]ref. [7]; [b]ref. [21]; [c]ref. [18]; [d]ref. [16]; [e]unpublished.

response to (−)-nicotine. The efficacies of agonists were compared by applying maximally effective concentrations of each one to the same oocyte in succession (Fig. 2). This confirmed the relative efficacies indicated by the normalised dose–response curves. In particular, DMPP responses were 20% of those of (−)-nicotine whereas AMP MeI gave 66% of the (−)-nicotine response. The plateau level of the AMP MeI dose–response curve (and hence the concentration used in Fig. 2) may be underestimated by the curve fit (Fig. 1) in the absence of data points for higher concentrations of the drug. Such values could not be obtained due to the limited availability and low solubility of AMP MeI. Efficacy comparisons were also carried out with EC_{50} concentrations of the agonists. These experiments confirmed that AMP MeI is at least three times more efficacious than DMPP.

Current–voltage relationships were determined for each agonist and the results are depicted in Fig. 3. The seven agonists behave similarly, responses decreasing as the membrane potential is stepped from −100 to −40

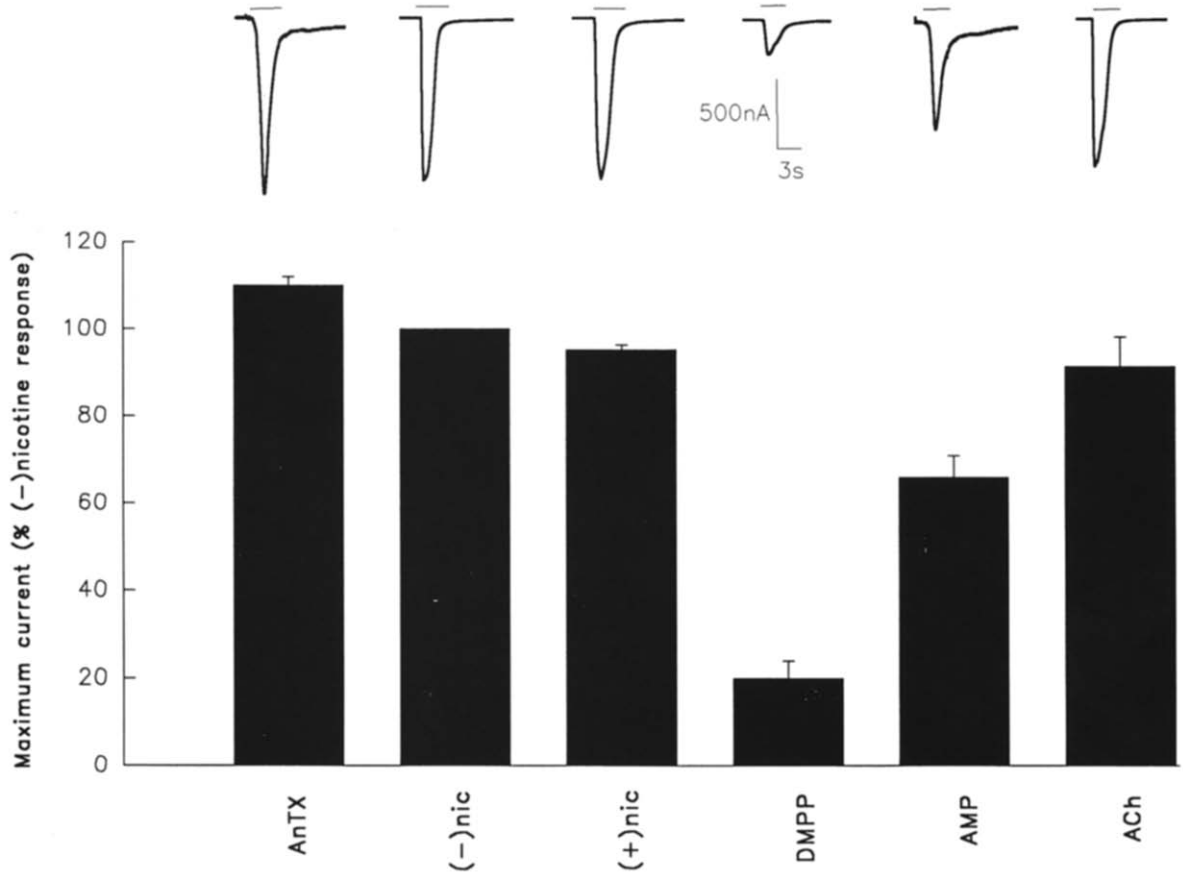

Fig. 2. Efficacy of agonists compared at maximally effective concentrations. Six agonists were tested sequentially on the same oocyte at maximally effective agonist concentrations: (+)-anatoxin-a (4 μM); (−)-nicotine (250 μM); (+)-nicotine (300 μM); DMPP (1 mM); AMP MeI (1 mM) and ACh (4 mM). Current responses were normalised with respect to currents evoked by 250 μM (−)-nicotine. Oocytes were clamped at −70 mV; 12 min intervals were allowed between agonist applications. Values are the mean ± S.E.M. from 5 individual oocytes. Insert: representative current traces.

