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Regular Article

Septal innervation regulates the function of α7 nicotinic receptors in CA1 hippocampal interneurons

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Abstract

The hippocampus receives substantial input from the medial septum/diagonal band of broca (MS/DB) via the fibria-fornix (FF). Projections from the MS/DB innervate hippocampal interneurons that express α7 nicotinic receptors and regulate excitation in principal cell populations. In the present report we used stereotaxic surgery, whole-cell patch clamping, and immunohistochemical techniques to evaluate the effects of FF and MS/DB lesions on α7 nicotinic receptors in stratum radiatum interneurons. Focal somatic application of ACh (1 mM) evoked methyllycaconitine (MLA)-sensitive currents that were markedly reduced following aspirative lesions of the FF. Reductions in current amplitudes were prevented or restored to levels not significantly different from controls following in vivo treatment with the α7-selective agonist GTS-21, and GTS-21 treatment did not change current amplitudes measured in tissue from unlesioned animals. MS/DB injections of the selective cholinergic neurotoxin 192 IgG-saporin did not affect α7 receptor currents, although MS/DB ChAT and hippocampal AChE immunolabeling were significantly reduced. In contrast, kainic acid lesions of the MS/DB, potentially more selective for GABAergic projection neurons, produced significant reductions in current amplitudes. These findings are the first to show functional changes in α7 receptors following hippocampal denervation and suggest that MS/DB hippocampal innervation regulates functional aspects of hippocampal α7 receptors. The results confirm hippocampal α7 nicotinic receptors as viable therapeutic targets in diseases that involve degradation of the septohippocampal pathway and may indicate that GABAergic MS/DB hippocampal input plays a more substantial role in the regulation of α7 nicotinic receptor function than MS/DB hippocampal cholinergic input.

Keywords: Alpha-7; Fimbria-fornix; Transection; Septohippocampal; Septum; Saporin; Kainic acid; GTS-21

Introduction

Nicotinic agonists can improve memory in aged animals and humans (Arendash et al., 1995; Levin and Torry, 1996; Prendergast et al., 1997; White and Levin, 2004) and enhance short-term memory in normal subjects (Socci et al., 1995; Prendergast et al., 1997). Twelve different genes have been cloned that code for neuronal nAChR subunits and may underlie the cognitive effects of nicotinic drugs. The α7 subtype has been strongly and consistently implicated in learning and memory as it is expressed at high levels in the hippocampus (Whiteaker et al., 1999), is highly permeable to calcium (Seguela et al., 1993), and may play a role in synaptic plasticity (Matsuyama et al., 2000; Ji et al., 2001). Selective agonists for this receptor subtype improve performance in a variety of memory-related behaviors across species, including rats, rabbits, and primates (Woodruff-Pak et al., 1994; Briggs et al., 1997; Van Kampen et al., 2004).

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In the hippocampus, functional hippocampal α7 receptors are most easily identified on the cell bodies of interneurons in stratum radiatum (Jones and Yakel, 1997; Alkondon et al., 1998; Frazier et al., 1998b) as well as on mossy cells and interneurons in the dentate gyrus (Jones and Yakel, 1997; Frazier et al., 2003). Important input to these neurons comes from the septohippocampal (SH) pathway. Both cholinergic and GABAergic cells located in the medial septum and diagonal band of Broca (MS/DB) send axons of through the fimbria-fornix (FF) to the hippocampus. Chemical lesions of both cholinergic and GABAergic MS/DB cells (Pang et al., 2001) and electrolytic lesions of the SH pathway (Levin et al., 1993; Robbins et al., 1997) produce learning and memory deficits, and it has been shown that FF lesions reduce hippocampal acetylcholine esterase (AChE)-positive nerve terminals (Larkfors et al., 1987; Duconseille et al., 1999; Lee et al., 2003) and hippocampal innervation from choline acetyltransferase (ChAT)-positive fibers (Blaker et al., 1988).

In the present study we tested the hypotheses that hippocampal innervation by MS/DB projections was necessary for normal α7 receptor function and that functional changes occurring as a result of denervation could be prevented or reversed with an α7-selective agonist. We show here that lesions of the FF produce marked reductions in the peak amplitude and net charge of ACh-evoked hippocampal α7 receptor currents without changing overall receptor density and that reductions in current magnitudes can be prevented or restored with in vivo treatments using GTS-21. Finally, we show that chemical lesions of MS/DB with kainic acid (KA) produce significant reductions in α7 receptor currents while selective cholinergic lesions of the MS/DB do not.

Materials and methods

Chemicals

GTS-21 (2,4dimethoxybenzylidene3-anabaseine, also DMXB) was supplied by Taiho Pharmaceuticals (Tokyo Japan). All other chemicals were purchased from Sigma (St. Louis MO).

General surgical procedures

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee and were in accord with the NIH *Guide for the Care and Use of Laboratory Animals*. Aspirative lesions and basal forebrain injections were produced using standard stereotaxic procedures under the general anesthetic isoflurane (2–4%) in combination with the analgesic Banamine (1–2 mg/kg).

Aspirative lesions of the fimbria-fornix

Male Sprague–Dawley rats (18–22 days old) were placed in an isoflurane-containing chamber for 5 min prior to mounting on a stereotaxic frame. Lack of hindlimb withdrawal reflex indicated readiness for making the initial incision, which followed the midline suture from just rostral to bregma, continuing back to lambda. Two small holes (0.5-mm diameter) were drilled immediately adjacent to each other inside of the right angle formed by the midline and bregma sutures (0.5 mm lateral to midline and 0.5 mm caudal to bregma). Using a dissecting microscope and fiber-optic lighting, a glass Pasteur pipette (0.5-mm tip) attached to a slight vacuum was lowered in 0.25 mm steps, which enabled slow removal of tissue continuing ventrally approximately 4.5–5 mm. The aspirative hole was then filled with small particles of Gelfoam (Pharmacia and Upjohn, Kalamazoo, MI) to control any bleeding and help maintain the structural integrity of the brain around the lesion site. The incisions were closed using surgical nylon and treated with Betadine in order to reduce the possibility of topical infection. Animals recovered from anesthesia under a heat lamp and were monitored for signs of pain. Additional analgesic injections were administered as necessary.

