A Rapid Increase in the Total Number of Cell Surface Functional GABA<sub>A</sub> Receptors Induced by Brain-derived Neurotrophic Factor in Rat Visual Cortex*

Yoshito Mizoguchi, Takashi Kanematsu, Masato Hirata, and Junichi Nabekura

From the ¥Cellular and Systems Physiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582 and the ¥Laboratory of Molecular and Cellular Biochemistry, Faculty of Dental Science, and Station for Collaborative Research, Kyushu University, Fukuoka 812-8582, Japan

The number of postsynaptic γ-aminobutyric acid type A (GABA<sub>A</sub>) receptors is a fundamental determinant of the variability of inhibitory synaptic responses in the central nervous system. In rat visual cortex, [3H]SR-95531 binding assays revealed that brain-derived neurotrophic factor (BDNF), one of the neurotrophins, induced a rapid increase in the total number of cell surface GABA<sub>A</sub> receptors, through the activation of Trk B receptor tyrosine kinases. We also demonstrated that BDNF rapidly induced a sustained potentiation of GABA<sub>A</sub> receptor-mediated currents, using nystatin-perforated patch clamp recordings, in visual cortical layer 5 pyramidal neurons freshly isolated from P14 rats. The potentiation was caused by the activation of Trk B receptor tyrosine kinase and phospholipase C-γ. In addition, intracellular Ca<sup>2+</sup> was important for the potentiation of GABA<sub>A</sub> responses induced by BDNF. The selective increase in mean miniature inhibitory postsynaptic (mIPSC) current amplitude without effects on mIPSC time courses supports the idea that BDNF rapidly induces an increase in the total number of cell surface functional GABA<sub>A</sub> receptors in visual cortical pyramidal neurons. These results suggest that BDNF could alter the number of cell surface GABA<sub>A</sub> receptors in a region-specific manner.

The γ-aminobutyric acid type A (GABA<sub>A</sub>) receptors are ligand-gated ion channels that mediate fast synaptic inhibition in the central nervous system (1). GABAergic transmission is characterized by high variability of synaptic responses attributable to many factors at both pre- and postsynaptic sites (2).

The elevation of intracellular Ca<sup>2+</sup> levels, leading to the activation of protein kinases or phosphatases, either potentiates (3–5) or suppresses (6–8) the postsynaptic GABA<sub>A</sub> receptor responses. These complex results suggest that region-specific phosphorylation or dephosphorylation processes regulate GABAergic function in a cell-specific manner.

* This work was supported by Grants-in-aid for Scientific Research on Priority Areas (C)-Advanced Brain Project (15018082) and Research Grants (15390065, 15650076) from the Ministry of Education, Culture, Sports, and Science and Technology, Japan to J. Nabekura. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 81-92-642-6090; Fax: 81-92-642-6094; E-mail: nabekuru@mailserver.med.kyushu-u.ac.jp.

§ The abbreviations used are: GABA<sub>A</sub>, γ-aminobutyric acid type A; BDNF, brain-derived neurotrophic factor; mIPSC, miniature inhibitory postsynaptic current; PLC, phospholipase C; BAPTA-AM, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetra (acetoxy-methyl) ester; P, postnatal day.

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.

This paper is available on line at http://www.jbc.org

Published, JBC Papers in Press, August 26, 2003, DOI 10.1074/jbc.M305872200

Vol. 278, No. 45, Issue of November 7, pp. 44097–44102, 2003
Printed in U.S.A.

Received for publication, June 4, 2003, and in revised form, August 25, 2003
Published, JBC Papers in Press, August 26, 2003, DOI 10.1074/jbc.M305872200

The number of postsynaptic GABA<sub>A</sub> receptors is a fundamental determinant of the variability of postsynaptic responses (2). The endocytosis of GABA<sub>A</sub> receptors occurs both in hippocampal (9) and in cortical (10) neurons. In contrast, the ubiquitin-like protein Plic-1 increases the number of GABA<sub>A</sub> receptors available for the recruitment to the plasma membrane (11). In hippocampus, insulin induces a rapid recruitment of functional GABA<sub>A</sub> receptors to the postsynaptic membranes through the activation of insulin receptor tyrosine kinase, thereby increasing the amplitude of GABA<sub>A</sub> receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) without effects on their time courses (12).

Brain-derived neurotrophic factor (BDNF), one of the neurotrophins, rapidly modulates postsynaptic GABA<sub>A</sub> receptor function. In hippocampus of postnatal day (P) 14 rat, BDNF acutely inhibits postsynaptic GABA<sub>A</sub> responses by elevating intracellular Ca<sup>2+</sup> levels via the activation of Trk B receptor tyrosine kinase and subsequent phospholipase C (PLC) γ phosphorylation (13, 14), but at P6, BDNF reversibly potentiates postsynaptic GABA<sub>A</sub> responses (14).

