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Epibatidine Induces Long-Term Potentiation (LTP) via Activation of $\alpha4\beta2$ Nicotinic Acetylcholine Receptors (nAChRs) In Vivo in the Intact Mouse Dentate Gyrus: Both $\alpha7$ and $\alpha4\beta2$ nAChRs Essential to Nicotinic LTP

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Abstract. Activation of nicotinic acetylcholine receptors (nAChRs) induces nocotinic long-term potentiation (LTPn) in vivo in the mouse dentate gyrus. We have found that $\alpha4\beta2$ nAChRs activated by epibatidine induce LTPn, the full size of which requires the involvement of $\alpha4\beta2$ and $\alpha7$ nAChRs, in the intact mouse dentate gyrus using extracellular recording techniques. Intraperitoneal application of epibatidine, a potent $\alpha4\beta2$ nAChR agonist, at 0.3 – 3.0 μg/kg induced a long-lasting increase similar to LTPn induced by choline, a selective $\alpha7$ nAChR agonist, and at 10 μg/kg caused a transient increase followed by a depression. The LTPn induced by epibatidine at 3.0 μg/kg or choline at 30 mg/kg was significantly suppressed by pre-treatment but not post-treatment with mecamylamine (0.5 mg/kg, i.p.), a non-selective neuronal nicotinic antagonist. Post-application of nicotine at 3.0 mg/kg enhanced epibatidine-induced LTPn to the same level of nicotine-induced LTPn, but post-application of epibatidine had no effect on nicotine-induced LTPn. Epibatidine-induced LTPn was additionally increased by post-application of choline, and vice versa, reaching the same level of nicotine-induced LTPn. The present study revealed that epibatidine induced the LTPn via $\alpha4\beta2$ nAChRs and that both $\alpha7$ and $\alpha4\beta2$ nAChRs were essential for full-sized LTPn, suggesting that both nAChRs play an important role in synaptic plasticity.

Keywords: epibatidine, choline, nicotine, nicotinic acetylcholine receptor, long-term potentiation (in vivo)

Introduction

Nicotinic acetylcholine receptors (nAChRs) are known to be involved in various complex cognitive functions such as attention, learning, memory consolidation, arousal, and sensory perception (1). Most of these data came from behavioral studies performed with nicotine or nicotinic receptor antagonists in humans and animals (2). Long-term potentiation (LTP) is a long-lasting increase in the efficacy of synaptic transmission (3) and is assumed to underlie plastic changes associated with learning and memory (4, 5). Thus, it is proposed that nAChRs may play crucial roles in LTP. Indeed, nicotine facilitates LTP induction in hippocampal slice preparations (6). Moreover, we showed that intraperitoneal application of nicotine caused a long-lasting potentiation, which was named nicotinic LTP (LTPn), in vivo in the intact mouse dentate gyrus (7), suggesting that the intact preparation is especially relevant because all of the normal neural connections of the hippocampal formation are preserved.

The predominant functional nAChR subtypes in the mammalian brain are composed of $\alpha7$ or $\alpha4\beta2$ subunits (8, 9). Both of these nAChRs appear to play important

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roles in cognitive function, particularly with regard to hippocampal involvement in memory: Learning and memory performance in variety of experimental animal studies have been improved by the $\alpha7$ nAChR-selective agonist GTS21 (10) and the $\alpha4\beta2$ nAChR-selective agonist ABT418 (11) and impaired by the non-selective nicotinic antagonist mecamylamine (2), the $\alpha7$ nAChR-selective antagonist methyllycaconitine and the $\alpha4\beta2$ nAChR-selective antagonist dihydro-β-erythroidine (12, 13). It is proposed that $\alpha7$ nAChRs exhibit low affinity for ACh and nicotine, rapidly desensitize, and are involved in phasic synaptic responses, whereas $\alpha4\beta2$ nAChRs exhibit a high affinity for ACh and nicotine, desensitize slowly, and are involved in tonic synaptic responses (8, 14, 15). Many studies have shown that pre- and postsynaptic $\alpha7$ nAChRs in the hippocampus can play significant roles in cholinergic modulation of the release of glutamate or γ-aminobutyric acid (GABA) in the hippocampus by mediating the release of both excitatory and inhibitory signals through a Ca^{2+}-dependent mechanism (14 – 18). Similarly, it has been shown that pre- and postsynaptic $\alpha4\beta2$ nAChRs located on only GABAergic interneurons in the hippocampus are involved in a disinhibitory mechanism, finally leading to activating glutamatergic neurons (15, 19, 20). These neuronal circuitries involving $\alpha4\beta2$ and $\alpha7$ nAChRs have important implications for neuronal plasticity. Indeed, activation of $\alpha7$ nAChRs facilitates LTP induction in the rat hippocampus (21) and produces LTP induction in the rat midbrain (22). We also showed that intraperitoneal application of choline, a selective $\alpha7$ nAChR agonist, induced smaller LTPn in the mouse dentate gyrus (7). However, there is no evidence about the involvement of $\alpha4\beta2$ nAChRs, which are the most abundant subtype of nAChRs in the brain, in LTP.