Septal injections

The general surgical procedures for medial septal injections were the same as those used for aspirative lesions (see above). Following the initial incision, a small hole (0.5-mm diameter) was drilled 1 mm rostral to bregma and 0.25–0.5 mm lateral to the midline in order to avoid rupture of the blood vessels underneath the midline suture. A 27-gauge needle attached to a 10-μl Hamilton syringe was lowered into the medial septum and stopped at a depth 6.5–7 mm ventral to the skull surface. 192 IgG-saporin (Advanced Targeting Systems, San Diego, CA) (1 μl of 1 μg/μl in 0.1 M phosphate-buffered saline (PBS, pH 7.4)) or kainic acid (Sigma, St. Louis, MO) (0.5 μl of 0.25–0.5 μg/μl in PBS) was delivered at 0.2 μl/min using a CMA/100 microinjection pump (CMA/Microdialysis, Solna, Sweden). Following the injection, the needle was left in place for 10 min in order to prevent the solution from rising upward through the injection tract.

Electrophysiology

Animals were anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ) and swiftly decapitated 7–10 days following either injection or lesion. Transverse (300 μm) whole brain slices were prepared using a vibratome (Pelco, Redding, CA) and a high Mg^{+}/low Ca^{2+} ice-cold ACSF containing (in mM) 124 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 2.5 $MgSO_4$, 10 D-glucose, 1 $CaCl_2$, and 25.9 $NaHCO_3$ saturated with 95% O_2–5%CO_2. Prior

to sectioning a single edge razor was used to make a longitudinal cut that separated the two hemispheres and allowed for keeping the lesioned and unlesioned sides separate. Slices were incubated at 30°C for 30 min and then left at room temperature until they were transferred to a submersion chamber (Warner Instruments, Hamden, CT) for recording. During experiments slices were perfused (2 ml/min) with normal ACSF containing (in mM) 126 NaCl, 3 KCl, 1.2 NaH_2PO_4, 1.5 $MgSO_4$, 11 D-glucose, 2.4 $CaCl_2$, 25.9 $NaHCO_3$, and 0.005–0.008 atropine sulfate saturated with 95% O_2–5% CO_2 at 30°C. Hippocampal stratum radiatum interneurons and CA1 pyramidal cells were visualized with infrared differential interference contrast microscopy (IR DIC) using a Nikon E600FN microscope. Whole-cell patch-clamp recordings were made with glass pipettes (3–5 MΩ) containing an internal solution of (in mM) 125 K-gluconate, 1 KCl, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 MgATP, 0.3 Na_3GTP, and 10 HEPES. Cells were held at −70 mV, and a −10 mV/50 ms test pulse was used to determine series and input resistances. Cells with series resistances >60 MΩ or those requiring holding currents >200 pA were not included in the final analyses. Local somatic application of acetylcholine (1 mM mixed in oxygenated ACSF) was done using glass pipettes identical to those used for patch clamping. Pipettes were attached to a picospritzer (General Valve, Fairfield, NJ) with Teflon tubing, and a series of applications was administered using 10–20 psi for 5–30 ms. In order to control between-group variability, and because this technique is not ideal for creating dose–response curves, for each cell we systematically ascertained the maximum response to 1 mM ACh by increasing the application times starting with 5 ms and augmenting by 2.5–5 ms until no further increase in peak was observed. Typical maximum effect stimuli were in the range of 7.5–20 ms delivered 20–30 μm from the cell body. Once the optimal stimulation parameters were established and responses remained stable, we delivered a series of four consecutive applications and averaged the resulting traces to yield a single value representing the average peak for an individual cell. Drug applications were separated by 30 s, which eliminated reductions in peak responses produced by receptor desensitization. In experiments using methyllycaconitine (MLA), baseline responses were recorded every 30 s for 3–5 min. MLA (50 nM) was then introduced into the ACSF using a syringe pump (Kd Scientific, Holliston, MA) loaded with a 2.5 mM solution that was diluted to the final concentration in the perfusion line prior to entering the recording chamber.

Signals were digitized using an Axon digidata1322A and sampled at 20 kHz (filtered at 1 kHz) on a Dell computer using Clampex version 8 or 9. Data analysis was done with Clampfit version 8 or 9, Excel 2000, and GraphPad/Prism version 3.02. Statistical analyses used two-sample one- and two-tailed Student's *t* tests as appropriate, assuming equal variances.

Histology

Lesions were confirmed with forebrain regions acquired during the dissection procedures for electrophysiological experiments. The forebrains were submersion fixed 48 h in PBS with 10% formaldehyde to which 30% sucrose was added for cryoprotection. The tissue was sectioned on a sliding microtome with a freezing stage at 50 μm. Sections were collected and stored briefly in PBS prior to mounting on glass slides. Each slide was dehydrated with 95% and 100% ethanol and then cleared with xylenes prior to cover slipping. Evaluations of lesions and injection sites were done using low power (1×–4×) objectives with standard bright-field microscopy. Data obtained from animals in which the forebrains did not clearly contain unilateral lesions of the fimbria/fornix and/or a visible needle tract in the medial septum were not included in the analyses.

Immunohistochemistry (AChE and ChAT staining)

Additional animals lesioned as those used for electrophysiological experiments were overdosed with pentobarbital (100 mg/kg) 1 week following MS/DB 192 IgG-saporin injections. They were perfused transcardially with a PBS wash followed by a 10% formaldehyde/PBS solution and postfixed in situ for 2 h at 4°C. The brains were removed and transferred to a 30% sucrose/10% formaldehyde PBS solution for 48 h prior to sectioning. Transverse 50-μm sections were cut using a sliding microtome with a freezing stage and transferred to polystyrene 24-well plates (NUNC 1147) containing 500 μl PBS in each well.

