

GABAergic stimulation switches from enhancing to repressing BDNF expression in rat hippocampal neurons during maturation in vitro

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SUMMARY

γ -aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the adult mammalian central nervous system. However, GABA depolarizes immature rat hippocampal neurons and increases intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$). Here we show, that GABA and the GABA_A receptor agonist muscimol induce c-Fos immunoreactivity and increase *BDNF* mRNA expression in embryonic hippocampal neurons cultured for 5 days. In contrast, after 3 weeks in culture, GABA and muscimol failed to induce c-fos and *BDNF* expression. Fura-2 fluorescence microscopy revealed that muscimol produces a dihydropyridine-sensitive transient increase in $[\text{Ca}^{2+}]_i$, comparable to the effect of the non-NMDA receptor agonist kainic acid in neurons cultured for 5 days, but not in 3-week-old cultures.

The increase in c-Fos immunoreactivity and *BDNF* mRNA levels by GABA were dependent upon the activation of voltage-gated Ca^{2+} channels, as shown using the L-type specific Ca^{2+} channel blocker nifedipine. The differential regulation of c-fos and *BDNF* expression by GABA and muscimol in developing and mature hippocampal neurons is due to a switch in the ability of GABA_A receptors to activate voltage-gated Ca^{2+} channels. These observations support the hypothesis that GABA might have neurotrophic effects on embryonic or perinatal hippocampal neurons, which are mediated by BDNF.

Key words: neurotrophins, GABA_A receptors, c-fos, Ca^{2+} channels, fura-2, hippocampus, rat

INTRODUCTION

Brain-derived neurotrophic factor (*BDNF*) is a member of the neurotrophin gene family (Barde et al., 1982; Leibrock et al., 1989), which to date comprises *BDNF*, nerve growth factor (*NGF*), neurotrophin-3, neurotrophin-4/5 and neurotrophin-6 (Barde, 1990; Snider, 1994; Götz et al., 1994). In vitro and in vivo studies have established that BDNF plays an important role in controlling neuronal survival and differentiation in the peripheral nervous system (Barde, 1990; Davies, 1994; Ernfors et al., 1994; Jones et al., 1994). In the brain, *BDNF* mRNA is expressed in virtually all regions, the highest levels being found in the hippocampus and cerebral cortex (Hofer et al., 1990). However, the function of *BDNF* is less clear in the brain, although BDNF has been shown to promote the survival of cultured dopaminergic neurons from the substantia nigra (Hyman et al., 1991), cultured cerebellar granule cells (Lindholm et al., 1993) and cortical neurons (Gosh et al., 1994). Recently, it has been demonstrated that BDNF regulates neuropeptide expression in interneurons of various brain areas, including neocortex and hippocampus (Jones et al., 1994; Nawa et al., 1994; Croll et al., 1994), suggesting a function for *BDNF* during maturation of the GABAergic system and its maintenance.

GABA acts as the main inhibitory neurotransmitter at central synapses in adult mammals. However, it depolarizes various types of developing neurons including spinal (Wu et al., 1992; Reichling et al., 1994), cerebellar (Connor et al., 1987), cortical (Yuste and Katz, 1991) and hippocampal neurons (Mueller et al., 1984; Ben-Ari et al., 1989; Cherubini et al., 1990; Hosokawa et al., 1994). In the developing rat hippocampus, GABA is responsible for the expression of so-called 'giant depolarizing potentials' (GDPs) in immature CA3 pyramidal neurons (Ben-Ari et al., 1989). These bicuculline-sensitive potentials are expressed only during early postnatal life (Ben-Ari et al., 1989). As a consequence of its depolarizing action, stimulation by GABA leads to a rise in $[\text{Ca}^{2+}]_i$, as shown for developing cortical neurons using slice preparation (Yuste and Katz, 1991; Lin et al., 1994), and in cultured hippocampal neurons (Segal, 1993).

In neurons, the expression of several immediate early genes, such as the proto-oncogene c-fos, is regulated by neuronal activity (Morgan and Curran, 1986; Sheng and Greenberg, 1990). In PC12 cells, depolarization by high KCl concentrations leads to an increase in c-fos expression, which is dependent on Ca^{2+} influx (Morgan and Curran, 1986). Moreover, both synaptic activation of NMDA receptors and seizures up-regulate c-fos expression in the rat hippocampus in

vivo (Morgan et al., 1987; Cole et al., 1989). Recently, evidence has been provided that distinct Ca^{2+} signalling pathways might be employed for the up-regulation of *c-fos*, depending upon whether Ca^{2+} enters the cells via voltage-gated Ca^{2+} channels or NMDA receptors (Bading et al., 1993).

Physiological and pathophysiological neuronal activity also increases the mRNA expression of two members of the neurotrophin gene family, *NGF* and *BDNF*, in hippocampal neurons in vitro and in vivo (Gall and Isackson, 1989; Zafra et al., 1990, 1991; Castrén et al., 1993; Lindefors et al., 1992; Berzaghi et al., 1993). Glutamatergic stimulation via non-NMDA and NMDA receptors as well as cholinergic activation via muscarinic receptors rapidly increases *BDNF* mRNA in the rat hippocampus (Zafra et al., 1990, 1991; Berzaghi et al., 1993). The increase in *BDNF* mRNA has been shown to be Ca^{2+} dependent and can be blocked by Ca^{2+} /calmodulin inhibitors (Zafra et al., 1992). In contrast, intraperitoneal injection of the GABA_A receptor agonist, muscimol reduces *BDNF* mRNA in adult rat hippocampal neurons (Zafra et al., 1991). On this basis, it has been suggested, that the interplay between excitatory and inhibitory activity determines the levels of *BDNF* expression (Zafra et al., 1991).

Given the depolarizing effect of GABA on developing hippocampal neurons, in the present study we have examined whether, in contrast to the adult situation, GABA is able to up-regulate the expression of *c-fos* and *BDNF* in developing neurons. We show that GABA and muscimol acting via GABA_A receptors have a differential effect on *BDNF* and *c-fos* expression in developing and mature cultured hippocampal neurons. Stimulation of *c-fos* and *BDNF* expression by GABA or muscimol in developing hippocampal neurons could be attributed to the ability of GABA_A receptors to activate voltage-gated Ca^{2+} channels, which is lost during maturation. These results provide a rational basis for the hypothesis that GABA might exert a neurotrophic effect on rat hippocampal neurons during embryonic and early postnatal development.

