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Pharmacological Characterization of a Nicotinic Autoreceptor in Rat Hippocampal Synaptosomes*

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The modulation of [^{3}H]ACh release by nicotinic compounds was studied in superfused rat hippocampal synaptosomes loaded with [^{3}H]choline. (−)-Nicotine (0.1–10 μM) evoked a dose-dependent increase in [^{3}H]ACh release; higher concentrations were less effective. Nicotine-evoked release was Ca^{2+}-dependent, and blocked by the nicotinic antagonists dihydro-β-erythroidine, mecamylamine, and pempidine. The α7-selective antagonist methyllycaconitine did not inhibit nicotine-evoked release when tested at 1 μM, although at 10 μM some attenuation of the response was observed. Six agonists tested were equally efficacious in stimulating [^{3}H]ACh release, as judged by the maximum responses, and gave the following EC_{50} values: (±)-epibatidine 0.12 μM; (+)-anatoxin-a 0.14 μM; (−)-nicotine 0.99 μM; (−)-cytisine 1.06 μM; ABT-418 2.6 μM; isoarecolone 43 μM. Each agonist generated a "bell-shaped" dose response curve, suggesting desensitisation at higher concentrations. This is supported by analysis of repetitive stimulation with (−)-nicotine and (−)-cytisine: S2/S1 ratios declined sharply with increasing concentration, whereas subsequent KCl-evoked release remained constant. These results are discussed in terms of possible nicotinic receptor subtypes that might be present on hippocampal nerve terminals.

KEY WORDS: Rat hippocampus; nicotinic autoreceptor; nicotinic receptor subtypes; acetylcholine release; epibatidine; (+)-anatoxin-a; (−)-nicotine; (−)-cytisine; ABT-418.

INTRODUCTION

The positive modulation of neurotransmitter release appears to be a widespread and potentially important role of presynaptic nicotinic acetylcholine receptors (nAChR) in the brain (1,2). The nicotinic stimulation of dopamine release from striatal nerve terminals in vitro has been particularly well documented (3–8). [^{3}H]Dopamine release is stimulated by a number of nicotinic agonists, typically having EC_{50} values in the sub- to low-micromolar range, and is blocked by competitive antagonists such as dihydro-β-erythroidine (DHβE), the non-competitive antagonists mecamylamine (4,5) and chlorisondamine (6) and the channel blocker histrionicotoxin (9). At high nicotine concentrations at least, nicotine-evoked [^{3}H]dopamine release is inhibited by neuronal bungarotoxin (5), but is insensitive to α-bungarotoxin (4,5) and low concentrations of methyllycaconitine (MLA) (10).

The nicotinic stimulation of [^{3}H]noradrenaline release from hippocampal preparations in vitro has recently been characterized (11,12), and found to differ

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Abbreviations: DHβE, dihydro-β-erythroidine; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor.

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from striatal dopamine release, studied in parallel, with respect to agonist potency and antagonist sensitivity. For example, nicotine was forty times more potent in releasing [^{3}H]dopamine than [^{3}H]noradrenaline. The competitive antagonists DHβE and MLA were more potent inhibitors of [^{3}H]dopamine release, compared with [^{3}H]noradrenaline release, whereas d-tubocurare and chlorisondamine displayed the reverse preference, and mecamylamine was equally effective on the two systems (11,12). These observations have been interpreted in favour of different nAChR subtypes on hippocampal noradrenergic and striatal dopaminergic terminals. Molecular cloning techniques have disclosed a plethora of nicotinic receptor subunits that are expressed in mammalian neurons (13). However, the combinations of subunits that constitute native pentameric nAChR are largely unknown, and there are insufficient pharmacological tools at present to adequately discriminate different subtypes. Nevertheless, sensitivity to neuronal bungarotoxin has been interpreted as implicating the α3 subunit in the regulation of striatal dopamine release (5). The pharmacological differences, and expression of candidate subunits in noradrenergic neurons as judged by in situ hybridization results for the locus coeruleus (14–16), has lead to the proposition that the α3β4 combination of nicotinic subunits may modulate noradrenaline release (12). The α7 subunit has been discounted in view of the insensitivity of these systems to α-bungarotoxin (4,5) and their low sensitivity to MLA (10,12).