For ChAT staining free-floating tissue sections were incubated sequentially in (1) 0.5% H_2O_2/PBS 10 min to quench endogenous peroxidative activity; (2) a blocking solution (3% goat serum, 0.3% Triton X-100, PBS) for 1 h at room temperature; (3) polyclonal anti-ChAT (Chemicon AB5042, 1:2500 in PBS) for 24 h; (4) biotinylated goat anti-mouse IgG (DAKO, 1:500 in PBS) for 24 h; (5) extravidin peroxidase (Sigma, 1:1000 in PBS); and (6) nickel-enhanced diaminobenzidine (DAB) (0.67 mg diaminobenzidine (Sigma), 0.13 μl 30% H_2O_2 per ml 80 mM sodium acetate buffer containing 8 mM imidazole and 2% $NiSO_4$) for 2–3 min. Sections were washed twice between incubations with PBS and ultimately dehydrated, mounted, and coverslipped.

For AChE staining, sections were reacted sequentially with (1) 200 μl/well of preincubation solution (5 mM sodium citrate, 1.9 mM cupric sulfate, 0.5 mM potassium ferricyanide, 0.078 M sodium acetate) for 10 min; (2) 200 μl/well of freshly prepared incubation buffer (5 mM sodium citrate, 1.9 mM cupric sulfate, 0.5 mM potassium ferricyanide, 0.063 M sodium acetate, 4.84 mM acetylthiocholine iodide, 0.4 mM ethopropazine), added to sections in preincubation solution, then microwaved over ice 200 W for 2 min; (3) 2× with 500 μl/well of 0.05 M Tris buffer (pH 7.6); (4) 500 μl/well of 0.1 M sodium acetate buffer; and (5)

350 μl/well of DAB solution (0.5 mg/ml diaminobenzidine, 2% $NiSO_4$, 0.045% H_2O_2, 0.0005% Triton X-100) for color development. Sections were rinsed again with acetate buffer, dehydrated, mounted, and coverslipped.

GTS-21 treatment

The non-surgical group (Fig. 3) began treatment on postnatal day 19 and the lesion group (Figs. 2C and D) began treatment approximately 12 h after surgery on postnatal day 19. Both groups received 1 mg/kg i.p. injections twice daily separated by approximately 8 h, for 7–10 days until being sacrificed for electrophysiological experiments. Control animals received the same injection regimen with identical volumes of 0.08% saline. To avoid experimenter bias, the animals were coded to disguise drug treatments throughout the data collection process.

Confirmation of lesions and experimental design

Unilateral aspirative lesions of the FF were confirmed using histological processing of forebrain tissue not used in electrophysiological experiments. We visualized each lesion and evaluated the extent of damage using standard bright-field microscopy. Lesions that did not completely eliminate the intended side or spare the majority of the contralateral FF were not used in the final analyses. Although removal of cortical regions located dorsal to the FF was necessary to lesion the FF, in animals with lesions that extended through the cortical region sparing the FF (data not shown) there were no significant effects. For lesion studies, each animal provided both control (contralateral to the lesion site) and experimental (ipsilateral to the lesion site) hippocampal tissue.

High affinity[^{3}H]MLA binding

Hippocampi were rapidly dissected and assayed for nicotine-displaceable, high-affinity [^{3}H]methyllycaconitine (MLA) binding using a modification of the procedure of Davies et al. (1999). The MLA concentration used in this study was 2.3 nM, a concentration that is selective for α7 receptors. Tissues were homogenized in 20 volumes of ice-cold Krebs Ringer HEPES buffer (KRH; in mM, 118 NaCl, 5 KCl, 10 glucose, 1 $MgCl_2$, 2.5 $CaCl_2$, and 20 HEPES; pH 7.5) with a Polytron (setting 4 for 15 s). After two 1-ml washes with KRH at 20,000×g, the membranes (90 μg protein) were incubated in 0.5 ml KRH with 2.3 nM [^{3}H]MLA (Tocris, Ellisville, MO) for 60 min at 4°C, ± 5 mM nicotine. Tissues were washed three times with 5-ml cold KRH by filtration through Whatman GF/C filters that had been preincubated for 30 min with 0.5% polyethylenimine. They were assayed for radioactivity using liquid scintillation counting. Nicotine-displaceable binding was calculated in triplicate in each experiment.

Results

Whole-cell recording parameters

For each cell we measured the current needed to hold at −70 mV (H_c), the whole-cell capacitance (C_m), the input resistance (R_m), and the access resistance (R_a). Data were pooled from all 239 cells to calculate the following means: H_c = −79.6 ± 10.7 pA; C_m = 76.9 ± 5.6 pF; R_m = 212.9 ± 18.8 MΩ; R_a = 28.1 ± 2.4 MΩ). Averaged whole-cell data for each treatment group are not reported below as we found no significant differences for any of these measurements except where otherwise noted.

ACh-evoked nicotinic receptor currents in hippocampal stratum radiatum interneurons under control conditions

As previously reported (Jones and Yakel, 1997; Alkondon et al., 1998; Frazier et al., 1998a) we found consistent expression of functional nAChRs on the cell bodies of hippocampal interneurons. The currents evoked by focal application of 1 mM ACh (Fig. 1A) were reduced to a level indistinguishable from system noise (RMS noise: 2.8 ± 0.36 pA, n = 5) following bath application of MLA (Fig. 1B). The time course for MLA blockade is illustrated in Fig. 1C. Although these data do not rule out expression of other nAChR subtypes on SR interneurons, it does suggest that those activated in our experimental conditions likely contained the α7 subunit.