MATERIAL AND METHODS

Cell culture

Hippocampal neurons were prepared from E17 rat embryos. Hippocampi were incubated for 20 minutes at 37°C in phosphate-buffered saline (PBS) without Ca^{2+} or Mg^{2+} , containing 10 mM glucose, 1 mg ml⁻¹ bovine serum albumin (Sigma, St. Louis, MO, USA), 1 µg ml⁻¹ DNase (Sigma) and 12 µg ml⁻¹ papain (Sigma), dissociated with a plastic pipette and centrifuged (5 minutes at 1000 revolutions per minute). Cells were resuspended in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Gibco, Paisley, UK). 5×10^5 cells were plated in 35 mm dishes (Falcon, Becton Dickinson, Plymouth, UK) and after 3 hours the medium was changed to defined medium as described previously (Zafra et al., 1990).

Calcium imaging

For measurement of $[\text{Ca}^{2+}]_i$ (Grynkiewicz et al., 1985) hippocampal neurons were cultured in coverglass chambers (Nunc, Wiesbaden, FRG). Cells were loaded for 40 minutes (37°C, 10% CO_2) with 2 µM fura-2/AM (Calbiochem, Bad Soden, FRG, 1 mM stock dissolved in DMSO/10% pluronic F-127, Molecular Probes, Eugene, OR, USA), rinsed and incubated in fresh culture medium for 10 minutes prior to the measurement. During $[\text{Ca}^{2+}]_i$ imaging, cells were kept in medium consisting of 142 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2 , 0.8 mM

MgSO_4 , 1 mM NaH_2PO_4 , 5 mM Glucose, 25 mM Hepes and 0.1% bovine serum albumin, adjusted to pH 7.4. Cells were visualized with a Zeiss Fluor 40×/1.30 oil objective using an inverted microscope (Axiovert 100, Zeiss, Jena, FRG). Fluorescence was determined at the

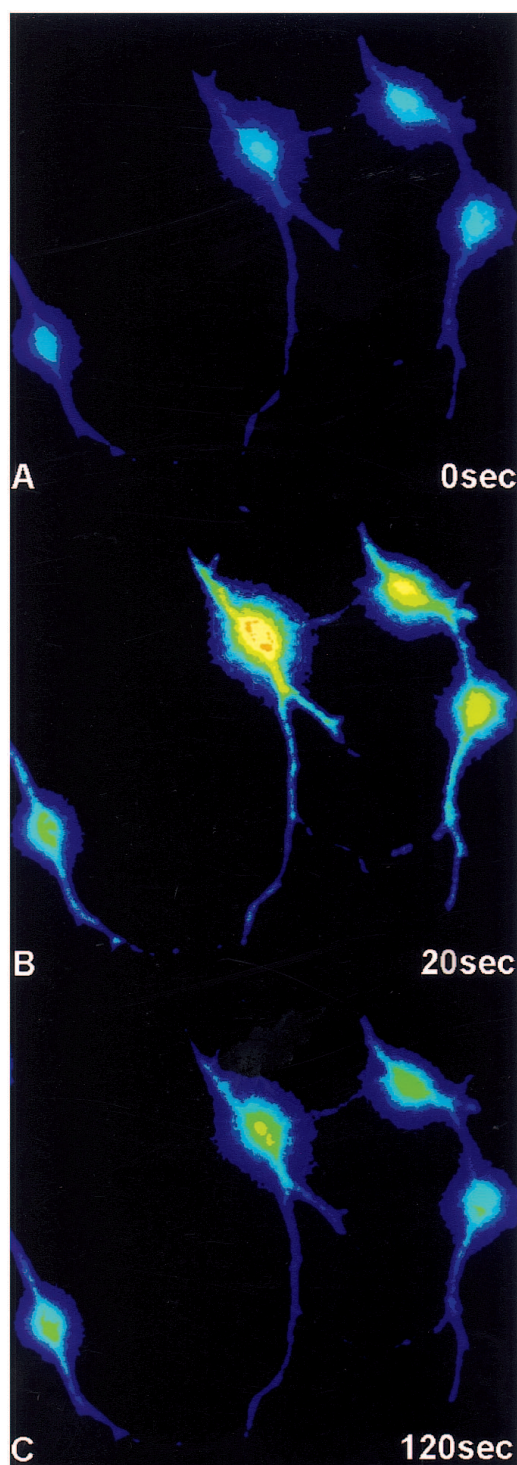
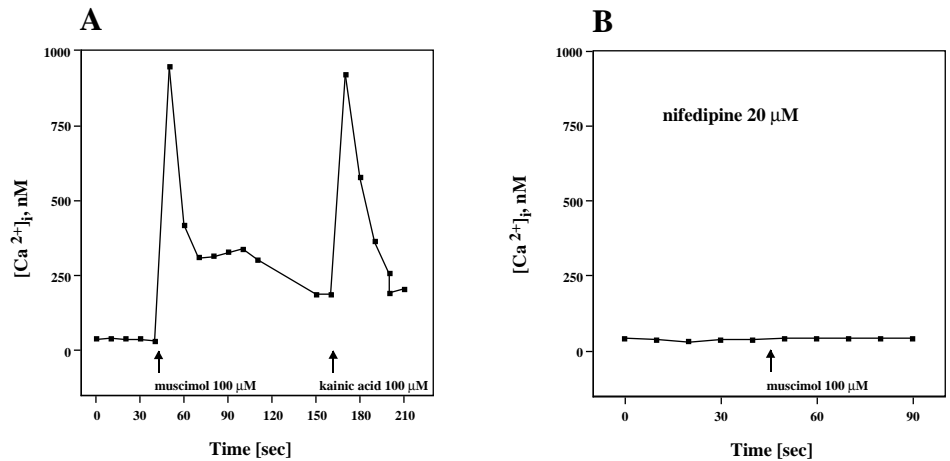


Fig. 1. GABA_A receptor stimulation induces a transient $[\text{Ca}^{2+}]_i$ increase in immature hippocampal neurons. (A) Ratio image of neurons which were cultured for 5 days. (B) Ratio image of these cells stimulated for 20 seconds with 1 µM GABA. (C) After 120 seconds $[\text{Ca}^{2+}]_i$ had almost recovered to basal levels. The sequence shows a uniform response in all neurons. Colour scale from dark blue (50 nM) to red (400 nM).