The alkaloid epibatidine, isolated from the skin of the Ecuadoran tree frog *Epipedobates tricolor* (23), is a potent agonist of $\alpha4\beta2$ nAChRs (24, 25). Animals treated with low doses of epibatidine display mecamylamine-sensitive responses commonly observed for other nicotinic agonists including hypomotility, hypothermia, and antinociceptin (26 – 28). Also, analgesic properties of epibatidine are likely mediated by interactions with $\alpha4\beta2$ nAChRs (29). Epibatidine has a very high-affinity for neuronal $\alpha4\beta2$ nAChRs in the mouse brain (30). Therefore, epibatidine as an agonist of $\alpha4\beta2$ nAChRs has the unique capability of providing substantial clues regarding the nAChR subtype subserving a nicotinic response.

Here, we aim to elucidate the following: i) LTPn induction via activation of $\alpha4\beta2$ nAChRs and ii) involvement of $\alpha7$ and $\alpha4\beta2$ nAChRs in LTPn, in vivo in the intact mouse dentate gyrus.

Materials and Methods

Experiments were performed on mice in vivo prepared as described previously (31). Animal care and handling were done strictly in accordance with the "Guidelines for Animal Experimentation at Kobe University Graduate School of Medicine." Briefly, C57BL/6 mice were anesthetized by injecting urethane (1.2 g/kg, i.p. followed by supplemental injections of 0.2 – 0.6 g/kg as needed) and placed in a stereotaxic apparatus. Body temperature was maintained at 37°C using a heated mat (BRC, Nagoya).

A glass recording electrode with 9 – 12-μm tip diameter, back-filled with 0.9% NaCl, was lowered to the cell body layer of the dentate granule cells. Initial responses were obtained using a cathodal stimulation (6.0 – 8.0 V, 0.1 Hz, 0.1-ms duration) of the perforant path. After electrode insertion and population responses were obtained, the preparation was allowed to stabilize for 60 min prior to baseline recording. Voltage was reduced so that the baseline spike amplitude was one-third the maximum asymptotic value. We plotted only the population spike without EPSP slope because the potentiated change of population spikes is similar to that of EPSP slope during LTP in this procedure (31). The amplitude of the population spike was measured from initial positivity to peak negativity. At 5-min intervals, the population spikes induced by 5 successive stimulations were averaged and analyzed with a personal-computer (PowerLab System; BRC).

Nicotine, mecamylamine, epibatidine, or choline was dissolved in sterile 0.9% saline. Nicotine, mecamylamine, epibatidine, choline, or saline at a volume of 0.1 ml was injected intraperitoneally at the indicated dose and time.

Data are expressed as the mean ± S.E.M. of n values. Multiple group means were compared using the Bonferroni/Dunn test or the Scheffé test following one-factor or two-factor factorial analysis of variance (ANOVA). Differences with a P value of less than 0.05 were considered significant.

(−)-Nicotine di-*d*-tartrate and mecamylamine HCL were obtained from RBI (Natik, MA, USA). (+)-Epibatidine hydrochloride and choline chloride were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of the highest purity commercially available.

Results

Epibatidine induces LTPn in vivo in the intact mouse dentate gyrus

The effects of epibatidine, a potent $\alpha4\beta2$ nAChRs

agonist, were examined at doses of 0.3, 3.0, and 10 μg /kg in vivo in the intact mouse dentate gyrus. Figure 1A shows the representative trace at the indicated dose and time. The responses for 120 min after epibatidine application at doses of 0.3 and 3.0 μg/kg (n = 5 for each group) were potentiated and maintained. Percent of baseline spike amplitude for recording after epibatidine application was 126.9 ± 8.7 for 0.3 μg/kg (n = 5) and 151.6 ± 16.7 for 3.0 μg/kg (n = 5) . The response that was potentiated to 155% of baseline spike amplitude 5 min after epibatidine application (10 μg/kg, i.p.) decayed to the level of the control group in 30 min (n = 5, Fig. 1B). Figure 1C shows the dose-dependent change in the population spike at 120 min after epibatidine application, and the mice receiving epibatidine (3.0 μg /kg, i.p.) showed a significant difference from the control group (P<0.05, Bonferroni/Dunn test, n = 5 for each group). At a dose of 30 μg/kg, death occurred in three of five mice. These mice showed a short period of gasping following tachypnea before death.