FF lesions reduced the magnitude of ACh-evoked α7 nicotinic receptor currents in hippocampal stratum radiatum interneurons

Lesion studies were done using within-animal controls, that is, each animal provided both control (contralateral to the lesion site) and experimental (ipsilateral to the lesion site) hippocampal tissue. Amplitudes of α7 receptor currents in hippocampal interneurons ipsilateral to the lesion site following focal somatic application of 1 mM ACh were significantly reduced (129.5 ± 29.1 pA, n = 20) ($P < 0.01$) compared to those recorded on the contralateral side (486.9 ± 111.6 pA, n = 17) (Figs. 2A and B). Net charge (pA·s) was also significantly reduced (3.9 ± 0.8 ipsilateral vs. 21.0 ± 6.9 contralateral) ($P < 0.01$). In order to test for potential differences produced by the surgical procedure alone, a group of littermates to the above animals were used as non-surgical controls (data not shown). Peak currents recorded ipsilaterally to lesions were significantly ($P < 0.01$) less than those recorded in the non-surgical group (582.1 ± 116.1 pA, n = 23) but currents recorded contralaterally to lesions were not significantly different from those in the non-surgical group, suggesting no general procedural effects. These results suggest that MS/DB hippocampal innervation regulates functional aspects of α7 receptors.

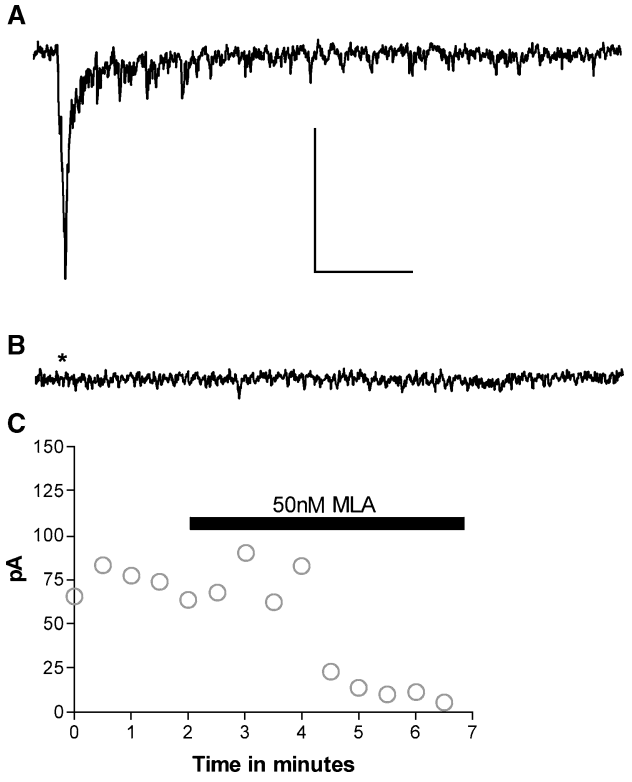

Fig. 1. Hippocampal stratum radiatum interneurons express somatic α7 nicotinic acetylcholine receptor currents that are sensitive to methyllycaconitine (MLA). (A) A whole-cell current produced by a 5 ms focal application of 1 mM ACh. (B) The same cell after bath application of 50 nm MLA (*the time that correlates with the original response shown in panel A). (C) A scatter plot showing the time course of blockade with MLA. It should be noted that the time lag between the onset of bath-applied MLA and the reduction in the response is related to solution exchange and does not reflect the time course of drug action (see Materials and methods). Scale bars are 50 pA and 100 ms.

FF lesions effects on the magnitude of ACh-evoked α7 nicotinic receptor currents were independent of α7 receptor binding in the hippocampus

Animals were lesioned in a manner identical to those used for electrophysiological studies and hippocampi were used for binding studies. The results indicated that the changes in functional responses were apparently not due to changes in total hippocampal receptor density, since there was no difference in high affinity MLA binding between tissues contralateral (10.6 ± 0.7 fmol/mg protein; $n = 3$) versus ipsilateral (8.9 ± 2.2 fmol/mg protein; $n = 3$) to the lesion site (not shown).

ACh-evoked α7 nicotinic receptor currents were not present in hippocampal CA1 pyramidal cells following lesions of the FF

Previous reports (Jones and Yakel, 1997; Alkondon et al., 1998; Frazier et al., 1998a) have shown no measurable currents produced by focal somatic application of ACh in CA1 pyramidal cells in normal animals. In order to test the hypothesis that there may have been a compensatory upregulation or increase in the functional expression of potential somatic pyramidal cell α7 receptors ipsilateral to lesion sites, we tested CA1 pyramidal cells for responses to ACh following lesions but found no measurable currents in 12 of 12 cells examined (data not shown).

GTS-21 treatment prevented a reduction in, or restored the amplitude of, ACh-evoked α7 nicotinic receptor currents following lesions of the FF

Animals with aspirative lesions produced using identical procedures to those above were treated with 1 mg/kg GTS-21 (IP, twice daily for 7–10 days until sacrifice for electrophysiology). As shown in Fig. 2, the amplitudes of α7 receptor responses in interneurons ipsilateral to the lesion site in GTS-21-treated animals were restored to levels not significantly different from those contralateral to the lesion site. Alternatively, GTS-21 may have prevented the decline in current amplitudes seen in lesioned animals not treated with GTS-21. Although the peak amplitudes of currents in cells recorded ipsilateral to the lesion site in animals receiving GTS-21 treatment (390.2 ± 89.3 pA, $n = 19$) tended to be less than those recorded in cells located contralateral to the lesion site (502.0 ± 105.2 pA, $n = 20$) (Figs. 2C and D), the difference was not statistically significant. Likewise there were no significant differences in net charge (pA·s) between cells recorded from the ipsilateral and contralateral hippocampi of GTS-21-treated animals (ipsilateral: 24.2 ± 7.3; contralateral: 35.4 ± 6.7; mean ± SEM). As shown in Fig. 2C (filled bar), peak currents recorded contralateral to the lesion site in GTS-21-treated animals were not significantly different than those recorded in animals not receiving GTS-21 treatment (Fig. 2A, filled bar) or from those in the non-surgical group (data not shown). These results suggest that GTS-21 activation of α7 receptors or other GTS-21-mediated effects produced a preventative measure, restoration, or compensatory change in the function or expression of α7 receptors following lesions of the FF.