In addition to these examples of nicotinic heteroreceptors, nicotinic autoreceptors have been proposed to regulate ACh release at motor nerve terminals (17), in cortical synaptosomes (18) and in cortical and hippocampal slices (19). nAChR identified by [^{3}H]agonist binding (and correlating with α4 and β2 subunits (20,21)) appear to be associated with cholinergic terminals in the cortex and hippocampus, because the loss of these binding sites in Alzheimer's disease parallels the loss of the presynaptic marker choline acetyltransferase (22). Thus nicotinic autoreceptors constitute a potential therapeutic target for enhancing cholinergic transmission in the earlier stages of this disease (23,24). Postsynaptic nAChR in the hippocampus may include the α7 subunit which preponderates in this brain region (23). The α4β2 candidate autoreceptor might be anticipated to differ in agonist sensitivity and antagonist profile from the heteroreceptors described above (although lesion studies indicate that [^{3}H]nicotine binding sites are also present on dopaminergic terminals in the striatum (25)). In the present study, we have examined the nicotinic stimulation of [^{3}H]ACh release from hippocampal synaptosomes, and have compared a number of novel agonists with respect to their potency and efficacy. A preliminary account of some of these findings has already appeared (26).

EXPERIMENTAL PROCEDURE

Agonist-Evoked [^{3}H]ACh Release from Hippocampal Synaptosomes. Highly purified synaptosomes were prepared from rat hippocampus by density gradient centrifugation on Percoll gradients as previously described (27,28). The F4 synaptosome fraction was washed twice in Krebs-bicarbonate buffer (NaCl, 118.5 mM; $NaHCO_3$, 24.9 mM; KCl, 2.4 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 2.5 mM; glucose, 10 mM, gassed with 95% O_2/5% CO_2, to give pH 7.4), and resuspended to a protein concentration of 1mg/ml. The synaptosomes were loaded with [^{3}H]choline by incubation for 30 min with 0.8 μM [^{3}H]choline (diluted with unlabelled choline to give a specific activity of 2 Ci/mmol). Aliquots (150 μl) were loaded into 6 perfusion chambers of a Brandell superfusion apparatus, and perfused with Krebs-bicarbonate buffer at 37°C, flow rate 0.25 ml/min. Three minute fractions of perfusate were collected and counted for radioactivity.

After a 45 min washout period, agonists were administered in Krebs-bicarbonate buffer as 20 s pulses (S1), separated from the bulk flow of the buffer by 10 s air bubbles (3). Dose response curves for agonists were determined by comparing up to six different agonist concentrations in parallel in a single experiment. A standard response was provided by challenging a parallel chamber with 5 μM nicotine. After an interval of 30 min for recovery of baseline, a second stimulation (S2) with 20 mM KCl in Krebs-bicarbonate buffer was given as an internal standard. In some experiments, S2 consisted of a second application of agonist, in which case a 20 mM KCl pulse was administered as S3, after a further 30 min interval. In these repetitive stimulation experiments, one chamber received a standard pulse of 1 μM nicotine as both S1 and S2. Comparison of responses to KCl given as S1 and S2 pulses was also made. The effect of antagonists was examined by introducing the drug into the perfusion buffer 3 fractions before the S2 agonist pulse (or, in some cases, 3 fractions before S1) and maintaining it throughout the remainder of the experiment.

Data Analysis. Evoked release was measured as the area under the peak above basal release. The cpm tritium was converted to pmol [^{3}H]ACh released/mg protein by reference to the specific activity of the [^{3}H]choline used to load the synaptosomes and the amount of tissue loaded into the perfusion chambers. We have previously confirmed that all of the evoked release corresponds to [^{3}H]ACh (28). A blank buffer pulse typically released tritium corresponding to only 10.4 ± 5.1 fmol [^{3}H]ACh/mg protein: this represents about 6% of the standard KCl response and 1% of the maximum response to nicotinic agonists. This was not subtracted from the data but results were normalised for variations in (i) [^{3}H]choline uptake between experiments: mean uptake was 99.8 ± 8.3 pmol [^{3}H]choline/mg protein/30 min (mean ± SEM for 24 experiments), and (ii) the standard 5 μM nicotine response.