Epibatidine-induced LTPn is inhibited by pre-treatment of mecamylamine

We used mecamylamine at a dose of 0.5 mg/kg as a non-selective nAChRs antagonist as shown in our previous report (7). Mecamylamine and epibatidine were applied to examine how the $\alpha 4\beta 2$ nAChRs are involved in LTPn in vivo in the intact mouse dentate gyrus. Figure 2A shows the representative trace at the indicated time. There was no significant difference in the responses of population spike between the pre- and post-treated period with mecamylamine (0.5 mg/kg, i.p., P>0.05, Scheffé test, n = 5; Fig. 2B). Treatment with mecamylamine (0.5 mg/kg, i.p.) 10 min before epibatidine (3.0 μg/kg, i.p.) gradually reduced LTPn to 119% of baseline spike amplitude for 2 h (Fig. 2B). There was a significant difference between the "mecamylamine + epibatidine" group, which means application of mecamylamine (0.5 mg/kg, i.p.) at 0 min and epibatidine (3.0 μg/kg, i.p.) at 10 min during the 15 – 120-min period, and the "epibatidine" group, which means application of epibatidine (3.0 μg/kg, i.p.) at

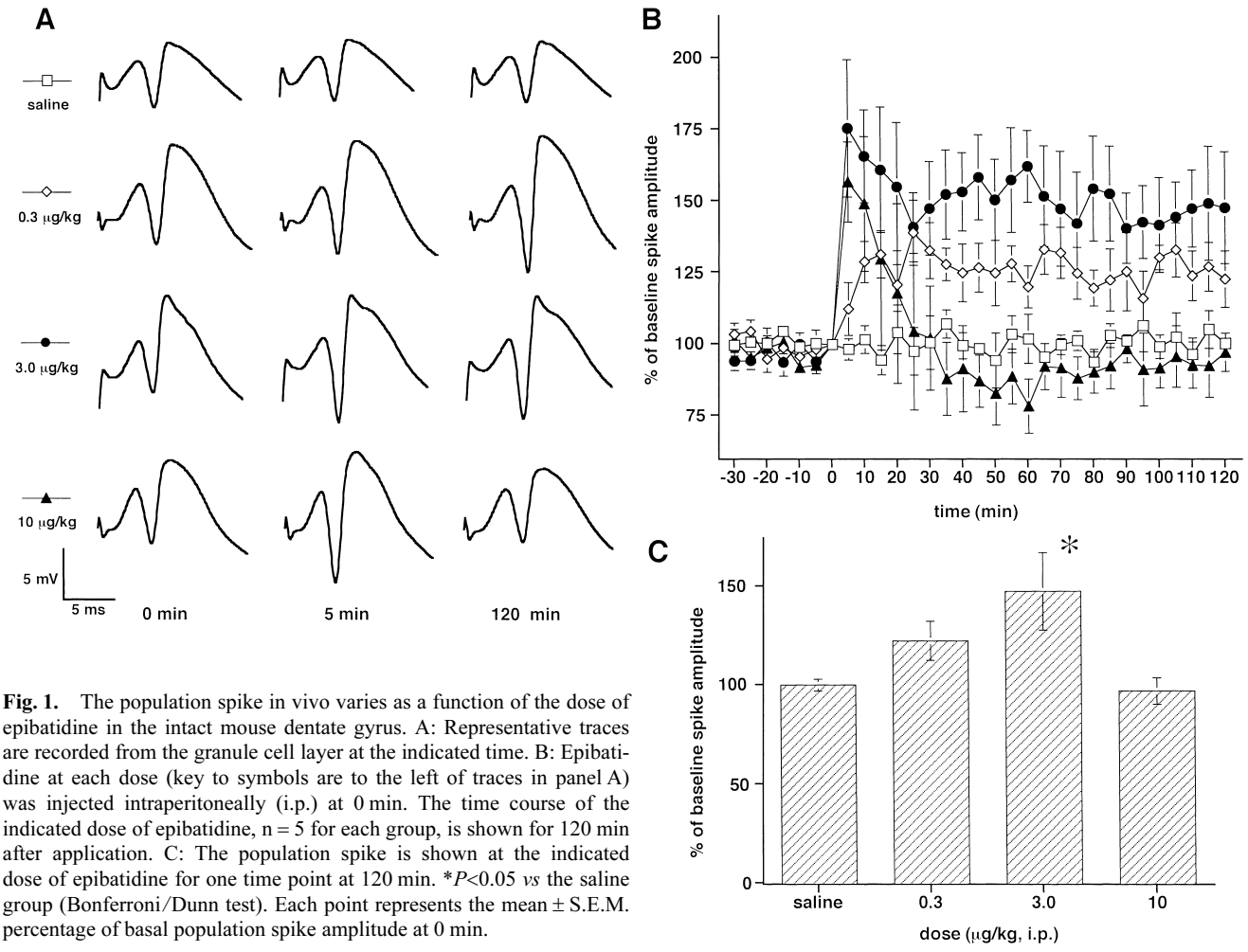

Fig. 1. The population spike in vivo varies as a function of the dose of epibatidine in the intact mouse dentate gyrus. A: Representative traces are recorded from the granule cell layer at the indicated time. B: Epibatidine at each dose (key to symbols are to the left of traces in panel A) was injected intraperitoneally (i.p.) at 0 min. The time course of the indicated dose of epibatidine, n = 5 for each group, is shown for 120 min after application. C: The population spike is shown at the indicated dose of epibatidine for one time point at 120 min. *P<0.05 *vs* the saline group (Bonferroni/Dunn test). Each point represents the mean ± S.E.M. percentage of basal population spike amplitude at 0 min.

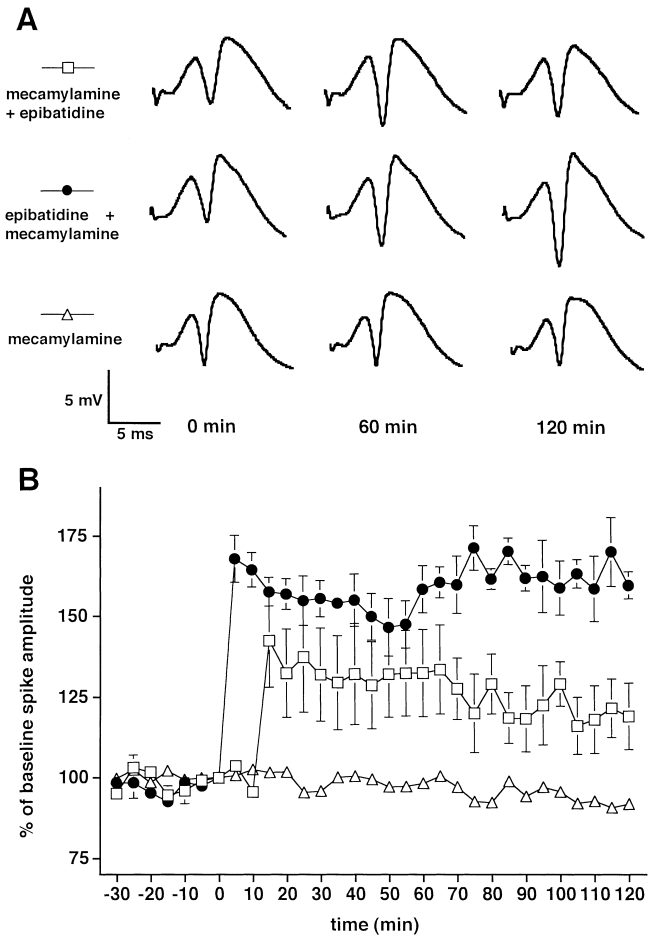