GTS-21 treatment did not produce changes in ACh-evoked α7 nicotinic receptor currents in non-surgical/control animals

In light of the results presented above, it became of interest to evaluate whether GTS-21 treatment would have a generalized effect, potentially increasing the amplitude of α7 receptor responses in cells from animals without lesions. As such, two groups of animals were established, one that received 1 mg/kg IP GTS-21 2× daily and one that received identical volumes of 0.08% saline. Although the GTS-21 treatment group showed peak amplitudes

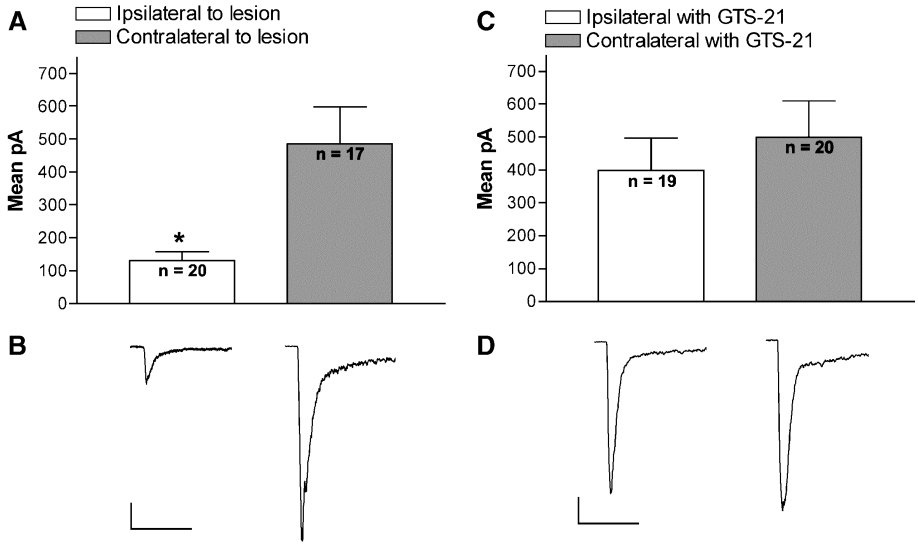

Fig. 2. Fimbria-fornix (FF) lesions produced a marked decrease in the amplitude of somatic α7 nicotinic receptor currents that was prevented or restored with GTS-21 treatment. (A) Bar graph showing the means and SEMs of ACh-evoked currents in cells recorded ipsilateral to lesion sites (open bar) and cells recorded contralateral to lesion sites (closed bar) (*statistical significance; $P < 0.01$). (B) Representative averaged ACh-evoked whole-cell current traces from a hippocampal interneuron located ipsilateral to the lesion site (left) and from one located contralateral to the lesion site (right). (C) Bar graph showing the means and SEMs of ACh-evoked currents in cells recorded ipsilateral to lesion sites (open bar) and cells recorded contralateral to lesion sites (closed bar) following treatment with GTS-21. (D) Representative averaged ACh-evoked whole-cell current traces from a hippocampal interneuron located ipsilateral to the lesion site (left) and from one located contralateral to the lesion site (right) following treatment with GTS-21 (scale bars: 100 pA and 100 ms).

(772.2 ± 177.7 pA, $n = 25$) that were somewhat greater than those recorded in the saline group (594.0 ± 138.7 pA, $n = 24$), this difference was not statistically significant (Figs. 3A and B). Likewise although the mean net charge value (pA·s) was also greater in cells from GTS-21-treated animals, this was not a significant difference (GTS-21: 31.2 ± 6.7 vs. saline: 22.7 ± 6.1; mean ± SEM). Neither GTS-21- nor saline-treated animals had current amplitudes different from control groups in other experiments (compare Figs. 2A, C, and 5A). In this experiment we did note a significant difference ($P < 0.01$) in input resistances between treatment group (GTS-21: 144.6 ± 7.4 MΩ; saline: 187.1 ± 10.6 MΩ). In order to investigate this anomaly further we performed Pearson's R tests on all treatment groups in order to evaluate the possibility that input resistances were correlated with peak ACh-evoked amplitudes. We found no significant correlations between input resistances and peak ACh-evoked amplitudes in any of the treatment groups.

Immunohistochemical confirmation of 192 IgG-saporin lesions

We confirmed the ability of 192 IgG-saporin to produce selective cholinergic lesions in a set of animals not used for patch clamp experiments. We found that the number of ChAT-immunoreactive neurons in the MS/DB (Fig. 4A) was clearly reduced (Fig. 4B) following intraseptal injections of 192 IgG-saporin (1 μg/μl). In addition, hippocampal tissue processed to evaluate AChE activity (Fig. 4C) showed substantial reductions in AChE-labeled fibers and cell density (Fig. 4D), consistent with a loss of cholinergic innervation. These results are in agreement with ones reported previously (Wiley et al., 1991; Book et al., 1992; Heckers et al., 1994).

