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Pharmacological characterization of nicotinic receptor-mediated acetylcholine release in rat brain—an in vivo microdialysis study

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Abstract

In vivo microdialysis was used to investigate nicotinic receptor-mediated acetylcholine release in the hippocampus, frontal cortex, and striatum of freely moving rats. Intraperitoneal administration of (−)-nicotine increased the release of acetylcholine in the hippocampus and frontal cortex but not in the striatum. (−)-Nicotine exhibited a bell-shaped dose–response relationship, and showed attenuation of response at the highest dose (5.0 mg/kg i.p.) in both the hippocampus and frontal cortex. In the hippocampus, (−)-nicotine (1.0 mg/kg i.p.)-induced increase of acetylcholine release was blocked by pretreatment with the centrally acting nicotinic receptor channel blocker, mecamylamine (1.0 mg/kg i.p.), but not by hexamethonium (5.0 mg/kg i.p.), suggesting that the effects of (−)-nicotine were mediated by the central nicotinic receptor. (*S*)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT-418, 1.0 and 5.0 mg/kg i.p.), reported to be a selective agonist for $\alpha4\beta2$ nicotinic receptor subunits, also enhanced the release of acetylcholine in the hippocampus, while 3-(2,4-dimethoxybenzlidene)-anabaseine (GTS-21, 1.0 and 5.0 mg/kg i.p.), which has high affinity for the $\alpha7$ nicotinic receptor subunit, was without effect. The natural alkaloids isolated from plants, (−)-cytisine and (−)-lobeline, had little effect on acetylcholine release from the hippocampus. A competitive antagonist for $\alpha4\beta2$ subunits of the nicotinic receptor, dihydro-β-erythroidine, and a partial agonist for the $\beta2$ subunit-containing nicotinic receptor, (−)-cytisine, inhibited (−)-nicotine-induced increase of acetylcholine release from the hippocampus, whereas a selective antagonist for the $\alpha7$ subunit, methyllycaconitine, and a partial agonist for the $\alpha3$ subunit-containing nicotinic receptor, (−)-lobeline, did not. These results indicate that there are certain differences among brain regions in the response of nicotinic receptor-mediated acetylcholine release and that (−)-nicotine-induced acetylcholine release in the rat hippocampus may be attributed to activation of the $\alpha4\beta2$ nicotinic receptor subunits.

Keywords: Nicotinic receptor; Acetylcholine release; Microdialysis; ABT-418; GTS-21; Dihydro-β-erythroidine; Methyllycaconitine; Brain; (Rat)

1. Introduction

In the past decade, it has become evident that there is a great diversity of nicotinic receptors in the central nervous system (review articles, Sargent, 1993; Decker et al., 1995). Several neuronal nicotinic receptor subunits have been cloned, and eight types of α subunits ($\alpha2$–$\alpha9$) and three types of β subunits ($\beta2$–$\beta4$) (Boulter et al., 1987; Nef et al., 1988; Wada et al., 1988; Couturier et al., 1990; Elgoyhen et al., 1994) have been identified. The neuronal nicotinic receptors are thought to be composed of α and β subunits (Anand et al., 1991; Cooper et al., 1991) and the most abundant nicotinic receptor in the central nervous system consists of $\alpha4$ and $\beta2$ subunits (Flores et al., 1992), while, in recombinant expression systems, $\alpha7$ as well as $\alpha8$ and $\alpha9$ subunits can form functional homooligomeric receptors (Couturier et al., 1990; Gerzanich et al., 1994; Elgoyhen et al., 1994). However, the functional significance of the nicotinic receptor subtypes formed by these subunits remains obscure.

Recently, neuronal nicotinic receptor agonists have attracted much interest as potential therapeutic agents for the treatment of Alzheimer's disease. Clinical studies have revealed that (−)-nicotine is effective to ameliorate memory and attention deficits in Alzheimer's disease patients (Newhouse et al., 1986; Sahakian et al., 1989; Jones et al., 1992). In animals, (−)-nicotine has been reported to show beneficial effects on memory in aged monkeys and to reverse spatial memory deficits in rats with an experimental lesion of the medial septal nucleus (Levin, 1992; Decker

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et al., 1995). In addition, the centrally acting nicotinic receptor channel blocker, mecamylamine, produces significant cognitive impairment that mimics certain aspects of Alzheimer's disease in young and elderly volunteers (Newhouse et al., 1994). Postmortem studies of Alzheimer's disease brain tissue demonstrated marked reductions of nicotinic receptors in both neocortex and hippocampus, consistent with the Alzheimer's disease pathology of neuronal degeneration (Araujo et al., 1988b). These findings point to the functional importance of nicotinic acetylcholine systems in cognitive functions.