EC_{50} values from dose-response curves were estimated by fitting the rising phase of the curve to the Hill equation. In the case of the steepest curves ((+)-anatoxin-a and (±)-epibatidine) estimates were made by visual inspection.

Materials. [Methyl-^{3}H]choline chloride (80 Ci/mmol) was from Amersham International, Aylesbury, Bucks., UK. (±)Epibatidine was from RBI Chemicals, Boston, USA. (−)-Nicotine, (−)-cytisine, mecamylamine and other drugs and chemicals were purchased from Sigma Chemical Co., Poole, Dorset, UK. (+)-Anatoxin-a was from Dr E. X. Albuquerque, isoarecolone was provided by Dr I. P. Stolerman and

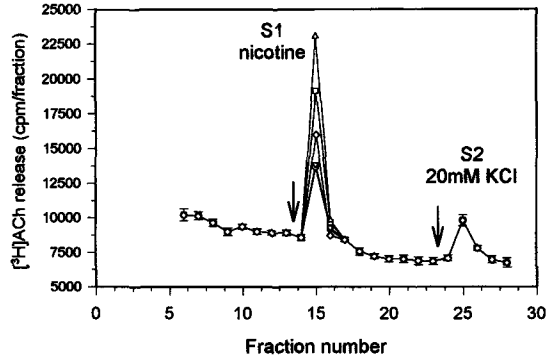

Fig. 1. Typical superfusion profile illustrating the dose-dependence of nicotine-evoked [³H]ACh release from hippocampal synaptosomes. Hippocampal synaptosomes were loaded with [³H]choline and superfused as described in the Methods. After a 45 min washout period, parallel chambers received a 20 s S1 pulse of 0.5 μM (○), 1.0 μM (◇), 5 μM (□), 10 μM (△) or 50 μM (Λ) nicotine, followed, 30 min later, by an S2 pulse of 20 mM KCl. Basal release and KCl-evoked release have been averaged from the five chambers; error bars indicate the SEM.

MLA was from Prof. M. Benn. ABT-418 (3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole was synthesised as previously described (30). Stock concentrations of drugs (1 mM) were made up in water and stored in aliquots at −20°C. Dilutions were prepared in the appropriate buffer prior to each experiment.

RESULTS

Highly purified synaptosomes prepared from rat hippocampus were loaded with [³H]choline and superfused with Krebs-bicarbonate buffer (27,29). Stimulation with low micromolar concentrations of nicotinic agonists increased the outflow of tritium in a concentration-dependent manner: this is illustrated in Fig. 1 with respect to the responses to (−)-nicotine. We have previously shown that all of the evoked release corresponds to [³H]ACh (28). At the low protein concentrations used, the maximum response to nicotinic stimulation was consistently about four-fold higher than the response elicited by a subsequent stimulation with 20 mM KCl (Fig. 1). This contrasts with the nicotinic stimulation of [³H]dopamine release from striatal synaptosomes, which is typically about one half to one quarter of that seen with 20-mM KCl [e.g. 3,5,7,31]. Nicotine-evoked [³H]ACh release was largely Ca^{2+}-dependent: omission of Ca^{2+} from the perfusion buffer and addition of 5mM EDTA reduced responses elicited by 1 μM (−)nicotine to 19.7 ± 7.7% of control (mean ± SEM from 3 experiments), compared with 18.8 ± 3.9% of control for KCl-evoked release.

Dose-response curves were determined for six nicotinic agonists (Fig. 2). In each case the dose-response curve was bell-shaped, showing marked attenuation of responses at higher agonist concentrations (Fig. 2). This made accurate assessment of EC_{50} values difficult, especially in the case of the steeper curves. Best estimates of EC_{50} values for the rising phase of the curves are documented in Table I. (±)-Epibatidine was the most potent agonist, with an EC_{50} value of 0.12 μM. It was only slightly more potent than (+)-anatoxin-a, although (±)-epibatidine consistently gave a shallow shoulder of release at lower concentrations. (−)-Nicotine and (−)-cytisine were about 7 times weaker than (+)-anatoxin-a, as judged by EC_{50} values (Table I). The dose-response curves for these two agonists were less sharp than that for (+)-anatoxin-a, and maximum responses were elicited by 10 μM agonist in both cases, compared with a maximally effective concentration of (+)-anatoxin-a of 0.5 μM. (−)-Nicotine and (−)-cytisine were essentially equipotent. ABT-418 showed half the potency of (−)-nicotine. Isoarecolone was the least potent agonist tested, with an EC_{50} value of 43 μM, and a maximum response elicited by 100 μM isoarecolone. However, from Fig. 2 it is clear that the six agonists examined were equally efficacious at stimulating [³H]ACh release, eliciting an average maximum response of 1.37 ± 0.04 pmol [³H]ACh released/mg protein (Table I).