Fig. 2. GABA_A receptor stimulation activates voltage-gated Ca²⁺ channels in immature hippocampal neurons. (A) 100 μ M muscimol and 100 μ M kainic acid increase [Ca²⁺]_i to a similar extent in neurons cultured for 5 days. (B) Pretreatment with 20 μ M nifedipine (15 minutes prior to stimulation) completely blocked the Ca²⁺ increase induced by muscimol.



excitation wavelengths of 340 and 380 nm with an ICCD camera (C2400-87, Hamamatsu, Joko-cho, Japan) and images were processed with the Argus 50/CA software (Hamamatsu) to calculate the respective fluorescence ratios. Corresponding [Ca²⁺]_i was estimated with the equation $[Ca^{2+}]_i = K_d (R - R_{min}) / (R_{max} - R) S_{f2}/S_{b2}$, where K_d is the dissociation constant for fura-2/Ca²⁺ (224 nM), R the determined ratio, R_{min} and R_{max} the ratios at zero and saturating [Ca²⁺]_i, respectively, and S_{f2}/S_{b2} the ratio of excitation efficiencies for free and bound fura-2 at 380 nm (Grynkiewicz et al., 1985).

c-Fos immunocytochemistry

Cultured hippocampal neurons were treated for 3 hours with 50 μ M GABA, 25 μ M muscimol or 25 μ M kainic acid. After fixation for 15 minutes in 4% paraformaldehyde in PBS, cells were rinsed with PBS and incubated for 30 minutes in TBS containing 0.5% Triton X-100 and 3% normal goat serum (Serva, Heidelberg, FRG). Cells were then incubated overnight at 4°C with polyclonal antibodies against c-Fos (1:2000; sc-52, Santa Cruz Biotechnology, CA, USA). Subsequently, cells were rinsed and incubated for 1 hour with a solution of anti-rabbit biotinylated antibodies (1:100; Vector Labs, Burlingame, CA, USA) followed by incubation with an avidin-biotin-horseradish peroxidase complex (1:100; Vector Labs) for 1 hour. Staining was developed with a TBS solution containing 0.05% diaminobenzidine (Sigma) and 0.01% hydrogen peroxide.

RNA analysis

Total RNA was extracted by the guanidinium thiocyanate method (Chomczynski and Sacchi, 1987), glyoxylated, fractionated on a 1% agarose gel and transferred to Hybond N filters (Amersham, Braunschweig, FRG). Filters were hybridized in a solution consisting of 50% deionized formamide, 6× SSC, 5× Denhardt's, 0.5% SDS, 50 mM NaH₂PO₄ (pH 7.0), 5 mM EDTA and 20 μ g ml⁻¹ salmon sperm DNA at 65.5°C to ³²P-labelled mouse *BDNF* or β -actin cRNA probes. Filters were washed twice for 10 minutes in 2× SSC, 0.1% SDS at 65.5°C and for 15 minutes in 0.2× SSC, 0.1% SDS at 72°C. Subsequently, filters were exposed to an imaging plate (Fuji Type BAS-III S, Fuji, Japan) or X-ray film (Fuji) for 24 hours and signals were analysed with a scanning device (Fujix Bas 1000). Two *BDNF* transcripts were detected at 4.2 and 1.6 kb. Both transcripts were regulated in the same manner. For quantification, the levels of both *BDNF* transcripts were normalized to the amount of β -actin mRNA per lane. The levels of β -actin mRNA were not changed by any of the experimental procedures.

Drugs

γ -aminobutyric acid, muscimol, kainic acid and bicuculline were obtained from Sigma, nifedipine from Biomol (Hamburg, FRG).

RESULTS

Increase in [Ca²⁺]_i in immature hippocampal neurons by GABA and muscimol

The effect of GABAergic stimulation on [Ca²⁺]_i in embryonic hippocampal neurons was studied by fura-2 fluorescence microscopy, 5 days after plating. The neurons had a basal

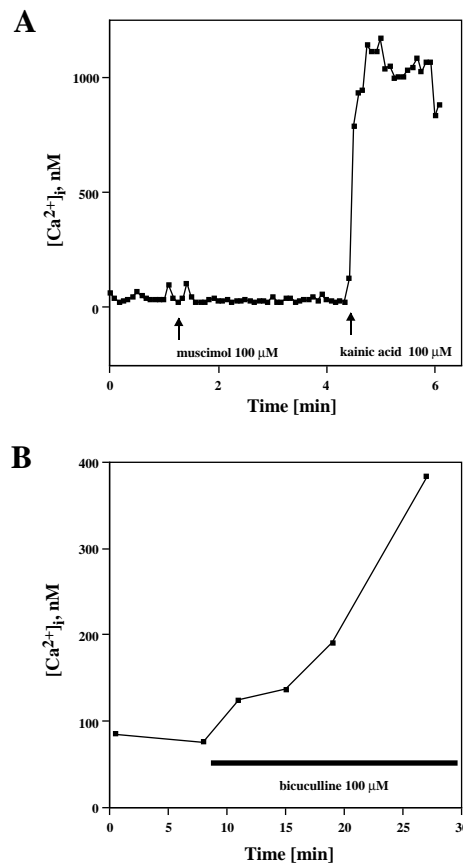


Fig. 3. GABA_A receptor activation produces only a minor rise in [Ca²⁺]_i, whereas GABA_A receptor blockade results in a high elevation of [Ca²⁺]_i in neurons cultured for 3 weeks. (A) 100 μ M muscimol did not induce an increase in [Ca²⁺]_i, whereas 100 μ M kainic acid produced a large response. (B) Treatment with 100 μ M bicuculline, a GABA_A receptor blocker led to a slow increase in [Ca²⁺]_i.