BDNF also alters the number of cell surface GABA<sub>A</sub> receptors. In cultured hippocampal (15) and cerebellar granule (16) cells, BDNF rapidly induces an internalization of postsynaptic cell surface GABA<sub>A</sub> receptors, through the Trk B receptor tyrosine kinase activation, thereby decreasing the amplitudes of mIPSCs.

In visual cortex, the timing of the critical period for ocular dominance columns formation (which is normally between P23 and P33) is determined, not simply by visual stimuli but also by the maturation of the inhibitory circuits themselves (17). Mice overexpressing BDNF exhibit a precocious maturation of GABAergic inhibition in visual cortex and accelerated decline of the critical period for ocular dominance plasticity (18).

In developing rat visual cortex, the expression of Trk B receptor is most prominent in layer 5 pyramidal neurons (19), and the levels of both BDNF mRNA (20) and BDNF protein (21) are highest in layers 2, 3, and 5. In layer 5 of rat visual cortex, the level of BDNF mRNA rapidly increases around P14 (when the eyes have just opened) (20) and thereafter decreases to reach a plateau level at P18 (22). Thus, increasing evidence suggests that BDNF has important roles in GABAergic transmission in developing visual cortex, but the potent mechanisms underlying these effects are unclear.

We tested the rapid effect of BDNF on the cell surface expression of GABA<sub>A</sub> receptors in visual cortex of P14 rats, using [3H]SR-95531 binding assays. [3H]SR-95531 binding assays revealed that BDNF induced a rapid increase in the total number of cell surface GABA<sub>A</sub> receptors in rat visual cortex, through the activation of Trk B receptor tyrosine kinases.
BDNF Increased Total Number of Cell Surface GABA<sub>A</sub> Receptors

We also demonstrated that BDNF rapidly induced a sustained potentiation of GABA<sub>A</sub> receptor-mediated currents, using nystatin-perforated patch clamp recordings, in visual cortical layer 5 pyramidal neurons isolated from P14 rats. The selective increase in mean mIPSC amplitude without effects on mIPSC time courses supports the idea that BDNF rapidly induces an increase in the total number of cell surface functional GABA<sub>A</sub> receptors in visual cortical pyramidal neurons.

EXPERIMENTAL PROCEDURES

All experiments conformed to the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan, and all efforts were made to minimize the number of animals used and their suffering.

<sup>1</sup>H[SR-95531 Binding Assays—Visual cortical cells were freshly isolated from rats using papain dissociation system (Worthington Biochemical Company, Lakewood, NJ) intended for the cell dissociation and culture procedures developed by Huettner and Baughman (23), by which high yields of viable and morphologically intact neurons were dissociated from visual cortex of P1–15 rats. In brief, 14-day-old Wistar rats were decapitated under pentobarbital anesthesia (50 mg kg<sup>−1</sup>, intraperitoneally). Brains were quickly removed and bathed in cold incubation solution bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Blocks of tissue comprising visual cortex were removed from the occipital cortex of both hemispheres and were gently minced or cut into small pieces. The tissue was placed in the Earle’s balanced salt solution, equilibrated with 95% O<sub>2</sub>, 5% CO<sub>2</sub> including papain (at a final concentration of 20 units ml<sup>−1</sup>) and DNase (0.005%). The mixed solution was incubated for 15 min at 37 °C. After albumin–ovoidoillaroid inhibitor was added to the mixed solution, the mixture was gently triturated with 10-ml pipette three times and centrifuged at 1,100 r.p.m. for 5 min. The supernatant was discarded, and the obtained visual cortical cells were immediately resuspended with external standard solution. Cell viability of the obtained visual cortical cells, determined by the trypan blue exclusion method (24), was 87.8 ± 2.5% (n = 4 rats). The trypan blue exclusion method was as follows. The cell suspension was incubated for 5 min in 0.4% trypan blue solution (at a final concentration of 0.2%; Sigma) and washed twice in external standard solution. In each experiment, five randomly chosen optical fields (at least 250 cells in each field) were analyzed by a phase contrast, bright field microscopy at low magnification. Viable cells are determined as dye-negative, whereas nonviable cells with damaged cell membrane are stained blue. Cell viability was expressed as a percent of the number of total viable (unstained) cells relative to the total (unstained and stained) cells. The final value was obtained as mean ± S.E. of four separate experiments.