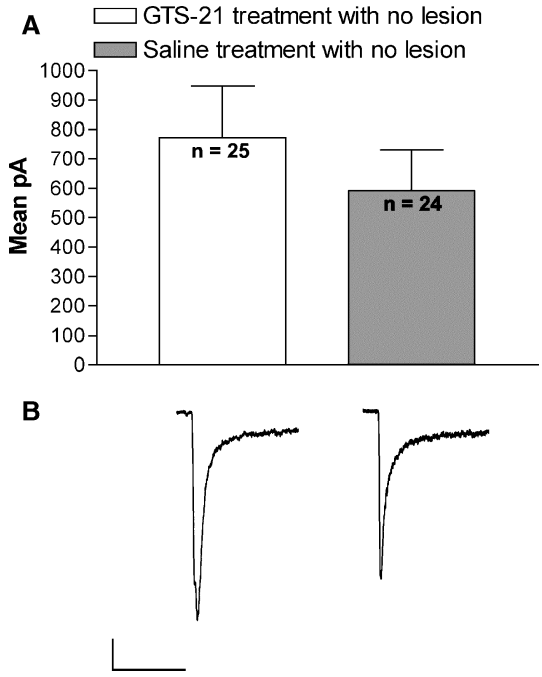

Fig. 3. GTS-21 treatment *did not* significantly increase α7 nicotinic receptor currents in animals without lesions. (A) Bar graph showing the mean and SEM of ACh-evoked currents in cells recorded from animals that received GTS-21 treatment (open bar) and cells recorded from control animals that received saline injections (closed bar). (B) Representative averaged ACh-evoked whole-cell current traces from an animal that received GTS-21 treatment (left) and from an animal that received saline injections (right) (scale bars: 100 pA and 100 ms).

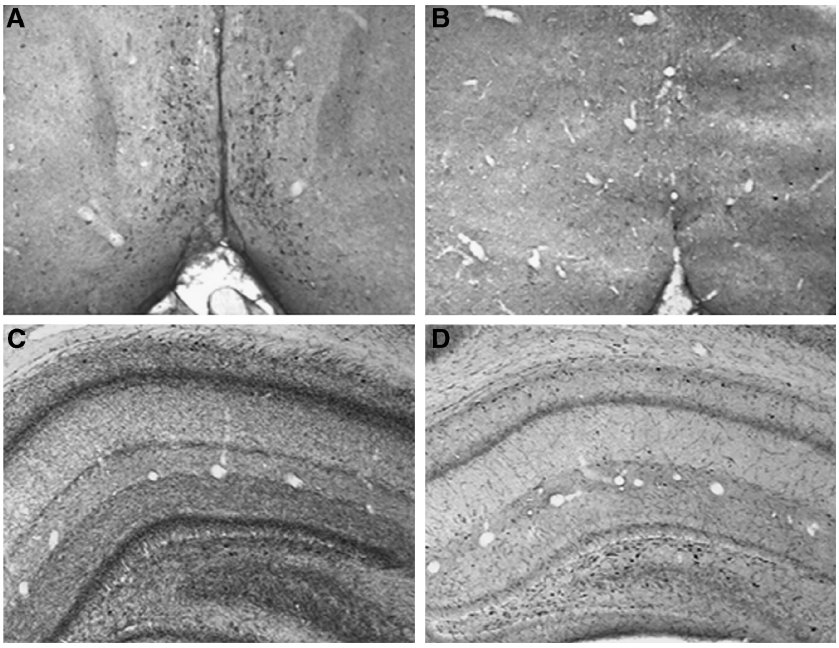

Fig. 4. Cholinergic markers are significantly reduced in the septum and hippocampus following intraseptal injections of 192 IgG-saporin. (A) Low magnification light field image (4×) of a transverse 50 μm tissue section showing ChAT-immunoreactive neurons in medial septum and diagonal band of Broca (MS/DB) in a control animal. Following 192 IgG-saporin lesions, the number of immunolabeled neurons in the MS/DB is substantially reduced (B). In a control brain (C), hippocampal AChE exhibits a distinct localization most dense near the cell body layers. More diffuse fibers course throughout the neuropil with laminar plexi at the distal border of stratum radiatum and in the dentate molecular layer. Following injection of 192 IgG-saporin (D), most AChE reactivity is lost from all hippocampal fields throughout the septal–temporal axis. AChE-containing intrinsic neurons can still be observed and probably contribute most of the remaining fiber labeling.

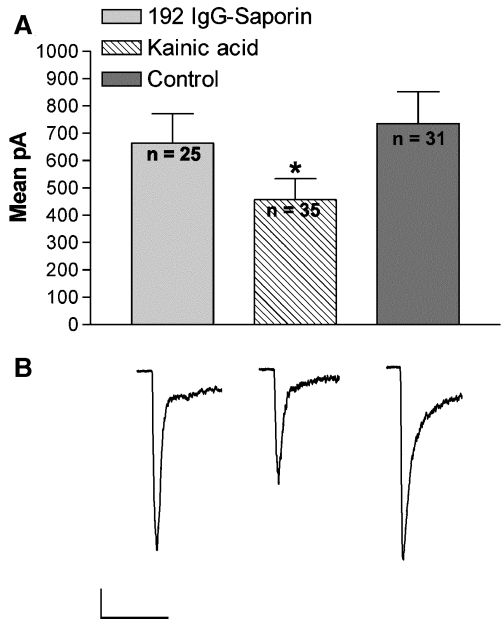

Fig. 5. Kainic acid lesions of the medial septum diagonal band (MS/DB) produced significant reductions in α7 nicotinic receptor currents while selective cholinergic MS/DB lesions did not. (A) Bar graph showing the means and SEMs of ACh-evoked currents in cells recorded from animals that received MS/DB injections of 1 μg/μl 192 IgG-saporin (open bar), kainic acid (hatched bar), and control animals (closed bar). (B) Representative averaged ACh-evoked whole-cell current traces from an animal that received an MS/DB injection of 192 IgG-saporin (left), kainic acid (center), and a control animal (right) (scale bars: 100 pA and 100 ms).