Fig. 2. $\alpha4\beta2$ nAChRs are involved in LTPn induction in vivo in the intact mouse dentate gyrus. A: Representative traces are recorded from the granule cell layer at the indicated time. Open triangles represent that mecamylamine (0.5 mg/kg) was injected intraperitoneally at 0 min ("mecamylamine group"). Open squares represent that mecamylamine (0.5 mg/kg) at 0 min and epibatidine (3.0 μg/kg) at 10 min were injected intraperitoneally ("mecamylamine + epibatidine group"). Closed circles represent that epibatidine (3.0 μg/kg) at 0 min and mecamylamine (0.5 mg/kg) at 60 min were injected intraperitoneally ("epibatidine + mecamylamine group"). B: The time course of mecamylamine, mecamylamine + epibatidine, and epibatidine + mecamylamine groups, n = 5 for each group, is shown for 120 min after mecamylamine or epibatidine application. Each point represents the mean ± S.E.M. percentage of basal population spike amplitude at 0 min.

0 min during the 5 – 120-min period (P<0.05, Scheffé test, n = 5 for each group; Table 1). The population spike of LTPn induced by epibatidine (3.0 μg/kg, i.p.) was not suppressed by post-application of mecamylamine (0.5 mg/kg, i.p.) at 60 min (Fig. 2B).

Nicotine produces further potentiation of epibatidine-induced LTPn

Epibatidine (3.0 μg/kg, i.p.) or nicotine (3.0 mg/kg, i.p.) was applied 60 min after nicotine or epibatidine application, respectively, to examine the in vivo involvement of $\alpha4\beta2$ nAChRs and other subtype in LTPn, in the intact mouse dentate gyrus. Figure 3A shows the representative trace at the indicated time. Application of nicotine at time 60 min caused further potentiation to 208% of the baseline spike amplitude that had already been potentiated to about 150% by epibatidine, whereas post-application of epibatidine had no effect on nicotine-induced LTPn (Fig. 3B).