The nicotinic character of evoked [³H]ACh release was confirmed with respect to (−)-nicotine. DHβE (1 μM) inhibited release evoked by 10 μM (−)-nicotine by 72% (Fig. 3a). KCl-evoked release was unaffected by the antagonist. To examine the possibility that the α7 subunit might contribute to the nAChR modulating [³H]ACh release, the Delphinium alkaloid MLA was tested as an antagonist (Fig. 3b). At 1 μM, MLA did not inhibit [³H]ACh release evoked by either 1 μM (−)nicotine or KCl, whereas 10 μM MLA produced a 46% inhibition of (−)-nicotine-evoked release with no significant effect on KCl-evoked release. The non-competitive antagonists mecamylamine (1 μM) and pempidine (10 μM) inhibited S1-nicotine-evoked [³H]ACh release by 37% and 54% respectively, and S2-nicotine-evoked release by 38% for both drugs (Fig. 3c). KCl-evoked release was insensitive to mecamylamine and pempidine.

It is evident from Fig. 3 that S2 responses to (−)-nicotine are greatly diminished, compared with S1. The effect of repetitive stimulation with agonist was examined over the full dose-response range (Fig. 4a). Whereas the S1 responses to (−)-nicotine resulted in the characteristic bell-shaped profile, S2 responses were greatly attenuated as the concentration of agonist increased. Calculated S2/S1 ratios are seen to decline over