Visual cortical cells were incubated in standard solution with vehicle (standard solution including BDNF or BDNF plus KN252a (200 nM) for 10 min at room temperature. The cells were washed with standard solution by centrifugation at 1,000 r.p.m. for 5 min and resuspended in ice-cold standard solution. The protein concentration of the cell suspension was measured using a Lowry method (25) with bovine serum albumin as the standard protein. Twenty μl of cell suspension was added to the assay buffer (50 μm Tris/Cl (pH 7.5), 150 mM NaCl, 1% bovine serum albumin) containing 20 nm <sup>1</sup>H[SR-95531, a competitive GABA<sub>A</sub> receptor antagonist (specific radioactivity 2,166.7 GBq mmol<sup>−1</sup>) (PerkinElmer Life Sciences) with or without 10 μM SR-95531, and incubated for 20 min at 4 °C (26). To calculate the specific binding, nonspecific binding values (determined in the presence of 10 μM SR-95531) were subtracted from the total binding values (in the absence of 10 μM SR-95531). For Scatchard analysis (27), assay buffers containing 5–30 nM <sup>1</sup>H[SR-95531 were used. Samples were filtered under negative pressure over Whatman GF/PC filters, which were then rapidly rinsed two times with 5 μl of ice-cold assay buffer. Radioactivity on filters was measured by liquid scintillation counting.

Electrophysiological Recordings—Visual cortical pyramidal neurons from 14-day-old Wistar rats were freshly dissociated using procedures similar to those described previously (14, 28). Briefly, rats were decapitated under pentobarbital anesthesia. Brains were quickly removed and transversely sliced at a thickness of 370 μm (VT-1000; Leica; Nussloch; Germany). The slices were kept in the incubation medium saturated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, at room temperature for 60 min. After 30 min of enzyme digestion, the slices were transferred into a 35-mm culture dish, and layer 5 of the visual cortex was identified under a binocular microscope (×20, SMZ-1; Nikon, Tokyo, Japan). A fire-polished glass pipette was touched lightly onto the surface of the region and was vibrated horizontally at 30–50 Hz for 5 min using an apparatus developed in our laboratory (29, 30). Slices were removed from the dish, and dissociated pyramidal neurons with a large pyramidal-shaped soma, a prominent apical dendrite, and a skirt of basal dendrites (31, 32) adhered to the bottom of the dish within 20 min (Fig. 3A).

Electrical measurements were performed using the nystatin-perforated patch recording method (14, 28, 33). All recordings were performed using voltage clamp at a holding potential of −50 mV, using patch clamp amplifier (EPC-7; List Biologic, Campbell, CA). Patch pipettes were made from borosilicate capillary glass tubes (G-1.5; Narishige, Tokyo, Japan) in two stages on a vertical pipette puller (PB-7; Narishige). The resistance between the patch pipette filled with the internal solution and the reference electrode in the normal external solution was 4–6 MΩ. Neurons were visualized with phase-contrast equipment on an inverted microscope (Diaphot; Nikon). Current and voltage were continuously monitored on an oscilloscope (VC-6725; Hitachi, Tokyo, Japan) and a pen recorder (Recti-Horiz-8K, Sanei, Tokyo, Japan) and recorded on a digital-audio tape recorder (RD-120TE, TEAC). Membrane currents were filtered at 1 kHz (E-5201A Decade Filter; NF Electronic Instruments, Tokyo, Japan), and data were digitized at 4 kHz. For mIPSCs recording, the extracellular solution was supplemented with 6-cyano-nitroquinoline-2,3-dione (10 μM) and 2-amino-5-phosphonopentanoic acid (20 μM). The mIPSCs were completely blocked by the competitive GABA<sub>A</sub> receptor antagonist, bicuculline (10 μM; data not shown). mIPSCs were detected and analyzed using MiniAnalysis software (Synaptosoft, NJ). All experiments were performed at room temperature (27 ± 1 °C). All data are expressed as mean ± S.E., and statistical analysis was performed using Student’s t test, with p < 0.05 being considered significantly different.

Solutions and Drugs—The ionic composition of the internal (patch pipette) solution was 40 mM methanesulfonic acid potassium salt, 110 mM KCl, 10 mM HEPES. The pH of internal solution was adjusted to 7.2 with Tris-OH. Nystatin (Sigma) was dissolved in acidic methanol at 10 mg ml<sup>−1</sup>. This stock solution was diluted with internal pipette solution just before use to a final concentration of 100–200 μM<sup>−1</sup>. The ionic composition of the incubation medium was 124 mM NaCl, 5 mM KCl, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 24 mM NaHCO<sub>3</sub>, 2.4 mM CaCl<sub>2</sub>, 1.3 mM MgSO<sub>4</sub>, and 10 mM glucose. Following equilibration with 95% O<sub>2</sub>, the pH was 7.4. The ionic composition of the external standard solution was 150 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM glucose, and 10 mM HEPES. The pH of the external standard solution was adjusted to 7.4 with Tris-OH. Drug solutions were applied, using the Y-tube perfusion system, allowing rapid exchange of the solution surrounding a cell within 20 ms (34–36).