The effects of 192 IgG-saporin and kainic acid MS/DB injections on ACh-evoked α7 nicotinic receptor currents

We found that midline cholinergic lesions of the MS/DB with 1 μg/μl 192 IgG-saporin did not produce significant reductions in α7 nicotinic receptor responses (Figs. 5A and B). Peak current amplitudes following 192 IgG-saporin were 670.8 ± 105.4 pA (n = 25) and 738.2 ± 121.7 pA (n = 31) in control cells. Net charge responses were 35.0 ± 5.4 pA·s following treatment and 34.3 ± 6.0 pA·s in controls. Overall, current amplitudes following 192 IgG-saporin showed no significant differences from other control groups (Figs. 2A and C; Fig. 3A; filled bars). While 192 IgG-saporin treatment was ineffective at modulating levels of functional α7 nAChRs, we found that low concentrations of KA (0.25–0.5 μg/μl) produced significant decreases in the amplitudes of ACh-evoked α7 nicotinic receptor currents (P < 0.05). Following MS/DB KA injections, peak responses were (464.0 ± 82.7 pA, n = 35) compared to controls (738.2 ± 121.7 pA, n = 31), and the net charge (pA·s) measures were reduced to 19.7 ± 2.8 in the KA-treated animals compared to 34.3 ± 6.0 in controls (P < 0.05) (Figs. 5A and B).

Discussion

Our data indicate that the level of functional α7 nAChR expression on hippocampal interneurons is regulated by

factors associated with the innervation from the basal forebrain. In hippocampi ipsilateral to lesions, nicotinic responses were only 20–25% as large as the responses of neurons located contralateral to lesions. Decreases in functional expression were not directly related to changes in receptor binding sites in the hippocampus. While specific loss of cholinergic innervation did not decrease α7 function, chronic activation with an α7-selective agonist allowed lesioned animals to maintain control levels of functional expression. These results could indicate that there is little tonic activation of α7 receptors in hippocampus from septal cholinergic innervation normally, since selective cholinergic lesions did not have the opposite effect from agonist treatment. They further point to an important role for non-cholinergic, potentially GABAergic, projection neurons from the basal forebrain in maintaining hippocampal α7 expression.

We found no change in hippocampal MLA binding following lesions of the FF. This observation is generally consistent with a previous study that failed to note any changes in cytisine or α-btx binding in the rat hippocampus following electrolytic lesions of the medial septum, despite clear functional differences in responses to nicotine (Frazier et al., 1996). These apparent discrepancies produced by changes in function with no clear change in binding may be due to structural or biochemical factors. Potential structural changes include the subcellular relocation of receptors or their redistribution across varying cell types. Biochemically, reductions in α7 receptor currents can result from alterations in the levels of endogenous α7 receptor modulators such as the prostaglandin precursor, arachidonic acid (Oz et al., 2003), or kynurenic acid (Hilmas et al., 2001). Both of these agents are potent endogenous inhibitors of α7 receptor currents, and levels of these inhibitory modulators could be increased following lesions of the FF. An alternative intracellular mechanism that may be involved is phospholipase C activation, which has been shown to inhibit α7 receptor function without changing density (Pardi and Margiotta, 1999). These biochemical changes could alter receptor structure, desensitization rates, or the percentage of activatable receptors, any of which could account for the present results.

Treatment with GTS-21 prevented a reduction in or produced a rescue of α7 receptor currents in FF-lesioned animals. These findings parallel those in other studies showing that functional changes following FF lesions can be reversed or restored with cholinergic treatment. For example, treatment with nicotine can reverse FF lesion-induced memory deficits (Brown et al., 2001), and septal fetal tissue transplants can restore the normal firing properties of pyramidal cells (Duconseille et al., 1999) following FF lesions. The anabaseine derivative GTS-21 has been shown to improve memory-related behaviors (Meyer et al., 1997; Meyer et al., 1998) and is a highly selective activator for the α7 nAChR (Meyer et al., 1997). Just as we have shown GTS-21 to preserve α7 nAChR function in hippocampal interneurons, other studies have shown it to have cytoprotective qualities, preventing neuronal death in many model systems including ischemia (Shimohama et al., 1998), excitotoxic insult (Akaike et al., 1994), axotomy (Martin et al., 1994), and trophic factor deprivation (Meyer et al., 1997; Meyer et al., 1998). Interestingly, it has been reported that there were no changes in hippocampal α-bungarotoxin binding following GTS-21 treatments similar to those in the current study (1 mg/kg IP for 14 days) (Meyer et al., 1997; Meyer et al., 1998), so that it is unlikely that the GTS-21 prevention of or restoration of reduced current amplitudes in our experiments occurred because of an increase in α7 receptor protein. Alternatively, current amplitude restorations may have been produced by GTS-21-mediated receptor activation or cytoprotective actions. It has been shown that low concentrations of GTS-21 produce α7 receptor activation with little residual inhibition (Meyer et al., 1998). Thus, activation of α7 receptors may have contributed to the recovery or preservation of function reported above. While the effects of any nicotinic agent may be confounded by possible receptor desensitization, as well as activation, this is less likely to be the case with α7 receptors that recover rapidly from desensitization and do not convert to high affinity states upon prolonged exposure to agonist. Since GTS-21 has a half life of only about 2 h (Mahnir et al., 1998), it is unlikely to accumulate in tissues, and behavioral effects of similar GTS-21 injections have been shown to be blocked by mecamylamine (Meyer et al., 1997; Woodruff-Pak, 2003), consistent with the production of receptor activation rather than desensitization-mediated effects. Regarding cytoprotective actions, although the exact intracellular mechanisms underlying GTS-21 cytoprotective properties are not known, it is likely that they involve calcium influx and downstream effects on calcium-sensitive transduction systems associated with neuroprotection including protein kinase C and tyrosine protein kinase. As it is well established that lesions of the FF produce a significant degeneration of MS/DB cells (for a review, see Varon et al., 1991), it appears possible that the sparing of MS/DB cells with axons that course outside of the FF through ventral SH pathway (Milner and Amaral, 1984), or the few (~10%) with axons that cross into the contralateral hippocampus, may have been sufficient to restore α7 receptor currents in GTS-21-treated animals.