Choline-induced LTPn is inhibited by pre-treatment of mecamylamine

Choline, an $\alpha7$ nAChR-selective agonist, induced a long-lasting potentiation similar to LTPn in a dose-dependent manner, and the percent of baseline spike amplitude for recording after choline application was 126.3 ± 14.6 for 3.0 mg/kg (n = 5), 160.7 ± 11.3 for 30 mg/kg (n = 5), and 163.4 ± 15.7 for 90 mg/kg (n = 5), as shown in our previous report (7). Mecamylamine and choline were injected intraperitoneally to examine how the $\alpha7$ nAChRs are involved in LTPn in vivo in the intact mouse dentate gyrus. Figure 4A shows the representative trace at the indicated time. Treatment with mecamylamine (0.5 mg/kg, i.p.) 10 min before choline (30 mg/kg, i.p.) reduced LTPn to 143% of baseline spike amplitude for 2 h (Fig. 4B). There was a significant difference between the "mecamylamine + choline" group, which means application of mecamylamine (0.5 mg/kg, i.p.) at 0 min and choline (30 mg/kg, i.p.) at 10 min during the 15 – 120 min period, and the "choline" group, which means application of choline

Table 1. Effects of pre-treated mecamylamine on LTPn induced by epibatidine or choline

Compounds	Period (min)	% of baseline spike amplitude
Epibatidine	5 – 120	151.6 ± 16.7
Mecamylamine + Epibatidine	15 – 120	127.9 ± 12.0*
Choline	5 – 120	160.7 ± 11.3
Mecamylamine + Choline	15 – 120	143.8 ± 4.6*

Mecamylamine (0.5 mg/kg) was administered 10 min before epibatidine (3.0 μg/kg) or choline (30 mg/kg) was injected intraperitoneally. *P<0.05 *vs* the epibatidine or choline group in the absence of mecamylamine in the different mice (Scheffé test). Means ± S.E.M. for five mice per group are shown.

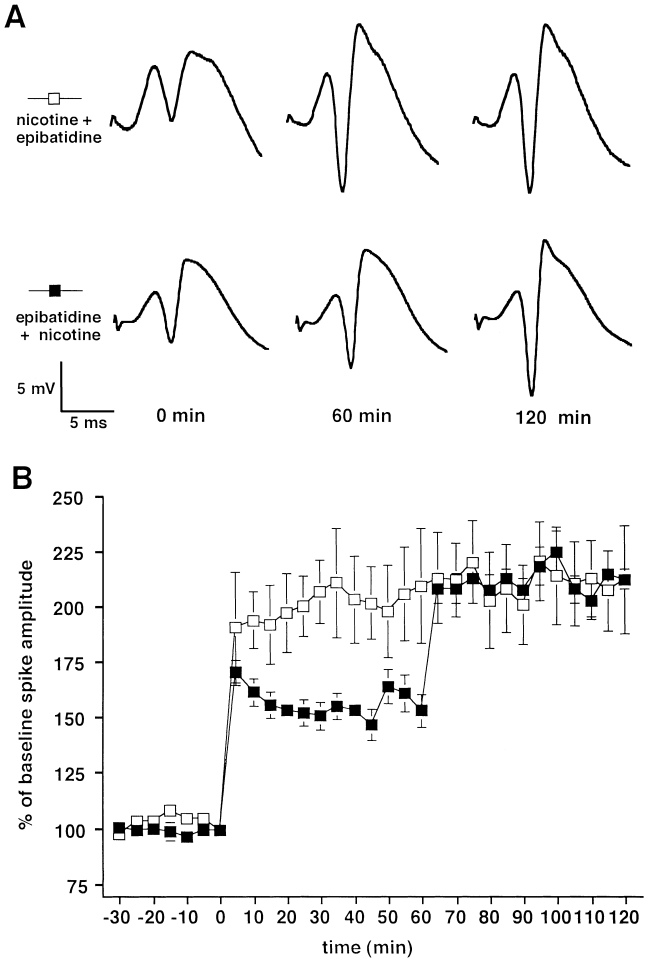

Fig. 3. Activation of $\alpha 4\beta 2$ nAChRs is not sufficient for full-sized LTPn in vivo in the intact mouse dentate gyrus. A: Representative traces are recorded from the granule cell layer at the indicated time. Open squares represent that nicotine (3.0 mg/kg) at 0 min and epibatidine (3.0 μg/kg) at 60 min were injected intraperitoneally ("nicotine + epibatidine group"). Closed squares represent that epibatidine (3.0 μg/kg) at 0 min and nicotine (3.0 mg/kg) at 60 min were injected intraperitoneally ("epibatidine + nicotine group"). B: The time course of nicotine and epibatidine + nicotine groups, n = 5 for each group, is shown for 120 min after nicotine or epibatidine application. Each point represents the mean ± S.E.M. percentage of basal population spike amplitude at 0 min.