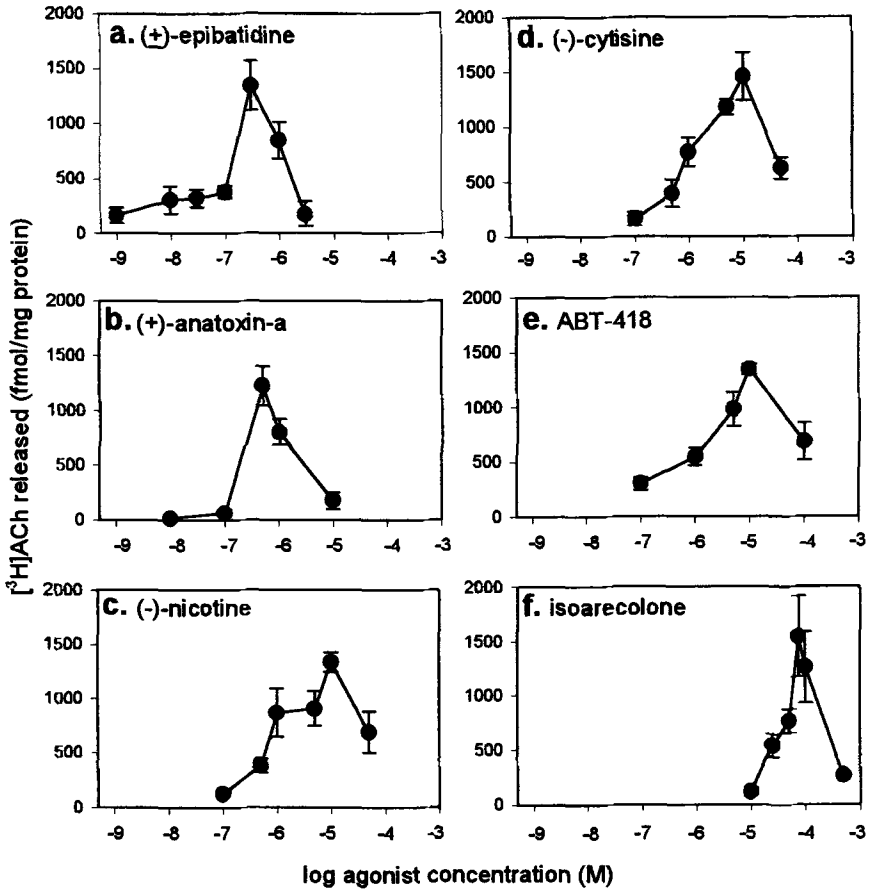

Fig. 2. Dose-response profiles for nicotinic agonist-evoked [³H]ACh release. Hippocampal synaptosomes were superfused as illustrated in Fig. 1. Parallel chambers received S1 pulses of increasing concentrations of agonist: a. (±)-epibatidine; b. (+)-anatoxin-a; c. (−)-nicotine; d. (−)-cytisine; e. ABT-418; f. isoarecolone. One chamber served as a standard, and was challenged with 5 μM nicotine. Evoked release above baseline was converted to fmol/mg protein by reference to the specific activity of the [³H]choline, and data were normalized for [³H]choline uptake and 5 μM nicotine standard, values are the mean ± SEM for at least 3 independent assays.

Table I. Potency of Nicotinic Agonists in Evoking [³H]ACh Release

Agonist	EC_{50} (μM)	Maximum release (fmol/mg protein)	Concn giving max response (μM)
(±)-Epibatidine	~0.12 ± 0.03	1344 ± 222	0.3
(+)-Anatoxin-a	~0.14 ± 0.04	1225 ± 177	0.5
(−)-Nicotine	0.99 ± 0.23	1335 ± 92	10
(−)-Cystine	1.06 ± 0.15	1456 ± 215	10
ABT-418	2.60 ± 0.12	1345 ± 51	10
Isoarecolone	43.2 ± 7.00	1546 ± 371	100

the concentration range studied (Fig. 4b). To confirm that this phenomenon is not peculiar to (−)-nicotine, a similar experiment was carried out with (−)-cytisine, which yielded almost identical results (Fig. 4b). In contrast, release stimulated by 20 mM KCl, given as an S3 pulse, was essentially constant (Fig. 4a). Moreover, KCl administered as consecutive S1 and S2 pulses gave an S2/S1 ratio of 1.09 (n = 9).

DISCUSSION

This study has demonstrated that nicotinic agonists can stimulate the release of [³H]ACh from hippocampal synaptosomes in a Ca^{2+}-dependent and DHβE-sensitive manner, consistent with the presence of presynaptic nAChR on hippocampal cholinergic terminals. These data compare favorably with the previous report of Araujo et al. (19) of the nicotinic stimulation of endogenous ACh release from cortical and hippocampal slices: the tetrodotoxin-insensitivity of release evoked by nicotinic agonists was interpreted in favor of cholinergic autoreceptors. The present examination of highly puri-