Previous in vitro studies have provided evidence that (−)-nicotine can enhance acetylcholine release by stimulating presynaptic nicotinic receptors in the cortex and hippocampus (Rowell and Winkler, 1984; Araujo et al., 1988a; Wilkie et al., 1996), but there are only a few studies that have addressed the in vivo ability of (−)-nicotine to increase acetylcholine release in these brain regions. In microdialysis studies, subcutaneous injection of (−)-nicotine significantly increased the release of acetylcholine from the rat cortex in a mecamylamine-sensitive manner (Summers et al., 1994; Summers and Giacobini, 1995). Since direct application of (−)-nicotine into the cortex through a microdialysis probe membrane increased acetylcholine release, it is likely that the stimulation of acetylcholine release by (−)-nicotine occurred via presynaptic nicotinic receptors (Summers and Giacobini, 1995). Thus, a role for nicotinic receptors in the modulation of acetylcholine release from cortex has been accepted, while the subunits of nicotinic receptors mediating acetylcholine release have not been definitively identified.

Regarding the several types of neuronal nicotinic receptor ligands recently discovered (see review, Brioni et al., 1996), extensive pharmacological and behavioral studies have been carried out on (*S*)-3-methyl-5-(1-methyl-2-pyrrolidnyl) pyrrolidinyl) isoxazole (ABT-418) (Garvey et al., 1994) and 3-(2,4-dimethoxybenzylidene) anabaseine (GTS-21) (Zoltewicz et al., 1993). ABT-418 selectively activates $\alpha 4\beta 2$ subunits more than $\alpha 3$ and $\alpha 7$ subunits (Arneric et al., 1994) and shows potent cognition-enhancing and anxiolytic properties in animal models, with low side-effects (Decker et al., 1994). In contrast, GTS-21 has selectively for the $\alpha 7$ subunit, which is preponderant in the hippocampus, and exerts cognition-enhancing activity in rats (Meyer et al., 1994) and cytoprotective effects in cells (Martin et al., 1994; Kihara et al., 1997). These ligands' pharmacological and neurochemical activities may be associated with release-enhancing effects, although there is no direct evidence for acetylcholine release in vivo with these nicotinic receptor ligands.

In the present study, therefore, we examined the effects of (−)-nicotine on acetylcholine release in three brain regions and compared the abilities of several types of nicotinic receptor ligands to affect acetylcholine release from rat hippocampus by using an in vivo microdialysis technique.

2. Materials and methods

2.1. In vivo microdialysis

Male Fischer-344 rats (9–10 weeks of age, b.wt 200–250 g, Charles River Japan Breeding Laboratories) were used. They were housed in a climate-controlled room (room temperature 23 ± 1°C and humidity 55 ± 5%) and allowed free access to food and water. The animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and a dialysis guide cannula (PC12, Carnegie Medicin, Sweden) was stereotaxically implanted into the right hippocampus (5.8 mm posterior and 5.0 mm lateral to the bregma and 3.0 mm below the skull surface), the right frontoparietal cortex (1.2 mm anterior and 5.0 mm lateral to the bregma and 3.0 mm below the skull surface), the right caudate-putamen (0.2 mm anterior and 3.0 mm lateral to the bregma and 4.0 mm below the skull surface), according to the rat brain atlas (Paxinos and Watson, 1986). The guide cannula, which was sealed with a dummy cannula, was fixed to the skull bone with an anchor screw and dental cement. After animals had been allowed to recover from the surgery for 1 day, they were placed individually in a lidless oval cage, which prevents the animals from bumping against the wall, and were connected to a wire extending from a swivel. A microdialysis probe (PC 12, 3-mm dialysis membrane, molecular cutoff 20 000, outer diameter 0.5 mm, Carnegie Medicin, Sweden) was inserted into the guide cannula with the animal conscious, without any more distress than that of the handling. Ringer's solution (147 mM NaCl, 4.0 mM KCl, 4.5 mM $CaCl_2$, pH 6.4) containing 10 μM physostigmine to block acetylcholinesterase activity was perfused at a flow rate of 2.0 μl/min with a microinjection pump (CMA/100, Carnegie Medicin, Sweden). After 3 h equilibration period, dialysates were collected in microtest tubes containing 10 μl of 50 pmol ethylhomocholine as the internal standard. Sampling was done every 20 min with a microfraction collector (CMA/140, Carnegie Medicin, Sweden). After the experiments, the brains were dissected out, and the location of the implantation site of the dialysis probe was verified visually.