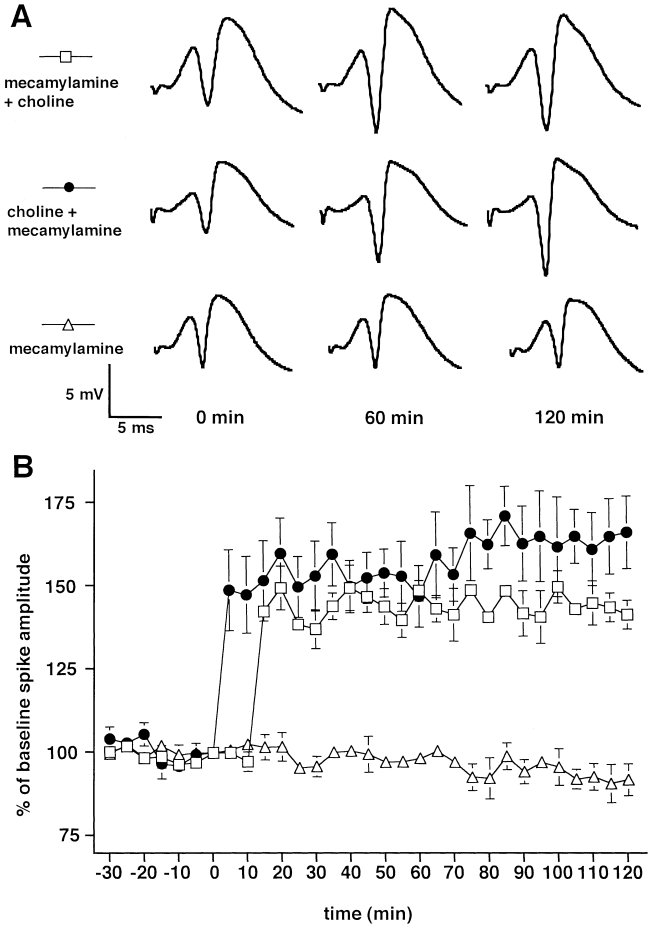

Fig. 4. $\alpha 7$ nAChRs are involved in LTPn induction in vivo in the intact mouse dentate gyrus. A: Representative traces are recorded from the granule cell layer at the indicated time. Open triangles represent that mecamylamine (0.5 mg/kg) was injected intraperitoneally at 0 min ("mecamylamine group"). Open squares represent that mecamylamine (0.5 mg/kg) at 0 min and choline (30 mg /kg) at 10 min were injected intraperitoneally ("mecamylamine + choline group"). Closed circles represent that choline (30 mg/kg) at 0 min and mecamylamine (0.5 mg/kg) at 60 min were injected intraperitoneally ("choline + mecamylamine group"). B: The time course of mecamylamine, mecamylamine + choline, and choline + mecamylamine groups, n = 5 for each group, is shown for 120 min after mecamylamine or choline application. Each point represents the mean ± S.E.M. percentage of basal population spike amplitude at 0 min.

(30 mg/kg, i.p.) at 0 min during the 5 – 120 min period (P<0.05, Scheffé test, n = 5 for each group; Table 1). The population spike of LTPn induced by choline (30 mg/kg, i.p.) were not suppressed by post-application of mecamylamine (0.5 mg/kg, i.p.) at 60 min (Fig. 4B).

Activation of both $\alpha 4\beta 2$ and $\alpha 7$ nAChRs produces the full-sized LTPn

Choline (30 mg/kg, i.p.) or epibatidine (3.0 μg/kg, i.p.) was applied 60 min after epibatidine or choline application, respectively, to examine the involvement of both $\alpha 4\beta 2$ and $\alpha 7$ nAChRs in LTPn in vivo in the intact mouse dentate gyrus. Figure 5A shows the representative trace at the indicated time. Application of choline or epibatidine at 60 min caused further potentiation to 207% or 213% of the baseline spike amplitude that had already been potentiated to about 150% by epibatidine or choline, respectively (Fig. 5B).