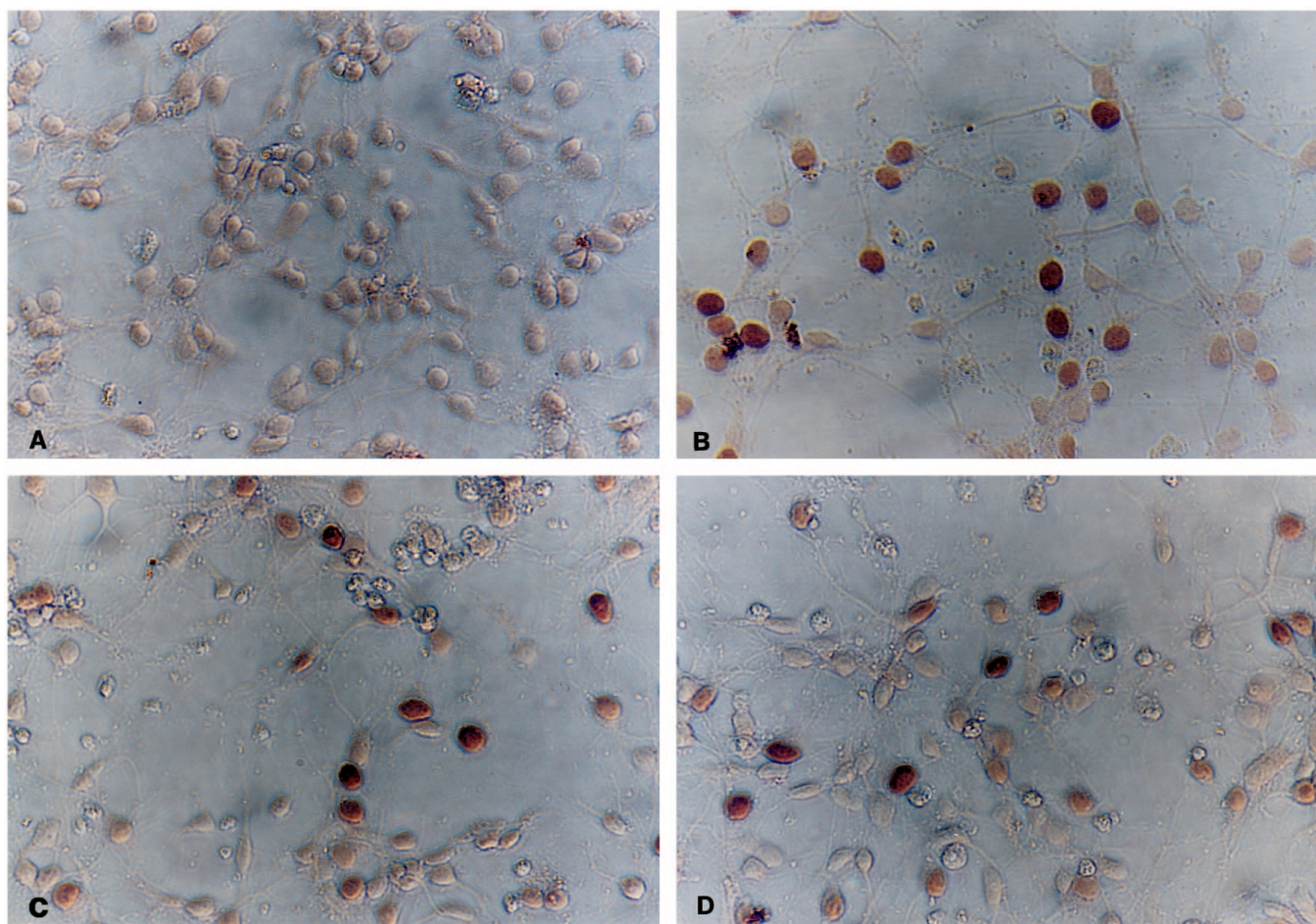


Fig. 4. Induction of c-Fos immunoreactivity by GABA_A receptor activation in immature hippocampal neurons. Neurons were cultured for 5 days and treated for 3 hours as indicated. (A) Control. (B) 50 μ M GABA. (C) 25 μ M muscimol. (D) 25 μ M kainic acid. An induction of c-Fos immunoreactivity is observed in response to both GABA_A receptor agonists and kainic acid.

[Ca²⁺]_i of 51 nM \pm 24 (s.d.; n >15). As shown in Fig. 1, virtually all neurons responded to 1 μ M GABA with a transient increase in [Ca²⁺]_i, peaking at 197 nM \pm 119 (n =21). Decreasing the concentration of GABA down to 100 nM still induced a significant [Ca²⁺]_i elevation (67 nM \pm 20; n =21), but the responsiveness varied markedly within a population of immature hippocampal neurons, many of them showing no change in [Ca²⁺]_i at this dose (not shown). 100 μ M bicuculline completely blocked the increase in [Ca²⁺]_i by GABA providing evidence for the involvement of GABA_A receptor activation (not shown). Moreover, the effect of GABA was mimicked by muscimol. The response to muscimol at the maximally effective concentration of 100 μ M peaked at 867 nM \pm 105, comparable to that elicited by 100 μ M kainic acid (621 nM \pm 278) (Fig. 2A). Pretreatment of the cells with 20 μ M nifedipine completely abolished the effect of muscimol on [Ca²⁺]_i, suggesting that the increase was mainly due to Ca²⁺ influx through L-type Ca²⁺ channels (Fig. 2B). Neurons, cultured for 15 days, had a slightly higher basal [Ca²⁺]_i (108 nM \pm 40) than the cells cultured for 5 days. Moreover, in the mature neurons oscillations in [Ca²⁺]_i were observed, indicating spontaneous synaptic activity in these cells (not shown). At this stage of development, however, treatment with 100 μ M muscimol had

only a minor effect on [Ca²⁺]_i (146 nM \pm 70), whereas 100 μ M kainic acid evoked a very prominent response (582 nM \pm 345) in these neurons, which recovered much slower than in neurons cultured for 5 days, indicating an increase in glutamate responsiveness. After 3 weeks in culture, application of muscimol had still a modest effect on [Ca²⁺]_i, raising [Ca²⁺]_i from 134 nM \pm 80 to 223 nM \pm 107 (Fig. 3A). 100 μ M bicuculline, however, induced a slow rise in [Ca²⁺]_i suggesting that endogenously released GABA, in fact, mediated inhibition (Fig. 3B). This indicates that the neurons had undergone a specific change in the Ca²⁺ response to GABA_A receptor activation.