The drugs used in the present study included GABA, 2-amino-5-phosphonopentanoic acid, 6-cyano-nitroquinoline-2,3-dione, bicuculline, diazepam, muscimol, and BAPTA-AM (Sigma), TTX (Wako, Tokyo, Japan), K252a, U73122, and KN-62 (Calbiochem). Drugs that are insoluble in water were first dissolved in dimethyl sulfoxide (Me<sub>2</sub>SO) and then diluted in the external solution. The final concentration of Me<sub>2</sub>SO was always less than 0.1% and did not affect neuronal responses observed in the present study. Human recombinant BDNF (Sigma) was dissolved (100 μg ml<sup>−1</sup>) in phosphate buffer solution containing 0.1% bovine serum albumin and stored below −20 °C. Before the experiment, this stock solution was diluted with external solution to obtain the final concentration (5 or 20 ng ml<sup>−1</sup>).

RESULTS

A Rapid Increase in the Total Number of Cell Surface GABA<sub>A</sub> Receptors Induced by BDNF in Rat Visual Cortex—We examined the expression of cell surface GABA<sub>A</sub> receptor α/β subunits of BDNF (20 ng ml<sup>−1</sup>) treated and -untreated cells in visual cortex of P14 rats, by measuring the binding of the hydrophilic GABA<sub>A</sub> receptor competitive antagonist <sup>1</sup>H[SR-95531. The hydrophilic nature of <sup>1</sup>H[SR-95531 restricts its binding to GABA<sub>A</sub> receptors on the cell surface (37, 38).

To examine whether BDNF modulates the cell surface GABA<sub>A</sub> receptor number or its affinity or both, total specific <sup>1</sup>H[SR-95531 radioligand binding activity expressed by visual cortical cells was determined by Scatchard analysis. In 20 ng/ml BDNF-treated cells, total binding (maximal binding capacity, B<sub>max</sub>) was increased by 2.25-fold as compared with the BDNF-untreated control cells (n = 4 rats), whereas the dissociation constant (K<sub>d</sub>) remained unchanged (n = 4; p > 0.05, Fig. 1). The values are summarized in Fig. 1B, and a representative...
BDNF Induced Rapid Increase in the Total Binding of Cell Surface GABA<sub>A</sub> Receptors in Rat Visual Cortex, Without Effects on GABA<sub>A</sub> Receptor Affinity. In A, a representative Scatchard plot is shown for BDNF-untreated (open squares) and -treated (filled circles) cells. For Scatchard analysis, assay buffers containing 6–30 nM <sup>3</sup>H[S]R-95531, a competitive GABA<sub>A</sub> receptor antagonist, were used. Each point is the mean of triplicate. B, a summary of the <sup>3</sup>H[S]R-95531 binding parameters (K<sub>A</sub> and B<sub>max</sub>) in BDNF-untreated (Control; n = 4) and -treated cells (BDNF; n = 4).

Scatchard plot is shown in Fig. 1A. This result suggests that BDNF induced a rapid increase in the total number of cell surface GABA<sub>A</sub> receptors in rat visual cortex, without effects on the receptor affinity.

BDNF specifically binds to Trk B, a neurotrophin receptor, which contains a catalytic domain of tyrosine kinase (39). The specific binding of 20 nM <sup>3</sup>H[S]R-95531 to BDNF-treated cells was significantly increased as compared with the control cells, by 72.6 ± 5.6% (n = 4 rats; p < 0.005). On the other hand, the inclusion of 200 nM K252a, a membrane-permeant inhibitor of Trk receptor tyrosine kinases (40, 41) to the BDNF-treated cells, blocked the elevation of <sup>3</sup>H[S]R-95531 binding (by 6.2 ± 2.4%, n = 4, Fig. 2). All together, these results suggest that BDNF induced a rapid increase in the total number of cell surface GABA<sub>A</sub> receptors in rat visual cortex, through the activation of Trk B receptor tyrosine kinases.

Rapid Potentiation of GABA<sub>A</sub> Responses Induced by BDNF in Rat Visual Cortical Pyramidal Neurons—We next tested whether BDNF rapidly potentiates GABA<sub>A</sub> responses in pyramidal neurons. Fresh pyramidal neurons were dissociated from layer 5 of the visual cortex isolated from P14 rats (Fig. 3A). Voltage clamp recordings were obtained at a holding potential of −50 mV using nystatin-perforated patch clamp recordings, which preserves Ca<sup>2+</sup> and other soluble intracellular constituents intact. The application of 10 µM GABA or muscimol, using the Y-tube perfusion system, to these neurons induced a GABA<sub>A</sub> receptor-mediated inward current (Fig. 3B, control), which was completely blocked by 10 µM bicuculline (n = 3, data not shown). In the presence of BDNF (5 ng ml<sup>−1</sup>), this GABA<sub>A</sub> response was potentiated in every neuron tested (by 71.6 ± 20.0%, n = 12; Fig. 3B), within 5 min of BDNF application, and in the longest recordings obtained, persisted more than 60 min after BDNF washout (Fig. 3C). A higher BDNF concentration (20 ng ml<sup>−1</sup>) caused an even more marked potentiation (by 154.3 ± 26.6%, n = 12). These results show that BDNF rapidly induces a sustained potentiation of GABA<sub>A</sub> receptor-mediated currents in rat visual cortical pyramidal neurons.