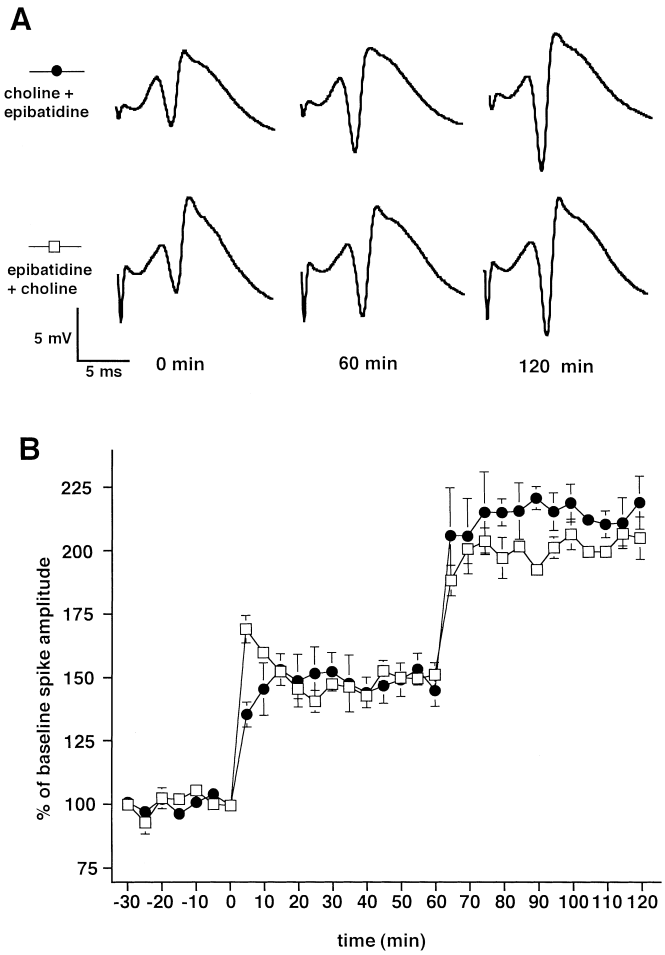

Fig. 5. Both $\alpha4\beta2$ and $\alpha7$ nAChRs are essential to full-sized LTPn in vivo in the intact mouse dentate gyrus. A: Representative traces are recorded from the granule cell layer at the indicated time. Open squares represent that epibatidine (3.0 μg/kg) at 0 min and choline (30 mg/kg) at 60 min were injected intraperitoneally ("epibatidine + choline group"). Closed circles represent that choline (30 mg/kg) at 0 min and epibatidine (3.0 μg/kg) at 60 min were injected intraperitoneally ("choline + epibatidine group"). B: The time course of epibatidine + choline and choline + epibatidine groups, n = 5 for each group, is shown for 120 min after epibatidine or choline application. Each point represents the mean ± S.E.M. percentage of basal population spike amplitude at 0 min.

Discussion

The present study demonstrates that the systemic application of epibatidine is sufficient to cause a long-lasting potentiation similar to LTPn and that both $\alpha7$ and $\alpha4\beta2$ nAChRs are essential for the establishment of full-sized LTPn in vivo in the intact mouse dentate gyrus. Ligand-binding studies indicate that two major types of functional nAChRs in the brain are formed by $\alpha4\beta2$ and $\alpha7$ subunits (32). Nicotine has different effects on the two major nAChR subtypes in the hippocampal neurons, which are composed of $\alpha7$ or $\alpha4\beta2$ subunits (8, 9). There are $\alpha7$ nAChRs on glutamatergic neurons and not only $\alpha7$ but also $\alpha4\beta2$ nAChRs on GABAergic neurons in the hippocampus (33). Activation of $\alpha7$ nAChRs on hippocampal glutamatergic neurons has been known to enhance the release of glutamate (18). Desensitization of $\alpha7$ nAChRs on GABAergic inhibitory interneurons would cause disinhibition of glutamatergic neurons (19). Because of their capacity to regulate neurotransmission in the hippocampus, $\alpha7$ nAChRs may play a role in LTP. Such a role has been shown by several studies (7, 21). Although the presence and properties of $\alpha4\beta2$ nAChRs on hippocampal glutamatergic neurons remain to be clarified, disinhibition of the glutamatergic neurons is considered to be accomplished by activation of $\alpha4\beta2$ nAChRs in intermediary GABAergic interneurons that synapse onto other GABAergic interneurons that in turn synapse onto glutamatergic neurons (19). Thus, it is proposed that activation of $\alpha4\beta2$ nAChRs may induce LTP via this disinhibitory mechanism.