2.2. Assay of acetylcholine in dialysates

The concentration of acetylcholine was determined by liquid chromatography with electrochemical detection. The liquid chromatography system consisted of an electrochemical detector (LC-4B, BAS) with platinum electrode coupled with an acetylcholine separation column (110×2.0 mm, BAS) followed by an immobilized enzyme column (7.0×2.0 mm, BAS) containing acetylcholinesterase and choline oxidase. The columns were maintained at a constant temperature of 35°C with a column heater (LC-22A, BAS). The detector was operated at a potential of 500 mV vs. Ag/AgCl. The mobile phase consisted of 50

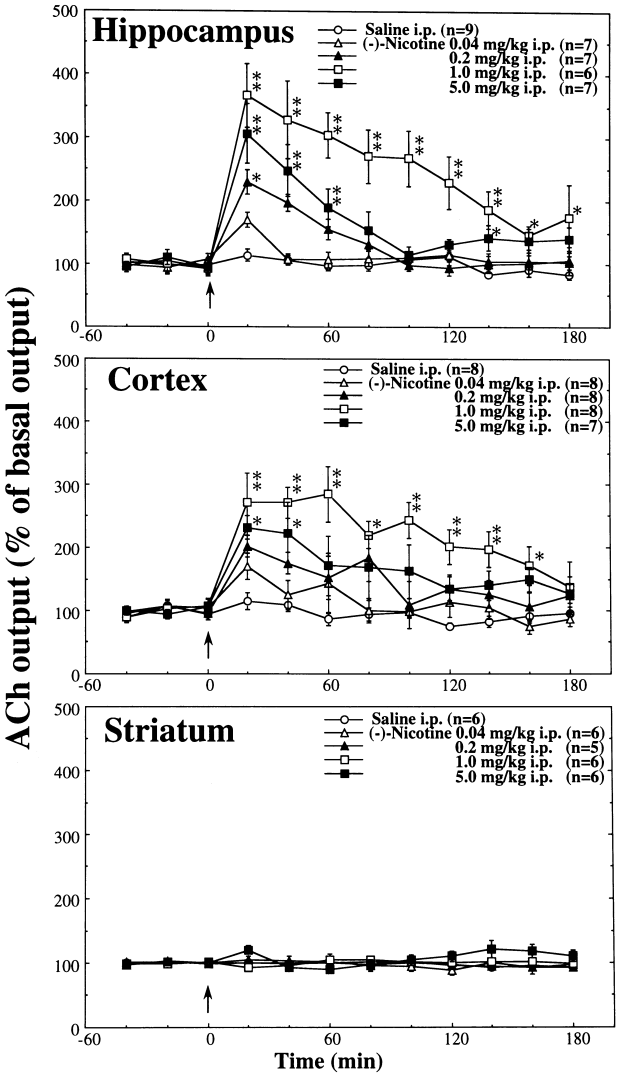

Fig. 1. Effects of (−)-nicotine on extracellular levels of acetylcholine in the rat frontal cortex, hippocampus and striatum. Data are expressed as percentages of the pre-injection basal level. Each symbol represents the mean ± S.E. for 5–9 rats. $* P < 0.05$, $** P < 0.01$; significantly different from saline group (Dunnett's multiple comparison test). Arrow indicates saline or (−)-nicotine injection.

mM Na_2HPO_4, 0.55 mM sodium octanesulfonate and 0.1 mM EDTA 2Na, and pH adjusted to 8.40 with orthophosphoric acid. The flow rate was maintained at 0.3 ml/min by a PM-60 pump (BAS). The concentration of acetylcholine was quantified by calculating the area under the curve, using an integrator (C-R4AX, Shimadzu, Japan).

2.3. Drugs

(−)-Nicotine ditartrate, dihydro-β-erythroidine hydrobromide and methyllicaconitine citrate were purchased from Research Biochemicals International (RBI, Natick, MA). Mecamylamine hydrochloride, hexamethonium chloride, (−)-cotinine, (−)-cytisine, (−)-lobeline hydrochloride and eserine (physostigmine) hemisulfate were purchased from Sigma (St. Louis, MO). (*S*)-3-Methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole (ABT-418) and 3-(2,4-dimethoxybenzlidene)-anabaseine (GTS-21) were synthesized at the Suntory Institute for Biomedical Research. All drugs were dissolved in saline and injected intraperitoneally (i.p.) in a volume of 0.1 ml/100 g b.wt. Each nicotinic receptor antagonist was administered 20 min before (−)-nicotine injection. All doses were calculated as the free base.