GABA_A receptor activation induces c-Fos immunoreactivity and *BDNF* mRNA expression in immature hippocampal neurons

An increase in [Ca²⁺]_i is known to activate a number of genes including the proto-oncogene *c-fos* (Morgan and Curran, 1986). Therefore we studied whether the [Ca²⁺]_i rise induced by GABA_A receptor stimulation in the immature hippocampal neurons would activate immediate early genes such as *c-fos*. 5 days after plating, neurons were treated for 3 hours with 50 μ M GABA, 25 μ M muscimol or 25 μ M kainic acid. All of these treatments dramatically increased c-Fos immunoreactivity,

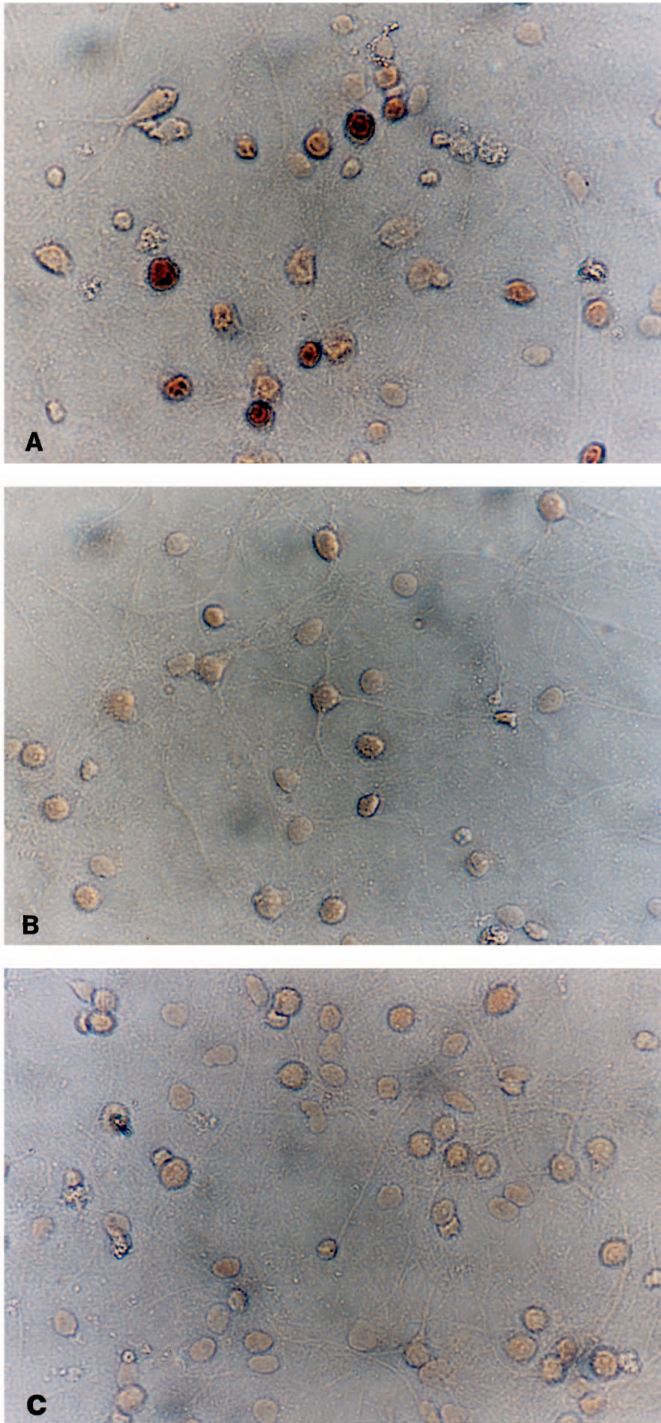


Fig. 5. The effect of GABA_A receptor activation on c-Fos immunoreactivity is blocked by 100 μ M bicuculline or 20 μ M nifedipine. Neurons were cultured for 5 days. Bicuculline or nifedipine treatment started 15 minutes before the stimulation with GABA. (A) 50 μ M GABA. (B) 100 μ M bicuculline + 50 μ M GABA. (C) 20 μ M nifedipine + 50 μ M GABA.

when compared to control cultures (Fig. 4). 100 μ M bicuculline blocked the induction of *c-fos* by GABA or muscimol (Fig. 5B). Moreover, when the neurons were pretreated with 20 μ M nifedipine, GABA and muscimol failed to induce *c-fos*,

showing that Ca^{2+} influx was indeed mediating the observed increase (Fig. 5C). In contrast to the effect of GABA_A receptor activation on *c-fos* expression in immature neurons, there was no induction of c-Fos immunoreactivity by GABA or muscimol 3 weeks after plating (Fig. 6). Kainic acid, however, clearly induced *c-fos* expression (Fig. 6D).

In addition to *c-fos*, GABA_A receptor activation might activate other genes such as *BDNF*, which is expressed in embryonic hippocampal neurons. Stimulation of hippocampal neurons 5 days after plating with 50 μ M GABA for 3 hours, resulted in an approximate 3-fold increase in the steady state levels of *BDNF* mRNA (Fig. 7A,D). The induction of *BDNF* mRNA by GABA was blocked by bicuculline and mimicked by muscimol (Fig. 7D) providing evidence that the effect was specifically mediated via GABA_A receptors. A very marked induction was already seen with a concentration of 1 μ M muscimol and was maximal at 25 μ M (Fig. 7B). *BDNF* mRNA expression increased within 1 hour, reached its maximum after 3 hours and remained above basal levels for more than 12 hours (Fig. 7C). *BDNF* mRNA expression was also up-regulated by 25 μ M kainic acid (Fig. 7D). As was observed for *c-fos*, the increase in *BDNF* mRNA by GABA and muscimol in these neurons was completely abrogated by 20 μ M nifedipine, demonstrating that Ca^{2+} influx mediated the effect of GABA_A receptor activation on *BDNF* expression (Fig. 7D). As shown in Fig. 8, after 2 weeks in culture, muscimol failed to induce *BDNF* mRNA expression. In contrast, kainic acid clearly up-regulated *BDNF* mRNA levels. Moreover, treatment with 100 μ M bicuculline dramatically increased *BDNF* mRNA levels in these cultures, in contrast to immature neurons, suggesting that endogenously released GABA acting via GABA_A receptors had a suppressive effect upon *BDNF* mRNA at this stage (Fig. 8).