Although none of compounds are selective for $\alpha4\beta2$ nAChRs, epibatidine has been shown to serve as a pharmacological tool to probe nAChR function further, especially as a novel nicotinic agonist for the study of $\alpha4\beta2$ nAChRs and to readily penetrate the blood-brain barrier (24, 25, 27, 28). In addition, the effects of epibatidine were short lived (27) and systemic administration of epibatidine at low doses (0.5 – 2 μg/kg) produced a transient increase of cardiorespiratory function, followed by a decrease and return to baseline (34). Taken together, it is suggested that the peripheral function affected by systemic application of epibatidine have no relation to the long-lasting change within the brain. Thus, we used epibatidine to study the involvement of $\alpha4\beta2$ nAChRs in LTP using our in vivo procedure. Our findings showed the long-lasting potentiating effects of epibatidine at 0.3 to 3.0 μg/kg in a dose-dependent manner, but a depression after transient potentiation at a higher dose of 10 μg/kg (Fig. 1), which may result from the desensitization of $\alpha4\beta2$ nAChRs by epibatidine exposure (19, 35). Our findings that the dose to reach the maximum % of baseline spike amplitude was 3.0 μg/kg for epibatidine and 3.0 mg/kg for nicotine are consistent with the observation that epibatidine was shown to be 300 to 1000 times more potent than nicotine in reduction of body temperature and locomotor activity in vivo in mice (28). Epibatidine is characterized by a higher efficacy at $\alpha4\beta2$ nAChRs than at $\alpha7$ nAChRs and by a much lower affinity for $\alpha7$ nAChRs than for $\alpha4\beta2$ nAChRs subunits (25, 36). Taken together with the observation that the $\alpha4\beta2$ subtype is the most abundant neuronal nicotinic receptor subtype in the brain (32), it appears that epibatidine is more selective

for the $\alpha4\beta2$ nAChR in the brain. Also, the effective dose range of epibatidine in this study is in accordance with that in the in vivo animal studies to investigate behavioral action via $\alpha4\beta2$ nAChRs (26 – 28). Thus, it is strongly suggested that epibatidine induces LTPn in vivo in the mouse dentate gyrus via activation of $\alpha4\beta2$ nAChRs. In combination with the GABAergic disinhibitory mechanism, $\alpha4\beta2$ nAChRs on GABAergic neurons might play an important role in epibatidine-induced LTPn.

The mean response was about 150% of the baseline spike amplitude for LTPn induced by epibatidine at 3.0 μg/kg (Fig. 1), about 160% for choline at 30 mg/kg, and about 213% for nicotine at 3.0 mg/kg (7). Also, application of nicotine in addition to choline induced further increase in the choline-induced LTPn to the same level as maximum response produced by only nicotine (7). Interestingly, this further increase was also shown by application of nicotine in addition to epibatidine and nicotine-induced LTPn occluded the potentiatiation via $\alpha4\beta2$ nAChRs by epibatidine (Fig. 3). Taken together, it is proposed that nicotine-induced LTPn are involved in activation of both $\alpha7$ and $\alpha4\beta2$ nAChRs. We showed that post-application of choline or epibatidine enhanced the smaller-sized LTPn induced by epibatidine or choline to the full-sized LTPn induced by nicotine, respectively (Fig. 5). Thus, it is suggested that both $\alpha7$ and $\alpha4\beta2$ nAChRs are required to establish the full-sized LTPn.

Mecamylamine is well-recognized to be a non-selective nAChRs antagonist, but the drug should be used with caution, since higher doses of the drug block NMDA-receptor ion channels (37) or inhibit ACh synthesis in rat brain (38). We, therefore, used mecamylamine at a dose of 0.5 mg/kg, the corresponding dose being effective in the in vivo systems (39, 40). Our previous study showed that pre-treatment of mecamylamine inhibited significantly but not completely LTPn induction (7). Similarly, LTPn induced by epibatidine or choline was significantly but not completely depressed by pre-treatment with mecamylamine, although mecamylamine caused no depression of the population spike after the establishment of LTPn induced by epibatidine or choline (Figs. 2 and 4). Thus, it is suggested that the induction but not expression or maintenance of LTPn is mediated by the activation of $\alpha4\beta2$ or $\alpha7$ nAChRs. Interestingly, the depression by pre-treatment with mecamylamine was greater in epibatidine-induced LTPn than choline-induced LTPn (Figs. 2 and 4 and Table 1), supported by the observation that mecamylamine is likely to act on $\alpha4\beta2$ nAChRs rather than $\alpha7$ nAChRs as a nAChR channel blocker (17, 24). Thus, it is suggested that epibatidine has a predominant agonistic effect on $\alpha4\beta2$ nAChRs in vivo in the mouse dentate gyrus.

To elucidate the precise properties of $\alpha7$ and $\alpha4\beta2$ nAChRs in synaptic plasticity, there are the following problems: 1) Several compounds are active on these receptors, but none of them are selective for a particular subtype. 2) Current knowledge of the gross regional localization of nAChR subtypes, which is mainly based on in situ hybridization studies, is not sufficient to define the neuronal circuits in which the nAChR subtypes are involved, and a more precise immunolocalization of the different subunits is needed. However, the facts described here provides evidence that $\alpha4\beta2$ nAChRs, similar to $\alpha7$ nAChRs, play an important role in synaptic plasticity.

Acknowledgments

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