2.4. Statistical analysis

The extracellular level of acetylcholine (pmol per 20 min dialysate sample) not corrected for its recovery through the dialysis probe was expressed as a percentage of pre-in-

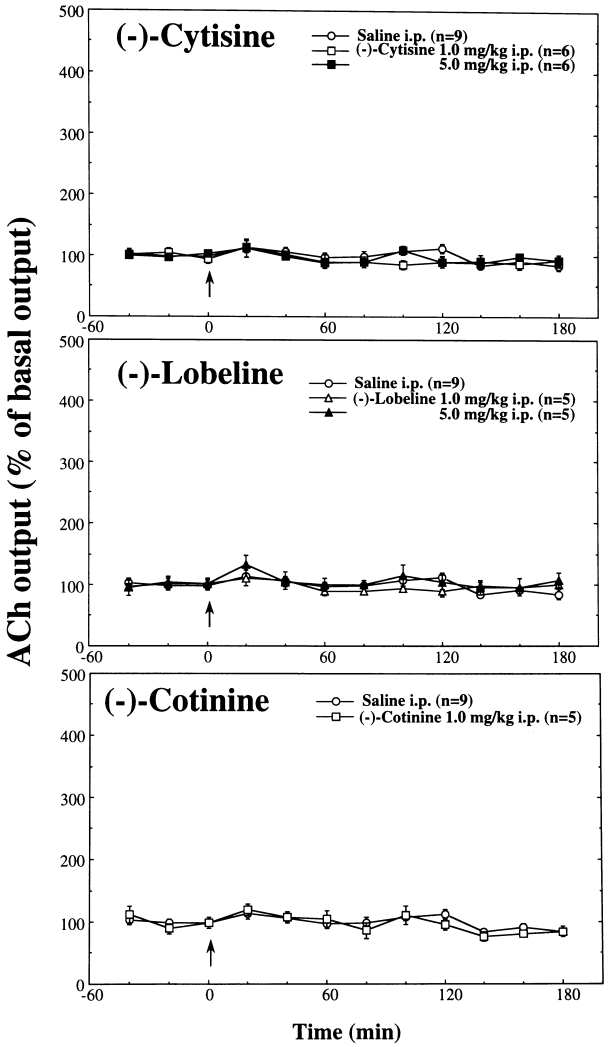

Fig. 2. Effects of (−)-cytisine, (−)-lobeline and (−)-cotinine on extracellular levels of acetylcholine in the rat hippocampus. Data are expressed as percentages of the pre-injection basal level. Each symbol represents the mean ± S.E. for 5–9 rats. Arrow indicates saline or drug injection. (−)-Cytisine (upper) and (−)-lobeline (middle) are the natural nicotinic receptor ligands isolated from plants and (−)-cotinine (lower) is the major metabolite of (−)-nicotine.

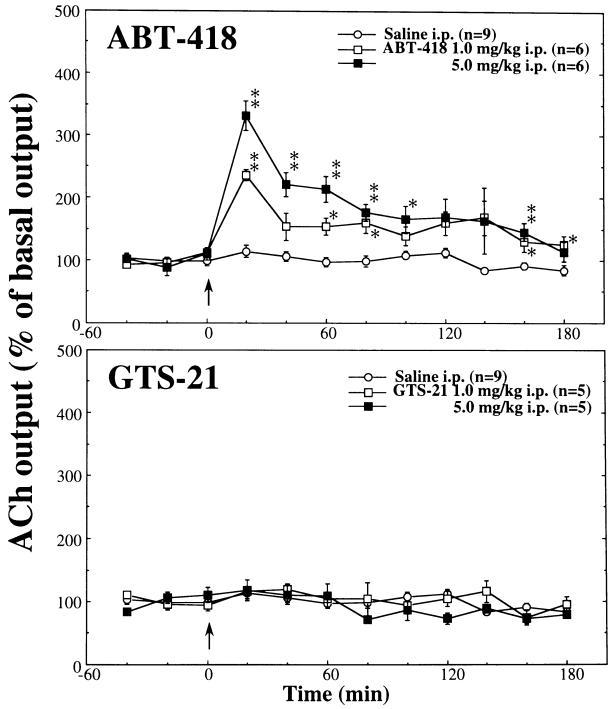

Fig. 3. Effects of ABT-418 and GTS-21 on extracellular levels of acetylcholine in the rat hippocampus. Data are expressed as percentages of the pre-injection basal level. Each symbol represents the mean ± S.E. for 5–9 rats. $*\ P < 0.05$, $**\ P < 0.01$; significantly different from saline group (Dunnett's multiple comparison test). Arrow indicates saline or drug injection. ABT-418 (upper) and GTS-21 (lower) are nicotinic receptor ligands which have high affinity for $\alpha 4\beta 2$ and $\alpha 7$ subunits, respectively.

jection baseline levels (average of the three samples prior to injection). Statistical analyses of the release rate data for multiple comparisons were performed by analyses of variance (ANOVA) followed by Dunnett's two-tailed multiple comparison test. A probability level of $P < 0.05$ was considered significant.