Intracellular Regulatory Pathway Involved in the Potentiating Effect of BDNF on GABA<sub>A</sub> Responses—In the presence of K252a (200 nM), BDNF (20 ng ml<sup>−1</sup>) failed to potentiate the GABA<sub>A</sub> responses in visual cortical pyramidal neurons (n = 5; Fig. 4A). Therefore, the absence of Trk B receptor tyrosine kinase activity was essential for the BDNF-induced potentiation of GABA<sub>A</sub> responses in these neurons (n = 5; Fig. 4B). In the presence of 150 µM BAPTA-AM, a membrane-permeant Ca<sup>2+</sup> chelator, BDNF (20 ng ml<sup>−1</sup>) failed to potentiate the GABA<sub>A</sub> responses (n = 5; Fig. 4C). In the presence of U73122 (5 µM), a membrane-permeant PLC-γ inhibitor (46),
Intracellular regulatory pathway involved in the potentiating effect of BDNF on GABA\textsubscript{A} responses in visual cortical pyramidal neurons. A, representative traces showing that, in the presence of K252a (200 nM), BDNF (20 ng ml\textsuperscript{−1}) failed to potentiate GABA\textsubscript{A} responses. B, time course of the effects of BDNF on GABA\textsubscript{A} receptor responses in the presence of K252a. C, time course of the effects of BDNF (20 ng ml\textsuperscript{−1}) on the GABA\textsubscript{A} responses, in the presence of BAPTA-AM (open squares) or 5 \(\mu\)M U73122 (filled squares). D, time course of the effects of BDNF on GABA\textsubscript{A} receptor responses in the presence of 5 \(\mu\)M KN-62 (open circles). In the inset, representative traces showing that, in the presence of KN-62 (a) and a response following the application of 20 ng ml\textsuperscript{−1} BDNF (b).

again BDNF (20 ng ml\textsuperscript{−1}), failed to potentiate the GABA\textsubscript{A} responses (n = 5; Fig. 4C).

In mouse cortical (5), rat cerebellar Purkinje (4), and immature rat hippocampal neurons (14), an increase in intracellular Ca\textsuperscript{2+} enhances postsynaptic GABA\textsubscript{A} responses via an activation of Ca\textsuperscript{2+}/calmodulin-dependent protein kinase 2 (CaMK-2). In the presence of KN-62 (5 \(\mu\)M), a membrane-permeant specific CaMK-2 inhibitor (48), BDNF (20 ng ml\textsuperscript{−1}), potentiated the GABA\textsubscript{A} responses.

These results suggest that BDNF potentiated GABA\textsubscript{A} responses through the activation of Trk B receptor tyrosine kinase and PLC-\(\gamma\). In addition, intracellular Ca\textsuperscript{2+} was important for the potentiation of GABA\textsubscript{A} responses induced by BDNF. In contrast, CaMK-2 was not involved in the sustained potentiation of GABA\textsubscript{A} responses induced by BDNF in rat visual cortical pyramidal neurons.

The application of K252a (200 nM) did not affect the GABA\textsubscript{A} responses potentiated by BDNF (20 ng ml\textsuperscript{−1}) (n = 3; Fig. 5A). In addition, either U73122 (5 \(\mu\)M) or BAPTA-AM (150 \(\mu\)M) failed to suppress the GABA\textsubscript{A} responses potentiated by BDNF (Fig. 5B).

Potentiation of mIPSCs by BDNF in Rat Visual Cortical Pyramidal Neurons—We next recorded mIPSCs in rat visual cortical pyramidal neurons at P14 to examine whether BDNF could also potentiate the response of GABA\textsubscript{A} receptors to released GABA in the synaptic cleft. We used an enzyme-free dissociation procedure so as to preserve functional presynaptic boutons adherent to the isolated neurons (49). BDNF (20 ng ml\textsuperscript{−1}) caused a significant increase, of approximately 78%, in the mean amplitude of mIPSCs (from −19.3 ± 2.2 pA to −34.3 ± 2.7 pA, p < 0.001, n = 5). BDNF increased mIPSC amplitude without affecting the mIPSC frequency (67.3 ± 2.3 events/3 min in control conditions and 68.0 ± 2.1 events/3 min during BDNF application, p > 0.1, n = 5). Fig. 6, A and B, show representative currents and corresponding amplitude histograms from one of these neurons. Thus, BDNF potentiates the GABA\textsubscript{A} receptor responses to both exogenously applied and released GABA in the synaptic cleft in rat visual cortical pyramidal neurons.