We investigated whether it was possible to discriminate between the relative impact of the cholinergic and other MS/DB projections with relatively selective lesions created by 192 IgG-saporin. Since GTS-21 treatments maintained α7 function, our initial expectation was that selective cholinergic lesions would decrease α7 function. However, we found no measurable effect of selective cholinergic lesions produced by MS/DB injections of 192 IgG-saporin. Current amplitudes were 14.2% less but not significantly different from those recorded in controls. While these findings indicate that selective cholinergic lesions do not produce

functional changes in ACh-evoked α7 receptor currents, the possibility that there might have been changes in other aspects of nicotinic receptor function cannot be ruled out. In any case, it seems unlikely that non-α7 AChRs would have been affected by the selective cholinergic lesions since it has been reported that there was no change in the number of hippocampal non-α7 nAChRs following saporin lesions of the basal forebrain, as measured by [^{3}H]-cytisine binding (Rossner et al., 1995). Likewise it has been shown that MS/DB injections of IgG-saporin lead to only very limited alterations in spatial learning and memory performances (Baxter and Gallagher, 1996; Pang et al., 2001) and that it is necessary to target both GABAergic and cholinergic MS/DB cell populations to produce spatial memory deficits (Pang et al., 2001) as severe as those obtained with a physical lesion.

In contrast to selective cholinergic lesions produced by 192 IgG-saporin, we found that MS/DB lesions produced by kainic acid (KA) significantly reduced the amplitude of ACh-evoked currents. Kainic acid has been used extensively for its ability to produce hippocampal epileptiform activity and excitotoxic degeneration. The excitotoxic effects of KA are produced by hyperactivation of neurons as a result of extended release of endogenous glutamate. Thus, it would appear that KA lesions are relatively non-selective and certain reports have confirmed this (Venero and Hefti, 1998) using relatively high concentrations (1.6 mg/ml). However, others have shown that septal injections of KA with the protocols we used reduce glutamic acid decarboxylase (GAD) but not ChAT (Malthe-Sorenssen et al., 1980; Walaas, 1981) suggesting that KA lesions can be selective for GABAergic neurons. More recently this has been demonstrated by Pang et al. (2001), who found that at concentrations used in the present study, MS/DB injections of KA spared cholinergic neurons but reduced the number of GABAergic projection neurons, as measured by parvalbumin immunolabeling. Because we used similar and sometimes even lower concentrations (0.25–0.5 mg/ml), it is likely that the lesions in the current study could have produced selective GABAergic cell loss. If KA produced selective lesions of MS/DB GABAergic cells, the present results would suggest that MS/DB GABAergic hippocampal innervation regulates functional aspects of α7 receptors or their expression in stratum radiatum interneurons. Alternatively, if KA produced a general excitotoxicity affecting multiple cell types, our data would argue that input from both cholinergic and GABAergic cells must be removed to affect the function or expression of α7 receptors.

Stimulation of septohippocampal GABAergic afferents directly inhibits the hippocampal interneurons they selectively innervate (Toth et al., 1997), ultimately producing a disinhibition of principal cells. Thus, permanent removal of MS/DB GABAergic input could produce a generalized disinhibition of hippocampal interneurons in vivo. This disinhibition could result in more depolarized resting potentials and higher interneuron firing rates, ultimately leading to changes in α7 receptor function. Measurements of holding current required to clamp cells in vitro at −70 mV showed control cells were slightly more depolarized at rest (−82.8 + 10.6 pA) than cells from kainic-acid-lesioned animals (−76.6 + 8.0 pA), but this difference was not statistically significant and does not rule out the possibility that changes in resting potentials could have occurred in vivo ultimately affecting α7 receptor function in vitro.

While it is interesting to speculate on potential direct effects of cholinergic and GABAergic signaling per se, it seems possible that the effects we observed were related to changes in hippocampal NGF levels. However, several reports have shown that following lesions of the septohippocampal pathway, there were increases in hippocampal NGF levels (Gasser et al., 1986; Korsching et al., 1986; Weskamp et al., 1986). In cultured hippocampal neurons (Kawai et al., 2002) and in PC12 cells (Henderson et al., 1994) NGF treatment has been shown to increase α7 receptor expression. Assuming the lesions in the current report produced increases in hippocampal NGF, a resulting increase in α7 receptor expression could not be detected with our binding assays. As such, it appears unlikely that a reduction in function could have resulted from lesion-induced increases in hippocampal NGF, but our data neither address this possibility nor rule it out.

In conclusion, our findings are the first to demonstrate specific changes in α7 receptor function following lesions of the FF and suggest that septal innervation regulates functional aspects of hippocampal α7 receptors rather than changes in receptor expression. The observation that treatment with the selective α7 receptor agonist GTS-21 restored current amplitudes in FF-lesioned animals, but did not alter the magnitude of ACh-evoked currents in animals without lesions, supports the concept that hippocampal α7 receptors may be viable targets for cholinergic therapeutic intervention in diseases that involve degradation of the septohippocampal pathway and suggests further that like other compounds, therapeutic effects of drugs targeting nicotinic α7 receptors may be most visible in compromised animals. Our results further support the idea that α7-mediated signals in the brain can be dynamically regulated, in either negative or positive directions. This may occur through increases or decreases of the impact of identified endogenous negative regulators or potentially by alterations in positive modulators. While as yet no endogenous positive modulators have been clearly identified in brain systems, the positive modulatory effects for agents such as certain serum albumins (e.g., BSA) (Conroy et al., 2003) and 5-hydrox-indole (Zwart et al., 2002) make it seem likely that such agents may exist in the brain or alternatively be developed as a new therapeutic approach for improving and maintaining cholinergic function in the aging or diseased brain.

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