3. Results

3.1. Effects of (−)-nicotine on acetylcholine release in the hippocampus, frontal cortex and striatum

The baseline levels of acetylcholine in the hippocampus, frontal cortex, and striatum were 1.62 ± 0.05 ($n = 162$), 4.10 ± 0.37 ($n = 39$), and 43.75 ± 2.27 pmol/20 min ($n = 29$), respectively. The effects of (−)-nicotine on extracellular levels of acetylcholine in the three brain regions were examined for the dose range of 0.04–5.0 mg/kg (Fig. 1). The dose–response curves for (−)-nicotine-induced acetylcholine release were bell-shaped. In the hippocampus, (−)-nicotine (0.2 and 1.0 mg/kg i.p.) significantly increased acetylcholine release in a dose-dependent manner, and the maximal response was observed at 1.0 mg/kg, reaching 2.0 to 3.5-fold over baseline, and lasted for 3 h after injection. However, the effect of (−)-nicotine at the highest dose (5.0 mg/kg) was considerably attenuated and had a shorter duration than that of the dose of 1.0 mg/kg. Similar results were obtained in the frontal cortex, although the effect of (−)-nicotine was slightly weaker than that observed in the hippocampus. (−)-Nicotine exerted no significant effect on acetylcholine release in the striatum.

3.2. Comparison of the effects of several nicotinic receptor agonists and (−)-cotinine on acetylcholine release in the hippocampus

The natural alkaloids isolated from plants, (−)-cytisine and (−)-lobeline, and synthesized nicotinic receptor ligands, ABT-418 and GTS-21, at the doses of 1.0 and 5.0 mg/kg i.p., were examined for their effects on acetylcholine release from the hippocampus. Both (−)-cytisine and (−)-lobeline showed high affinity for nicotinic receptors in the rat brain [^{3}H](−)-nicotine binding assay (Anderson and Arneric, 1994; Decker et al., 1995; Tani et al., 1997). These alkaloids, however, had no significant

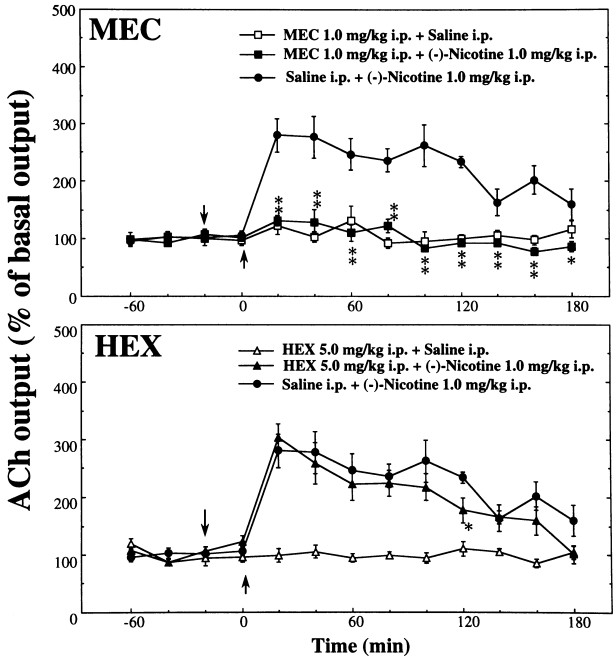

Fig. 4. Effects of mecamylamine (MEC) and hexamethonium (HEX) on (−)-nicotine-induced increase of extracellular levels of acetylcholine in the rat hippocampus. Data are expressed as percentages of the pre-injection basal level. Each symbol represents the mean ± S.E. for 6 rats. $*\ P < 0.05$, $**\ P < 0.01$; significant difference between saline +(−)-nicotine group and antagonist+(−)-nicotine group (Dunnett's multiple comparison test). Arrow indicates saline or drug injection, and each antagonist was administered 20 min before (−)-nicotine or saline injection. Mecamylamine (upper) and hexamethonium (lower) are nicotinic receptor channel blockers, centrally and peripherally acting, respectively.