We compared the actions of BDNF on mIPSCs with a well-established GABA\textsubscript{A} receptor modulator, the benzodiazepine, diazepam. Diazepam (1 \(\mu\)M), which increased the affinity of GABA\textsubscript{A} receptors (50), prolonged the mIPSC decay without markedly affecting peak amplitude (n = 5; Table I and Fig. 6C). In contrast, BDNF caused a significant increase in mean mIPSC amplitude and no change in either the mIPSC rise time or the decay time constant (n = 5). If GABA released from a single vesicle is sufficient to nearly saturate the cluster of postsynaptic GABA\textsubscript{A} receptors in our experimental condition, then one possible reason for the increase in mIPSC amplitude...
is that BDNF induces a rapid increase in the total number of cell surface GABA<sub>A</sub> receptors in rat visual cortical pyramidal neurons.

**DISCUSSION**

The present experiments demonstrated that BDNF induced a rapid increase in the total number of cell surface GABA<sub>A</sub> receptors in visual cortex of P14 rats, through the activation of Trk B receptor tyrosine kinases. In visual cortical pyramidal neurons isolated from P14 rats, BDNF rapidly induced a sustained potentiation of GABA<sub>A</sub> receptor-mediated currents. The potentiation was caused by the activation of Trk B receptor tyrosine kinase and PLC-γ. In addition, intracellular Ca<sup>2+</sup> was important for the potentiation of GABA<sub>A</sub> responses by BDNF. The selective increase in mean mIPSC amplitude without effects on mIPSC time courses supports the idea that BDNF rapidly induces an increase in the total number of cell surface functional GABA<sub>A</sub> receptors in visual cortical pyramidal neurons.

In the binding assays, we used visual cortical cells as preparations, which include not only pyramidal neurons but also interneurons or glial cells. A rapid increase in the total number of cell surface GABA<sub>A</sub> receptors induced by BDNF that we observed might not simply reflect the BDNF effect on pyramidal neurons.

In hippocampus at P6, Ca<sup>2+</sup>/calmodulin-dependent protein kinase 2 (CaMK-2) plays important roles in the reversible potentiation of postsynaptic GABA<sub>A</sub> responses by BDNF (14). In visual cortical pyramidal neurons isolated from P14 rats, CaMK-2 was not involved in the sustained potentiation of GABA<sub>A</sub> responses induced by BDNF (Fig. 4D). Thus, different intracellular regulatory pathways seem to underlie the potentiating effect of BDNF on GABA<sub>A</sub> responses in hippocampus of P6 rat and visual cortex at P14.

In rat hippocampus at P14, BDNF acutely inhibits postsynaptic GABA<sub>A</sub> responses by elevating postsynaptic Ca<sup>2+</sup> levels via the activation of Trk B receptor tyrosine kinase and PLC-γ (13, 14). A similar transduction mechanism seems to underlie the potentiating effect of BDNF on GABA<sub>A</sub> responses observed in isolated visual cortical pyramidal neurons. However, the activation of the Trk B-PLC-γ pathway was not important for the maintenance of the sustained potentiation of GABA<sub>A</sub> responses induced by BDNF (Fig. 5). This also supports the idea that BDNF increases the total number of functional GABA<sub>A</sub> receptors in visual cortical pyramidal neurons.

In cultured hippocampal (15) and cerebellar granule (16) cells, BDNF induces a rapid down-regulation of cell surface GABA<sub>A</sub> receptors. We proposed an opposite effect of BDNF in visual cortex, i.e. a rapid increase in the total number of cell surface GABA<sub>A</sub> receptors. Thus, in the CNS, BDNF may rapidly increase or decrease the cell surface expression of GABA<sub>A</sub> receptors through the activation of Trk B receptor tyrosine kinases in a region-specific manner.

In our experimental conditions, GABA released from a single vesicle is likely to be sufficient to saturate the cluster of postsynaptic GABA<sub>A</sub> receptors, as reported at other central nervous system synapses (51, 52). However, the degree of receptor occupancy and the use of benzodiazepines for determining postsynaptic GABA<sub>A</sub> receptor occupancy remain controversial. In layer 5 pyramidal neurons of visual cortex, GABA<sub>A</sub> receptors were not shown to be saturated by the synthetically released GABA (53, 54), but in layers 2 and 3, GABA<sub>A</sub> receptors were fully occupied (54). Zolpidem, a benzodiazepine, enhances the amplitudes of mIPSCs if applied at room temperature (22–25 °C) but not at physiological temperature (35 °C), suggesting that zolpidem cannot be used as a tool to determine the GABA<sub>A</sub> receptor occupancy at physiological temperature (53).

The variation in the GABA concentration in the synaptic cleft underlies the amplitude variability of postsynaptic responses (55). There might be differences in the GABA concentration in the synaptic cleft between our experimental conditions and those in previous reports.

**CONCLUSIONS**

BDNF induced a rapid increase in the total number of cell surface GABA<sub>A</sub> receptors in visual cortex of P14 rats, through the activation of Trk B receptor tyrosine kinases. In visual cortical pyramidal neurons isolated from P14 rats, BDNF rapidly induced a sustained potentiation of GABA<sub>A</sub> receptor-mediated currents. The potentiation was caused by the activation of Trk B receptor tyrosine kinase and PLC-γ. In addition, intracellular Ca<sup>2+</sup> was important for the potentiation of GABA<sub>A</sub> responses induced by BDNF. The selective increase in mean mIPSC amplitude without effects on mIPSC time courses supports the idea that BDNF rapidly induces an increase in the total number of cell surface functional GABA<sub>A</sub> receptors in visual cortical pyramidal neurons.