DISCUSSION

The aim of this study was to investigate the effect of GABA_A receptor activation on gene expression in immature rat hippocampal neurons. Since it was shown that GABA depolarizes immature hippocampal neurons (Ben-Ari et al., 1989; Cherubini et al., 1990; Fiszman et al., 1990) leading to Ca^{2+} influx (Segal, 1993), we have examined here the effect of GABA on the expression of two genes, *c-fos* and *BDNF*, which are regulated in an activity-dependent manner in hippocampal neurons (Morgan and Curran, 1986; Zafra et al., 1990). The main finding of this study was the observation that immature hippocampal neurons respond to GABA_A receptor activation by a transient Ca^{2+} influx, which up-regulates c-Fos immunoreactivity and *BDNF* mRNA expression. The causal link between the Ca^{2+} influx and the increased *c-fos* and *BDNF* expression was demonstrated by the finding that treatment with nifedipine, which blocks L-type voltage-gated Ca^{2+} channels, completely abolished the transient $[\text{Ca}^{2+}]_i$ rise and also abrogated both the increase in c-Fos immunoreactivity and the up-regulation of *BDNF* mRNA. In the course of the maturation of these neurons, however, Ca^{2+} transients and also the increase in c-Fos immunoreactivity and *BDNF* mRNA expression in response to GABA_A receptor activation disappear. Moreover, as shown by bicuculline, endogenously released GABA suppressed *BDNF* mRNA expression at this stage. Muscimol,