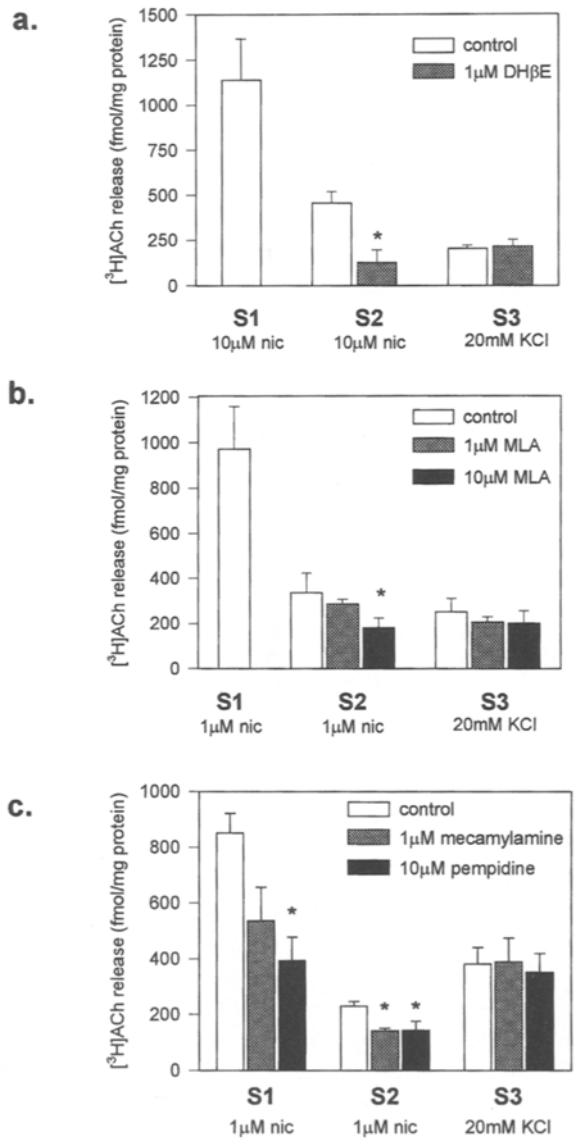

Fig. 3. The effect of nicotinic antagonists on nicotine-evoked [^{3}H]ACh release. Hippocampal synaptosomes were loaded with [^{3}H]choline and superfused as described in the Methods. (a) DHβE (1 μM) was administered three fractions before the S2 pulse of 10 μM nicotine, and maintained throughout the rest of the experiment. (b) MLA (1, 10 μM) was administered three fractions before the S2 pulse of 1 μM nicotine, and maintained throughout the rest of the experiment. (c) Mecamylamine (1 μM) or pempidine (10 μM) was introduced into the perfusion buffer three fractions before the S1 pulse of 1 μM nicotine, and maintained throughout the rest of the experiment. Values are the mean ± SEM from at least 3 independent assays. *significantly different from corresponding control, $p < 0.05$, Student *t*-test.

fied synaptosome preparations, in which synaptic interactions have been disrupted, reinforces that view.

The six nicotinic agonists compared in the present study exhibited potencies that ranged from 0.1 μM to 43 μM, with a rank order of potency:

(±)-epibatidine > (+)-anatoxin-a > (−)-nicotine = (−)-cytisine > ABT-418 >> isoarecolone

Epibatidine is currently the most potent nicotinic agonist known (32). Its 10-fold higher potency, relative to (−)-nicotine, for stimulating [^{3}H]ACh release is low compared with its potency ratios in other systems, e.g. (±)-epibatidine is 150-fold more potent than (−)-nicotine in stimulating [^{3}H]dopamine release from striatum (33). (+)-Anatoxin-a was almost as potent as (±)-epibatidine in stimulating [^{3}H]ACh release. Its EC_{50} value of 0.14 μM is similar to its potency in stimulating [^{3}H]dopamine release from striatal synaptosomes (7,12). (−)-Nicotine and (−)-cytisine were essentially equipotent and about 2.5 times more potent than the novel nicotinic ligand ABT-418. In various in vitro assays of nAChR function, the potency of ABT-418 ranged from equipotent with (−)-nicotine to 10 times less potent (34,35). The least potent agonist tested was isoarecolone, with a potency 40-fold less than that of (−)-nicotine. This is consistent with the low potency of this ligand in other in vitro and in vivo nicotinic assays (12,36), and contrasts with the higher potency shown by its methiodide salt [see 24].

Examination of the dose-response data (Fig. 2) reveals two significant features: firstly, all of the agonists examined were equally efficacious with respect to the maximum response elicited (Table I). Secondly, each of the dose-response curves is markedly "bell-shaped". This characteristic was also seen in the dose-response relationships for methylcarbamylcholine- and nicotine-evoked release of endogenous ACh from hippocampal slices (19), but is not obvious in dose-response curves for nicotinic agonist-evoked striatal [^{3}H]dopamine release or hippocampal [^{3}H]noradrenaline release, which tend to plateau (5,7,21,34). Does this signify that the hippocampal nicotinic autoreceptor is a different nAChR subtype than the heteroreceptors modulating catecholamine release? The α7-type nAChR can be excluded because nicotine-evoked [^{3}H]ACh release is insensitive to α-bungarotoxin (19) and low concentrations of MLA (Fig. 3). The 46% inhibition by 10 μM MLA resembles the sensitivity of [^{3}H]dopamine release (10) and is compatible with an IC_{50} of 3 μM for blockade of heterologously expressed human α4β2 nAChR (J. P. Sullivan, unpublished observation). In situ hybridisation studies indicate that the medial septum and diagonal band of Broca, which contain the cell bodies of cholinergic neurons that innervate the hippocampus, express α4 > β2 = α2 >> α3 subunits (14). Notably, no specific hybridisation of the β4 subunit mRNA was detected in the