In visual cortex, BDNF plays important roles in the formation of ocular dominance columns (56, 57). Mice overexpressing BDNF exhibit a precocious maturation of GABAergic inhibition in the visual cortex and an accelerated decline of the critical period for ocular dominance plasticity (which is normally between P23 and P33) (18). Both the onset and the close of this critical period are determined, not simply by visual stimuli, but also by the maturation of the inhibitory circuits themselves (17, 47, 58, 59). A rapid increase in the total number of cell surface GABA<sub>A</sub> receptors induced by BDNF in visual cortex of P14 rats may be important for the maturation of the inhibitory circuits and the development of visual cortex.

**Acknowledgment**—We thank A. Moorhouse for critical comments.

**REFERENCES**

1. Macdonald, R. L., and Olsen, R. W. (1994) *Ann. Rev. Neurosci.* **17**, 569–602.
2. Cherubini, E., and Conti, F. (2001) *Trends Neurosci.* **24**, 155–162.
3. Hashimoto, T., Ishii, H., and Omohri, H. (1996) *J. Physiol. (Lond.*) **497**, 611–627.
4. Kano, M., Kano, M., Fukunaga, K., and Konnerth, A. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13351–13356.
5. Aguayo, L. G., Espinoza, F., Kunos, G., and Satin, L. S. (1998) *Pfluegers Arch.* *Eur. J. Physiol.* **435**, 382–387.
6. Inoue, M., Oomura, Y., Yakuubushi, T., and Akaike, N. (1986) *Nature* **323**, 156–158.
7. Chen, Q. X., Stelzer, A., Kay, A. R., and Wong, R. K. S. (1990) *J. Physiol. (Lond.*) **420**, 207–221.
8. Smart, T. G. (1997) *Curr. Opin. Neurobiol.* **7**, 358–367.
9. Kittler, J. T., Delmas, P., Jovanovic, J. N., Brown, D. A., Smart, T. G., and Moss, S. J. (2000) *J. Neurosci.** **20**, 7972–7977.
10. Kilman, V., van Rossum, M. C. W., and Purrigiano, G. G. (2002) *J. Neurosci.* **22**, 1328–1337.
11. Bedford, F. K., Kittler, J. T., Muller, E., Thomas, P., Uren, J. M., Merlo, D., Wisden, W., Triller, A., Smart, T. G., and Moss, S. J. (2001) *Nat. Neurosci.* **4**, 908–916.
12. Wan, Q., Xiong, Z. G., Man, H. Y., Ackerley, C. A., Brauniton, J., Lu, W. Y., Becker, L. E., Macdonald, J. F., and Wang, Y. T. (1997) *Nature* **388**, 686–690.
13. Tanaka, T., Saito, H., and Matsuki, N. (1997) *J. Neurosci.* **17**, 2959–2966.
14. Miazguchi, Y., Ishihashi, H., and Nabekura, J. (2003) *J. Physiol. (Lond.*) **548**, 703–709.
15. Briniger, I., Penschuck, S., Berninger, B., Benson, J., and Frischy, J. M. (2001) *Eur. J. Neurosci.* **13**, 1320–1326.
16. Cheng, Q. and Yeh, H. H. (2003) *J. Physiol. (Lond.*) **548**, 711–721.
17. Henoch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S., and Kash, S. F. (1996) *Science* **273**, 1504–1508.
18. Huang, Z. J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M. F., Maffei, L. and Tonegawa, S. (1999) *Cell* **96**, 739–755.
19. Cellerino, A., Maffei, L., and Domenici, L. (1996) *Eur. J. Neurosci.* **8**, 1190–1197.
20. Bozzi, Y., Pizzorusso, T., Cremisi, F., Rossi, M. F., Barsacchi, G., and Maffei, L. (1995) *Neuroscience* **99**, 1133–1144.
21. Tropea, D., Capossi, S., Tongiorgi, E., Giannotta, S., Cattaneo, A., and Domenici, L. *Eur. J. Neurosci.* **13**, 709–721.
22. Capossi, S., Tongiorgi, E., Cattaneo, A., and Domenici, L. (1999) *Neuroscience* **95**, 1033–1040.
23. Haentser, J. E., and Baughman, R. W. (1986) *J. Neurosci.* **6**, 3044–3060.
24. Michaelis, R. L., and Rothman, S. M. (1990) *J. Neurosci.* **10**, 283–292.