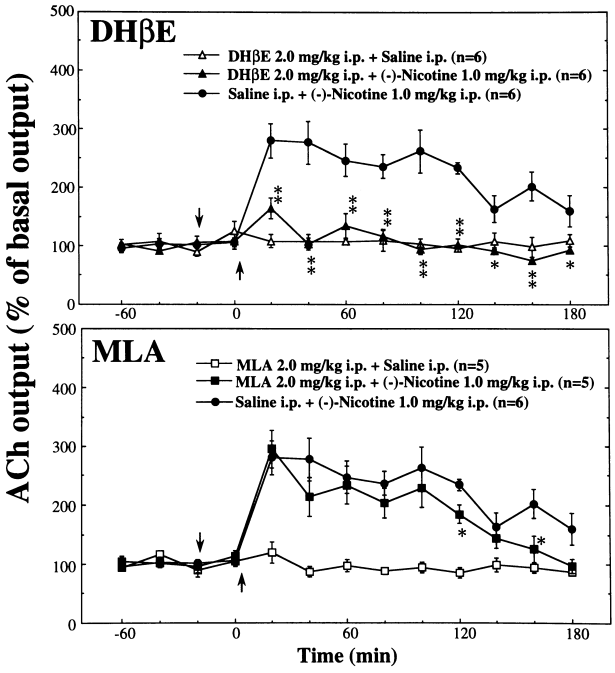

Fig. 5. Effects of dihydro-β-erythroidine (DHβE) and methyllycaconitine (MLA) on (−)-nicotine-induced increase of extracellular levels of acetylcholine in rat hippocampus. Data are expressed as percentages of the pre-injection basal level. Each symbol represents the mean ± S.E. for 5–6 rats. $*\ P < 0.05$, $**\ P < 0.01$; significant difference between saline + (−)-nicotine group and antagonist + (−)-nicotine group (Dunnett's multiple comparison test). Arrow indicates saline or drug injection, and each antagonist was administered 20 min before (−)-nicotine or saline injection. Dihydro-β-erythroidine (upper) and methyllycaconitine (lower) are competitive nicotinic receptor antagonists for $\alpha 4\beta 2$ and $\alpha 7$ subunits, respectively.

effects on acetylcholine release in the hippocampus (Fig. 2). In addition, (−)-cotinine (1.0 mg/kg i.p.), the major metabolite of (−)-nicotine, also had no significant effect on acetylcholine release (Fig. 2). GTS-21 (1.0 and 5.0 mg/kg i.p.), reported to be selective for the $\alpha 7$ subunit, did not show any significant effect on acetylcholine release in the hippocampus, whereas ABT-418 (1.0 and 5.0 mg/kg i.p.), a selective agonist for $\alpha 4\beta 2$ subunits, did have a significant, dose-dependent effect (Fig. 3).

3.3. Effects of nicotinic receptor channel blockers on the (−)-nicotine-induced increase of acetylcholine release in the hippocampus

The effects of mecamylamine and hexamethonium on the (−)-nicotine (1.0 mg/kg i.p.)-induced increase of acetylcholine release are shown in Fig. 4. The increase in acetylcholine release caused by (−)-nicotine administration was completely blocked by pretreatment with the centrally acting nicotinic receptor channel blocker, mecamylamine (1.0 mg/kg i.p.), but not by the peripherally acting one, hexamethonium (5.0 mg/kg i.p.).

3.4. Effects of competitive nicotinic receptor antagonists on the (−)-nicotine-induced increase of acetylcholine release in the hippocampus

As shown in Fig. 5, a competitive antagonist for $\alpha 4\beta 2$ subunits of nicotinic receptor, dihydro-β-erythroidine (2.0 mg/kg i.p.), did not itself affect acetylcholine release, but it significantly inhibited the effect of (−)-nicotine (1.0 mg/kg i.p.). Since dihydro-β-erythroidine at 0.5 mg/kg i.p. administered 20 min prior to the injection of (−)-nicotine was ineffective to antagonize the (−)-nicotine-induced increase of acetylcholine release (data not shown), the antagonizing effect of dihydro-β-erythroidine was dose-dependent. In contrast, the selective antagonist for the $\alpha 7$ nicotinic receptor subunit, methyllicaconitine (2.0 mg/kg i.p.), had little effect on the (−)-nicotine-induced increase of acetylcholine release.