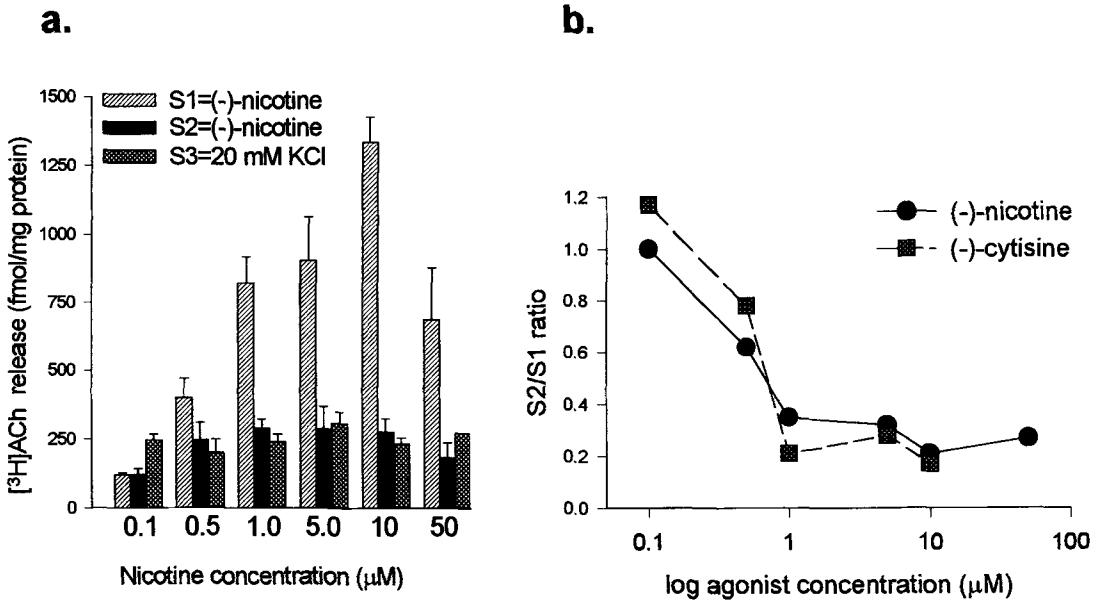

Fig. 4. Comparison of repetitive agonist stimulation. Hippocampal synaptosomes were superfused as described in the Methods. Each chamber received replicate stimulations with the same concentration of agonist as S1 and S2 pulses, separated by a 30 min recovery period, followed by an S3 pulse of 20 mM KCl after a further 30 min. (a) Release data for nicotine- and KCl-evoked [^{3}H]ACh release. Each bar represents the mean ± SEM from at least 3 determinations. (b) The change in S2/S1 ratio as a function of (−)-nicotine (●) or (−)-cytisine (■) concentration.

septum (15). The low agonist potency of agonists for the α3β4 nAChR and its relative insensitivity to DHβE (37) also argue against this subtype of nAChR in the mediation of [^{3}H]ACh release.

The loss of [^{3}H]agonist binding sites in Alzheimer's disease (22) and the enrichment of [^{3}H]nicotine binding sites on hippocampal synaptosomes (27) favours the α4β2 subunit combination. Anatoxin-a and (−)-nicotine give dose-response curves for [^{3}H]ACh release (Fig. 2) which resemble (in both potency and "bell"-shape) those for Rb^+ flux into M10 cells expressing chick α4 and β2 subunits (38). Recently, expression of pairwise combinations of rat nicotinic subunits in *Xenopus* oocytes and examination over an extended concentration range has shown the α4β2 combination to yield a "bell-shaped" dose-response curve, in contrast to α3β2 and α2β2 (39). However, in *Xenopus* oocytes expressing rat α4 and β2 nicotinic subunits, (−)-cytisine is a partial agonist (40), and this has been attributed to the β subunit (41). (−)-Cytisine also shows low efficacy in some brain preparations [e.g. 6,11,42] and this property has been taken to support the participation of a α4β2 nAChR. Yet in the present study, (−)-cytisine appears as efficacious as the other agonists. This would seem to argue against the α4β2 combination. However (−)-cytisine appears to be a full agonist at *chick* α4β2 nAChR expressed in *Xenopus* oocytes (43) or mammalian cells (26,44), and is equipotent with (−)nicotine in these preparations, despite having higher affinity in ligand binding assays (4,5,43). Post-translational modifications or the incorporation of additional types of nicotinic subunit may render the rat α4β2 nAChR sensitive to (−)-cytisine in hippocampal nerve terminals. In the chick, the α5 subunit is associated with α3 and β4 subunits to form functional nAChR (45). However, α5 expression was not detected in the septum (16) so this subunit is not likely to contribute to the autoreceptor discussed here. The participation of the α2 subunit (14) remains a possibility.