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol.*
26. Günther, U., Benson, J., Benke, D., Fritschy, J. M., Reyes, G., Knoflach, F., Crestani, F., Aguzzi, A., Arigoni, M., Lang, Y., Bluethmann, H., Möhler, H., and Lüscher, B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7749–7753
27. Zivin, J. A., and Waud, D. R. (1982) Life Sci. 30, 1407–1422.
28. Mizoguchi, Y., Monji, A., and Nabekura, J. (2002) Eur. J. Neurosci. 16, 761–794.
29. Rhee, J. S., Ishibashi, H., and Akaike, N. (1999) J. Neurochem. 72, 800–807.
30. Kakazu, Y., Uchida, S., Nakagawa, T., Akaike, N., and Nabekura, J. (2000) J. Neurophysiol. 84, 251–258.
31. Peters, A., and Kara, D. A. (1985) J. Comp. Neurol. 234, 218–241.
32. Nabekura, J., Omura, T., Okabe, A., Furuta, A., Iwaki, T., Okabe, C. S., Fukuda, A., and Akaike, N. (2002) J. Neurosci. 22, 4412–4417.
33. Bourguignon, J. J., Schlewer, G., McLikian, A., Chantreaux, D., Molimard, J. C., Heaulme, M., Chambon, J. P., Bizière, K., and Wermuth, C. G. (1985) Pharmacologist 27, 518.
34. Alicke, B., and Schwartz-Bloom, R. D. (1995) J. Neurochem. 65, 2808–2811.
35. Thoenen, H. (1995) Science 269, 570–573.
36. Aigner, L., and Schwartz-Bloom, R. D. (1995) Oncogene 2, 371–381.
37. Knüsel, B., and Hefti, F. (1992) J. Neurochem. 59, 1987–1996.
38. Widmer, H. R., Koplan, D. R., Bahn, S. J., Beck, R. D., Hefti, F., and Knüsel, D. (1993) J. Neurochem. 60, 2111–2123.
39. Patapoutian, A., and Reichardt, L. F. (2001) Curr. Opin. Neurobiol. 11, 272–280.
40. Berninger, B., Garcia, D. E., Imagaki, N., Hahnel, C., and Lindholm, D. (1993) NeuroReport 4, 1303–1306.
41. Mizoguchi, Y., and Nabekura, J. (2003) NeuroReport 14, 1481–1483.
42. Yule, D. I., and Williams, J. A. (1992) J. Biol. Chem. 267, 13830–13835.
43. Feldman, D. E. (2000) Nat. Neurosci. 3, 293–294.
44. Tokumitsu, H., Chijiwa, T., Hagimura, M., Minamitani, A., Terasawa, M., and Hidaka, H. (1990) J. Biol. Chem. 265, 4315–4320.
45. Akaike, N., and Nabekura, J. (1996) J. Neurosci. 19, 2843–2851.
46. Yule, D. I., and Williams, J. A. (1992) J. Biol. Chem. 267, 13830–13835.
47. Feldman, D. E. (2000) Nat. Neurosci. 3, 293–294.
48. Alicke, B., and Schwartz-Bloom, R. D. (1995) J. Neurochem. 65, 2808–2811.
49. Thoenen, H. (1995) Science 269, 570–573.
50. Aigner, L., and Schwartz-Bloom, R. D. (1995) Oncogene 2, 371–381.
51. Knüsel, B., and Hefti, F. (1992) J. Neurochem. 59, 1987–1996.
52. Widmer, H. R., Koplan, D. R., Bahn, S. J., Beck, R. D., Hefti, F., and Knüsel, D. (1993) J. Neurochem. 60, 2111–2123.
53. Patapoutian, A., and Reichardt, L. F. (2001) Curr. Opin. Neurobiol. 11, 272–280.
54. Berninger, B., Garcia, D. E., Imagaki, N., Hahnel, C., and Lindholm, D. (1993) NeuroReport 4, 1303–1306.
55. Mizoguchi, Y., and Nabekura, J. (2003) NeuroReport 14, 1481–1483.
56. Yule, D. I., and Williams, J. A. (1992) J. Biol. Chem. 267, 13830–13835.
57. Feldman, D. E. (2000) Nat. Neurosci. 3, 293–294.
58. Tokumitsu, H., Chijiwa, T., Hagimura, M., Minamitani, A., Terasawa, M., and Hidaka, H. (1990) J. Biol. Chem. 265, 4315–4320.
59. Akaike, N., and Nabekura, J. (1996) J. Neurosci. 19, 2843–2851.
60. Yule, D. I., and Williams, J. A. (1992) J. Biol. Chem. 267, 13830–13835.
61. Alicke, B., and Schwartz-Bloom, R. D. (1995) J. Neurochem. 65, 2808–2811.
62. Thoenen, H. (1995) Science 269, 570–573.
63. Aigner, L., and Schwartz-Bloom, R. D. (1995) Oncogene 2, 371–381.
64. Knüsel, B., and Hefti, F. (1992) J. Neurochem. 59, 1987–1996.