3.5. Effects of (−)-cytisine and (−)-lobeline on the (−)-nicotine-induced increase of acetylcholine release in the hippocampus

(−)-Cytisine and (−)-lobeline have often been regarded as nicotinic receptor agonists, and it was recently reported that (−)-cytisine was a partial agonist for $\beta 2$

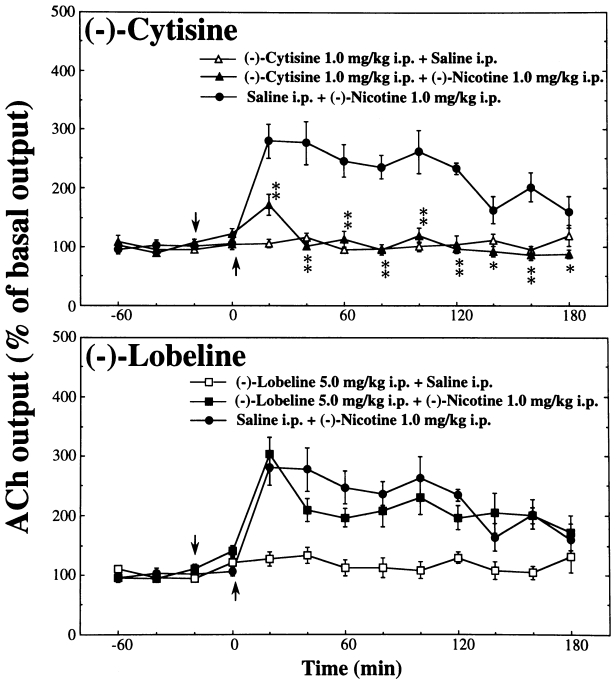

Fig. 6. Effects of (−)-cytisine and (−)-lobeline on (−)-nicotine-induced increase of extracellular levels of acetylcholine in rat hippocampus. Data are expressed as percentages of the pre-injection basal level. Each symbol represents the mean ± S.E. for 6 rats. $*\ P < 0.05$, $**\ P < 0.01$; significant difference between saline + (−)-nicotine group and antagonist + (−)-nicotine group (Dunnett's multiple comparison test). Arrow indicates saline or drug injection, and each antagonist was administered 20 min before (−)-nicotine or saline injection. (−)-Cytisine (upper) and (−)-lobeline (lower) are recently described partial agonists for $\beta 2$ and $\alpha 3$ subunit-containing nicotinic receptor, respectively.

subunit-containing nicotinic receptors, and its inhibitory efficacy for $\alpha4\beta2$ subunits was greater than that for $\alpha3\beta2$ subunits (Papke and Heinemann, 1994), whereas (−)-lobeline might be a partial agonist for $\alpha3$ subunit-containing nicotinic receptors (Decker et al., 1995). Therefore, the antagonistic properties of these natural alkaloids were tested. As shown in Fig. 6, the increase in acetylcholine release by (−)-nicotine (1.0 mg/kg i.p.) in the hippocampus was significantly blocked by pretreatment with (−)-cytisine (1.0 mg/kg i.p.), but not by (−)-lobeline (5.0 mg/kg i.p.).

4. Discussion

In the present study, (−)-nicotine caused an enhancement of acetylcholine release from the hippocampus and frontal cortex in a dose-dependent manner up to 1.0 mg/kg after its systemic administration. Thus, we confirmed the ability of (−)-nicotine to stimulate acetylcholine release in the hippocampus and frontal cortex under conscious and freely moving conditions. The effects of (−)-nicotine at the highest dose (5.0 mg/kg) tested had a shorter duration in both the hippocampus and frontal cortex. These results suggest that desensitization of the nicotinic receptors may occur at high doses of (−)-nicotine, as previous in vitro studies had indicated (Wilkie et al., 1993; Wilkie et al., 1996). On the other hand, (−)-nicotine (0.04–5.0 mg/kg i.p.) did not affect acetylcholine release from the striatum, which is consistent with results of in vitro receptor binding studies, demonstrating that nicotinic receptors (probably $\alpha4\beta2$ subunits) are found in the cortical and hippocampal areas of rat brain but not on cholinergic nerve endings of the striatum (Araujo et al., 1988a; Lapchak et al., 1989).

The effects of several nicotinic receptor ligands such as (−)-cytisine, (−)-lobeline, ABT-418, and GTS-21 on acetylcholine release from the hippocampus were examined at the doses of 1.0 and 5.0 mg/kg i.p. in the present study. Although (−)-cytisine and (−)-lobeline had high affinities for the rat brain nicotinic receptors in [^{3}H](−)-nicotine and [^{3}H](−)-cytisine binding assays (Anderson and Arneric, 1994; Decker et al., 1995; Tani et al., 1997), these alkaloids showed no significant effects on acetylcholine release. It was reported that (−)-cytisine and (−)-lobeline had, in part, (−)-nicotine-like pharmacological properties. For example, (−)-cytisine stimulated both noradrenaline and dopamine release from rat brain slices (Sacaan et al., 1995) and (−)-lobeline had the (−)-nicotine-like activity of exerting an anxiolytic effect on the elevated plus-maze test (Brioni et al., 1993). However, these alkaloids were less effective to stimulate rubidium efflux from mouse brain synaptosomes, which is thought to result from activation of the putative $\alpha4\beta2$ subunits of nicotinic receptors (Marks et al., 1993; Court et al., 1994). Thus, the (−)-nicotine-like pharmacological activity of these alkaloids is not attributable to agonist effects on $\alpha4\beta2$ subunits of nicotinic receptors.