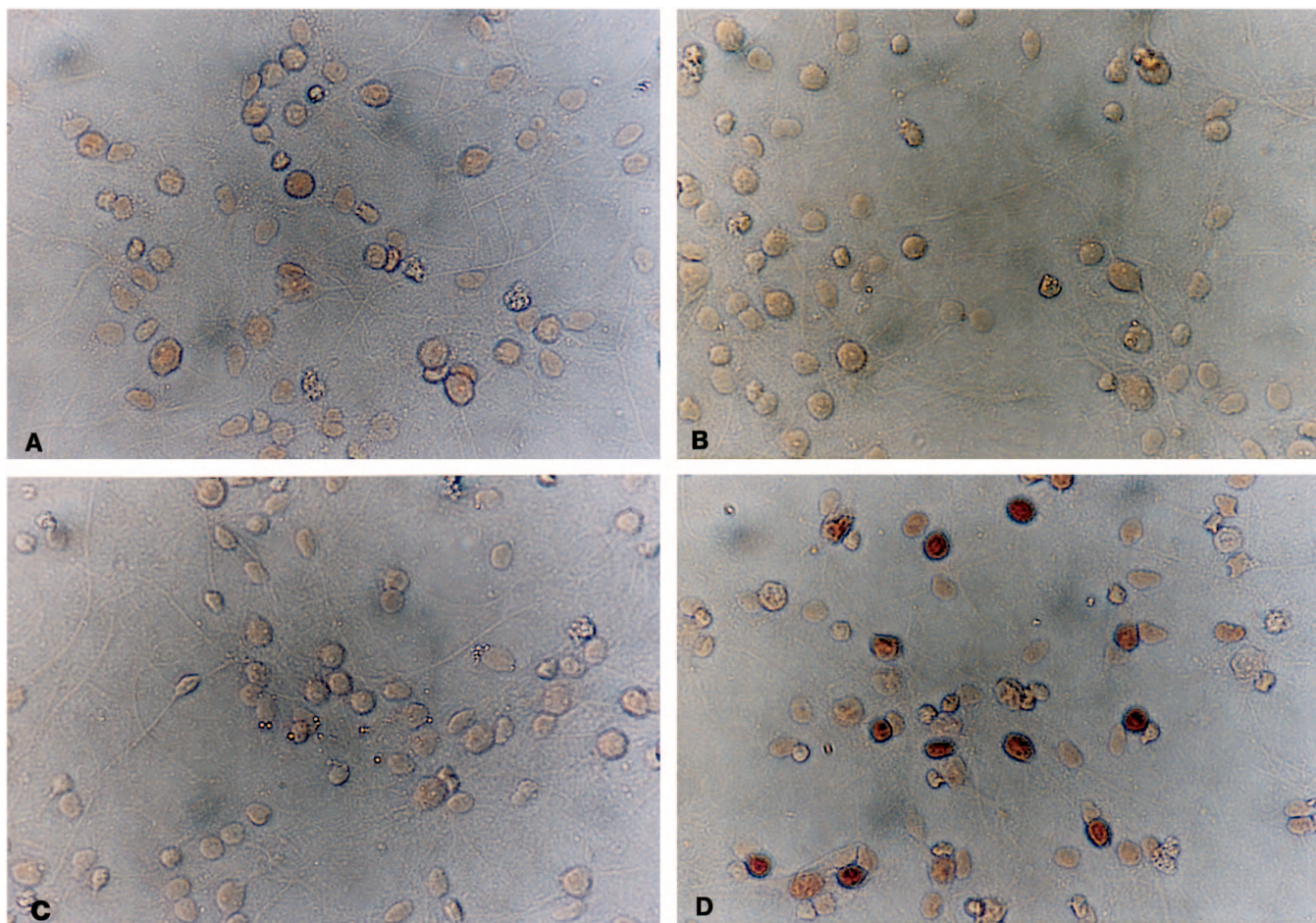


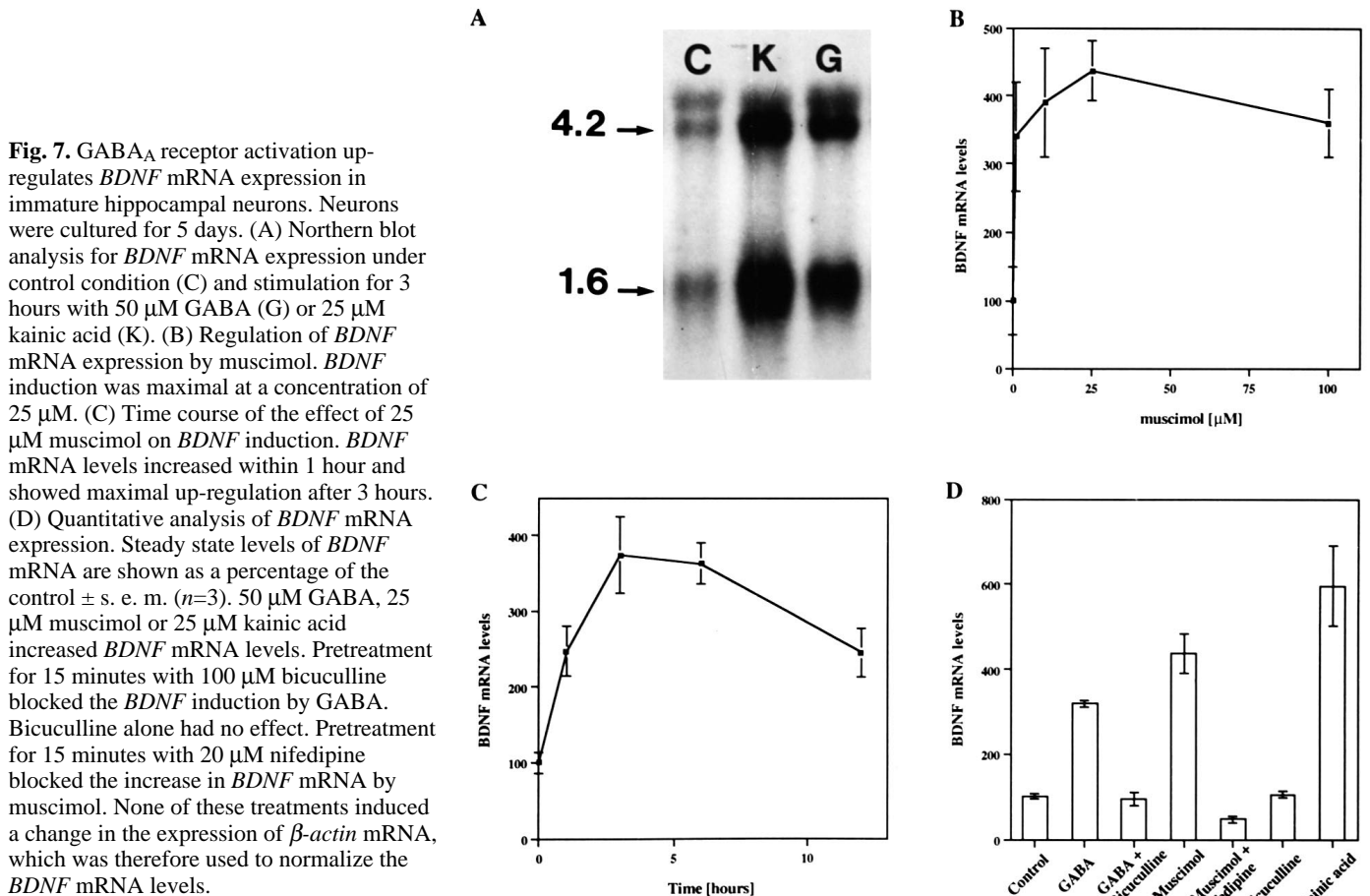
Fig. 6. GABA_A receptor activation fails to induce c-Fos immunoreactivity in mature hippocampal neurons. Neurons were cultured for 3 weeks. (A) Control. (B) 50 μ M GABA (C) 25 μ M muscimol. (D) 25 μ M kainic acid.

however, failed to reduce *BDNF* mRNA expression below the basal level, in contrast to observations made in vivo (Zafra et al., 1991). However, *BDNF* expression might be already in a maximally repressed state under control conditions due to the endogenous GABAergic activity in mature hippocampal cultures. Therefore, GABA_A receptor activation differentially regulates the expression of *c-fos* and *BDNF* in immature and mature neurons, due to a switch in the ability of GABA to activate Ca²⁺ channels during development.

In adult neurons GABA_A receptor activation leads to hyperpolarization (Mody et al., 1994), thereby reducing Ca²⁺ currents. During development, however GABA depolarizes various types of neurons such as spinal (Wu et al., 1992; Reichling et al., 1994), cerebellar (Connor et al., 1987), cortical (Yuste and Katz, 1991) and hippocampal neurons (Mueller et al., 1984; Ben-Ari et al., 1989; Cherubini et al., 1990; Hosokawa et al., 1994) leading to the activation of voltage-gated Ca²⁺ channels. Upon maturation of these neurons, however, GABA loses the ability to depolarize these cells and Ca²⁺ transients are no longer observed (Ben-Ari et al., 1989; Lin et al., 1994; Wang et al., 1994). These effects are clearly GABA_A receptor mediated as they are mimicked by the GABA_A receptor agonist muscimol, but not by the GABA_B receptor agonist baclofen (Lin et al., 1994; Reichling et al.,

1994). Since muscimol precisely mimicked and bicuculline completely blocked the increase in c-Fos immunoreactivity and *BDNF* mRNA expression induced by GABA in immature hippocampal neurons, we assume, that these effects of GABA are mediated by GABA_A but not by GABA_B receptors. The GABA_A receptor complex controls Cl⁻ conductance, leading to a Cl⁻-dependent inward current after channel opening (Mody et al., 1994). It has been suggested that a difference in the Cl⁻ gradient due to a reversed Cl⁻ membrane transport might be responsible for the depolarizing action by GABA in immature neurons (Misgeld et al., 1986; Cherubini et al., 1991; Reichling et al., 1994). Interestingly, the subunit composition of GABA_A receptors in developing and in adult brain seems to differ, which suggests that the embryonic and early postnatal GABA_A receptor complexes may have different properties compared to adult ones (Poulter et al., 1992; Fritschy et al., 1994).