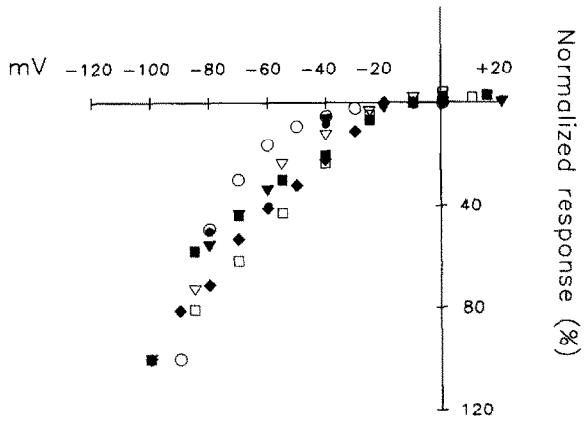

Fig. 3. Current–voltage relationships for six agonists. Applications (3 s) of an agonist were given at 12 min intervals while stepping the holding potential from −100 mV to +20 mV. Current responses were normalised with respect to the response observed at −100 mV. Agonist concentrations approximated to their $EC_{[50]}$ concentrations: ■-■, (+)-anatoxin-a 5×10^{-7} M; ▼-▼, cytisine 5×10^{-6} M; ●-●, (−)-nicotine 2.5×10^{-5} M; ▲-▲, (+)-nicotine 4×10^{-5} M; ▲-▲, DMPP 6×10^{-5} M; ◆-◆, AMP MeI 1×10^{-4} M; ○-○, ACh 2×10^{-4} M.

mV, followed by marked inward rectification at more positive potentials.

4. DISCUSSION

These results extend the quantitative agonist profile of the α7 nAChR. EC_{50} values for (−)-nicotine and ACh are in good agreement with those previously described [3] but cytisine was more potent in the present study. In addition, (+)-anatoxin-a is shown to be the most potent agonist, as it is at other nAChR [17], while (+)-nicotine is two-fold weaker than the naturally occurring enantiomer. Such slight stereoselectivity in favour of (−)-nicotine is a property of αBgt-sensitive sites in *Torpedo* and brain [18], and is in marked contrast to αBgt-insensitive nAChR which show a 100-fold preference for (−)-nicotine [19]. Interestingly, DMPP was a relatively potent agonist at α7 nAChR but displayed only 20% of the efficacy of (−)-nicotine. Bertrand et al. [3] reported that DMPP elicited no significant currents in oocytes expressing α7. Current responses were somewhat larger in the present study and this may have enabled the detection of DMPP-evoked responses. An α7 subunit recently cloned from rat brain [2] also showed anomalous dose-dependency for DMPP, and raised the suggestion that it might have channel blocking activity. Visual inspection of (−)-nicotine and DMPP-evoked current traces (Fig. 1) shows no obvious differences between these two agonists in the rates of activation or desensitisation, but the fast desensitisation shown by α7 nAChR and the limits of resolution imposed by the whole cell recording technique make it difficult to discern the mode of action of DMPP. Analysis is further complicated by the contribution to the α7 responses of a secondary Ca^{2+}-activated chloride current [2]. Single channel analysis will be necessary to determine the precise mechanism that accounts for the small responses observed with DMPP. The structurally related compound AMP MeI was at least 3 times more efficacious than DMPP and may be a fully effective agonist. Thus the common piperazine group is insufficient to explain the results observed with DMPP.

In Table I, the EC_{50} values for agonists are compared with their K_i values for binding to [^{125}I]αBgt sites in rat brain membranes, previously determined in this laboratory. A similar rank order is observed in the two series, but K_i values are approximately five-fold lower than EC_{50} values. However, concordance is not expected as equilibrium binding assays are likely to reflect binding to the desensitised state of the nAChR, and this is associated with higher affinity [20]. The agonists showing greatest deviation from the general relationship between EC_{50} and K_i are (+)-nicotine, which is rather more potent than predicted by the binding data, and ACh, which is less potent than expected. These discrepancies might arise from species differences between chick and rat, or may reflect the absence of additional subunits in the reconstituted nAChR compared to the native receptor. However, αBgt-sensitive nAChR characterised in rat hippocampal neurons (Type IA) [11] have a sensitivity to ACh comparable to that of α7, and the rank order of agonist potencies is similar. Nevertheless an important difference between the native and reconstituted nAChR is that DMPP is as efficacious as ACh at nAChR in hippocampal cells [11]. Secondly, current–voltage relationships for agonists in these neurons may differ from those of α7 nAChR: rectification of α7 currents at about −30 mV (Fig. 3) agrees well with the data of Couturier et al. [1], whereas αBgt-sensitive nAChR in hippocampal neurons have a slightly positive reversal potential and may [10,12] or may not [11] show rectification. Additional subunits in the native protein could account for these differences. Co-expression studies are now required to examine these more complex properties of nicotinic currents, to determine if they are influenced by structural subunits.

Acknowledgements: We are grateful to Marc Ballivet for the α7 cDNA, to Edson Albuquerque for the (+)-anatoxin-a, to Chuck Spivak and Ian Stolerman for the AMP MeI, and to Daniel Bertrand for advice on the electrophysiology and helpful discussions on the manuscript. This study was supported by Shell UK and R.J. Reynolds Tobacco Co. M.A. was the recipient of an EMBO Fellowship.

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