While the etiology of Alzheimer's disease remains unknown, neuropathologic and biochemical analyses of Alzheimer's disease brains have revealed deficits in several neurotransmitter systems (Price, 1986). A defect in the cholinergic system, reduced acetylcholinesterase activity in the cerebral cortex and hippocampus, impairment of choline uptake and acetylcholine synthesis and release, mainly seem to be present in Alzheimer's disease (Bowen et al., 1976). Therefore, a dose-dependent enhancement of acetylcholine release in the hippocampus and cortex induced by (−)-nicotine and ABT-418 may contribute to improving the deficit of cognitive functions in Alzheimer's disease. Extensive pharmacological studies of ABT-418 and GTS-21 have suggested the possibility that they would be effective as treatments for Alzheimer's disease (see review, Brioni et al., 1996). Although GTS-21 improved a variety of learning and memory tasks with attenuated adverse effects in rodents (Meyer et al., 1994; Arendash et al., 1995), this compound was devoid of any effect on acetylcholine release in the present study. However, this nicotinic receptor ligand enhanced long-term potentiation in rat hippocampal slices in a mecamylamine-sensitive manner (Hunter et al., 1994) and prevented β-amyloid-induced cytotoxicity in cultured rat cortical neurons (Kihara et al., 1997). Therefore, cognitive-enhancing effects of GTS-21 may be involved in mechanisms other than acetylcholine release.

Three types of nicotinic receptor antagonists were used to characterize the nicotinic receptor-mediated acetylcholine release from rat hippocampus in this study. Mecamylamine and hexamethonium are non-competitive antagonists, as they block the ion channel associated with the nicotinic receptor, and are considered to be centrally and peripherally acting antagonists, respectively. The increase in acetylcholine release induced by (−)-nicotine was completely blocked by pretreatment with mecamylamine, but not with hexamethonium, suggesting that the (−)-nicotine-induced acetylcholine release was mediated by central nicotinic receptors. On the other hand, dihydro-β-erythroidine and methyllicaconitine were reported to be competitive nicotinic receptor antagonists. Dihydro-β-erythroidine showed more potent blocking activity on whole cell currents generated by activating $\alpha4\beta2$ subunits than on currents involving other subunits such as $\alpha3\beta4$ (Alkondon and Albuquerque, 1993). Studies with *Xenopus* oocytes expressing the various nicotinic receptor subunits also demonstrated that dihydro-β-erythroidine was more effective on the $\alpha4\beta2$ subtype than on $\alpha3\beta2$, $\alpha3\beta4$, or $\alpha2\beta2$ subunit combinations (Luetje and Patrick, 1989). Methyllicaconitine was reported to display high potency and selectivity for the $\alpha7$ subunit of the nicotinic receptor (Alkondon et al., 1992). While these competitive antagonists did not themselves affect acetylcholine release, (−)-nicotine-induced acetylcholine release in the rat hippocam-

pus was blocked by pretreatment with dihydro-β-erythroidine but not with methyllicaconitine in the present study. Furthermore, (−)-cytisine was an effective blocker of the (−)-nicotine-induced increase of acetylcholine release, whereas (−)-lobeline was not. These natural alkaloids were regarded as nicotinic receptor agonists, based on results of several pharmacological and behavioral studies. However, recent studies have revealed that (−)-cytisine and (−)-lobeline appear to be partial agonists for $\alpha4\beta2$ subunits (Papke and Heinemann, 1994) and the $\alpha3$ subunit of nicotinic receptors (Decker et al., 1995), respectively. These results together indicate that neither the $\alpha7$ nor $\alpha3$ subunit, but rather the $\alpha4\beta2$ subunits of nicotinic receptor, may be involved in (−)-nicotine-induced acetylcholine release in the rat hippocampus, although it is premature to say whether or not the stimulation of acetylcholine release by (−)-nicotine occurred via presynaptic nicotinic receptors.

In summary, the (−)-nicotine-induced increase of acetylcholine release appears to be brain-region specific, since systemically administered (−)-nicotine increased the release of acetylcholine in the hippocampus and frontal cortex but not in the striatum. Furthermore, our comparison of several types of nicotinic receptor agonists and antagonists that affect acetylcholine release in the rat hippocampus provide evidence that the (−)-nicotine-induced increase in acetylcholine release may be mediated by $\alpha4\beta2$ subunits of nicotinic receptors.

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