Activation of GABA_A receptors may be of functional importance during neuronal maturation and differentiation. Embryonic hippocampal neurons express GABA_A receptors as early as day E15 (Poulter et al., 1992), as shown by in situ hybridization for various GABA_A receptor subunits. Moreover, these subunits form functional receptors, since E17 embryonic hippocampal neurons respond to muscimol at low nanomolar concentrations (Fiszman et al., 1990). In the early postnatal



hippocampus, glutamatergic synaptic transmission seems to be low (Hosokawa et al., 1994). Excitatory potentials at this stage might instead be mediated via GABAergic activity. Ben-Ari and colleagues previously described the occurrence of so-called 'giant depolarizing potentials' (GDPs) in the CA3 subfield of the rat hippocampus in vitro (Ben-Ari et al., 1989; Cherubini et al., 1990; Hosokawa et al., 1994). These GDPs are likely to be mediated by GABA_A receptor activation as they are blocked by bicuculline, and the corresponding currents reverse at the same potential as produced by exogenously applied GABA. (Ben-Ari et al., 1989). These potentials can be observed only until the postnatal day 7, after which the first inhibitory potentials appear, indicative of the maturation of the GABAergic system (Ben-Ari et al., 1989).

BDNF mRNA is highly expressed in the adult rat hippocampus. Moreover, the level of expression is developmentally regulated (Maisonpierre et al., 1990) and depends upon cholinergic and glutamatergic afferent inputs, such as those arising from the medial septum or the entorhinal cortex (Lindfors et al., 1992; Berzaghi et al., 1993). Systemic injection of kainic acid or intraventricular injection of NMDA rapidly up-regulate *BDNF* mRNA expression in vivo (Zafra et al., 1990; Berzaghi et al., 1993). In the developing hippocampus, however, kainic acid does not increase *BDNF* expression before postnatal day 15, whereas NMDA readily up-regulates *BDNF* mRNA synthesis at earlier ages (Dugich-Djordjevic et al., 1992; Berzaghi et al., 1993). This study demonstrates that

in hippocampal neurons, GABA_A receptor activation up- or down-regulates *BDNF* mRNA expression, depending upon the developmental stage of the neurons. It can be speculated,

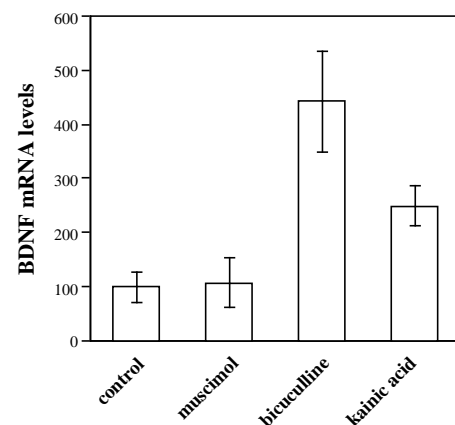


Fig. 8. GABA_A receptor activation suppresses *BDNF* mRNA expression in mature hippocampal neurons. Neurons were cultured for 15 days. Steady state levels of *BDNF* mRNA are shown as a percentage of the control \pm s. e. m. ($n=3$). In contrast to 5 days old cells, 25 μ M muscimol did not increase *BDNF* mRNA expression. 100 μ M bicuculline, however, greatly up-regulated *BDNF* mRNA steady state levels. *BDNF* mRNA levels were normalized to the amount of β -actin mRNA.

therefore, that GABAergic activity might enhance *BDNF* mRNA expression at early stages and thus contribute to the low level of *BDNF* mRNA during early development (Maisonpierre et al., 1990). With the establishment of mature GABAergic synapses mediating inhibition, GABA, however, starts to down-regulate *BDNF* mRNA levels as has been demonstrated in the adult (Zafra et al., 1991).

It has been suggested that GABA exerts a neurotrophic effect on embryonic neurons (Cherubini et al., 1991; Barbin et al., 1993). For example, GABA influences the expression of GABA receptors in rat cerebellar granule neurons (Meier et al., 1987). Moreover, GABA stimulates cytokinesis in early spinal neurons (Behar et al., 1993). Interestingly, it has been reported that blocking the endogenous GABAergic activity in cultured hippocampal neurons by bicuculline leads to reduction of neurite arborization (Barbin et al., 1993). As shown here, GABA regulates the expression of *c-fos*, which is known to enhance transcription of different genes (Sheng and Greenberg, 1990) and to mediate the increase in Ca^{2+} currents in PC12 cells by NGF (Cavalié et al., 1994). The regulation of *BDNF* expression by GABA, might directly regulate neuronal differentiation and survival.

Hippocampal neurons express the BDNF receptor TrkB and respond to BDNF in vitro and in vivo (Berninger et al., 1993; Ip et al., 1993; Marsh et al., 1993; Ernfors et al., 1994; Jones et al., 1994). In cultured hippocampal neurons BDNF induces TrkB autophosphorylation on tyrosine residues, thereby eliciting downstream signalling events like MAP kinase activation and $[\text{Ca}^{2+}]_i$ increase (Berninger et al., 1993; Marsh et al., 1993). Moreover, BDNF regulates neuropeptide and NT-3 mRNA expression in hippocampal neurons in vivo (Croll et al., 1994; Nawa et al., 1994; Lindholm et al., 1995). Although the hippocampal formation seems to be largely intact in mice deficient for the *BDNF* gene, the expression of *calbindin*, *parvalbumin* and *neuropeptide Y* in GABAergic interneurons is reduced, suggesting that these interneurons might be affected (Jones et al., 1994). Therefore, BDNF may be involved in the maturation of the GABAergic system in the hippocampus. These results, together with our observation that GABA up-regulates *BDNF* in embryonic hippocampal neurons, suggest an important interaction between BDNF and the neurotransmitter GABA in early brain development. BDNF induced by GABA might stabilize synaptic contacts, promote differentiation and, in conjunction to other growth factors, support neuronal survival.

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