The EC_{50} values for agonist-evoked [^{3}H]ACh release are similar to those reported for [^{3}H]dopamine release from striatal synaptosomes, although some variation occurs between laboratories (5,6,7,12,35). This relatively high sensitivity to agonists, the partial agonist properties of (−)-cytisine (11,12) and evidence from lesion studies for the presence of [^{3}H]nicotine binding sites on striatal dopaminergic terminals (25) has raised the possibility that the nicotinic heteroreceptor in this system may be comprised of α4 and β2 subunits (12). However, the sensitivity of high agonist concentrations to neuronal bungarotoxin (5) and the sustained dose-response curves are more compatible with α3-containing nAChR; heterogeneity of nAChR regulating striatal [^{3}H]dopamine release has been proposed, to reconcile these data (46). Until the subunit compositions of endogenous nAChR

are established, and definitive subtype-selective probes become available, these attempts to assign subunit compositions to nAChR mediating various nicotinic functions will remain largely speculative.

The "bell-shaped" dose-response curves for agonist-evoked [^{3}H]ACh release suggest desensitisation at higher agonist concentrations (47). This is supported by the greatly attenuated S2/S1 ratios (Fig. 4). Depletion of the releasable pool of [^{3}H]ACh does not appear to be a major contributor: basal release between the S1 and S2 pulses accounted for less than 10% of the total accumulated radioactivity in the synaptosomes, and the maximum evoked release in response to S1 was only 3% of the total radioactivity. Moreover, KCl-evoked responses were constant, irrespective of the magnitude of the preceding nicotinic response (Fig. 4) and repetitive KCl stimulation produced no attenuation of S2/S1 ratios. Thus pool depletion does not explain the diminished S2/S1 responses for (−)-nicotine and (−)-cytisine (unless KCl and nAChR provoke the release of transmitter from different intracellular pools). It would have been of interest to examine this phenomenon with the more potent agonists (±)-epibatidine and (+)-anatoxin-a, which generated sharper "bell-shaped" curves (Fig. 2). However, the very "sharpness" of their curves precluded this experiment, as only a couple of concentrations were capable of eliciting release and this number was insufficient for a meaningful comparison.

Desensitization of nicotine-evoked [^{3}H]dopamine release from striatal synaptosomes has been subjected to detailed scrutiny (31,48). In this system, repetitive stimulation also resulted in reduced responses, with S2/S1 ratios of about 0.2 in response to micromolar (−)-nicotine concentrations. But the time course for recovery from desensitisation in the striatal preparations predicts that at least 80% recovery should have occurred by the end of the 30 min interval between S1 and S2 pulses in the present experiments. As this was clearly not the case (Fig. 4), these results also point to differences between striatal nicotinic heteroreceptors and hippocampal autoreceptors: these might be gross differences in subunit composition of the nAChR or subtle differences in the cellular mechanisms influencing desensitisation, such as phosphorylation events.

In summary, this study has demonstrated the nicotinic modulation of [^{3}H]ACh release from hippocampal synaptosomes, consistent with the presence of nicotinic autoreceptors on cholinergic terminals. Agonist potencies and "bell-shaped" dose response curves resemble those of nAChR comprised of α4 and β2 subunits. However, the high efficacy of (−)-cytisine is difficult to reconcile with results from α4β2 nAChR expressed in *Xenopus* oocytes: the subunit composition of the nicotinic autoreceptor remains equivocal. The "bell-shaped" dose response curves signify profound desensitisation of the nicotinic autoreceptor at higher agonist concentrations and on repetitive stimulation. This tendency has implications for the utility of therapeutic agonists targeted at this nAChR.

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