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Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors

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The mRNAs of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) exhibit a similar, though not identical, regional and cellular distribution in the rodent brain. *In situ* hybridization experiments have shown that BDNF, like NGF, is predominantly expressed by neurons. The neuronal localization of the mRNAs of these two neurotrophic molecules raised the question as to whether neuronal activity might be involved in the regulation of their synthesis. After we had demonstrated that depolarization with high potassium (50 mM) resulted in an increase in the levels of both BDNF and NGF mRNAs in cultures of hippocampal neurons, we investigated the effect of a large number of transmitter substances. Kainic acid, a glutamate receptor agonist, was by far the most effective in increasing BDNF and NGF mRNA levels in the neurons, but neither *N*-methyl-D-aspartic acid (NMDA) nor inhibitors of the NMDA glutamate receptors had any effect. However, the kainic acid mediated increase was blocked by antagonists of non-NMDA receptors. Kainic acid also elevated levels of BDNF and NGF mRNAs in rat hippocampus and cortex *in vivo*. These results suggest that the synthesis of these two neurotrophic factors in the brain is regulated by neuronal activity via non-NMDA glutamate receptors.

Key words: BDNF/hippocampus/kainic acid receptors/ mRNA regulation/NGF

Introduction

BDNF and NGF are members of a gene family with ~50% amino acid identity (Leibrock *et al.*, 1989). The molecules exhibit strictly conserved domains which contain the six cysteine residues involved in the stabilization of the three dimensional structure of NGF and BDNF, a prerequisite for their biological activity. However, there are also variable domains in BDNF and NGF which determine their different neuronal specificity (Linsay *et al.*, 1985; Johnson *et al.*, 1986; Barde, 1989; Rodriguez-Tebar *et al.*, 1989). Moreover, the difference between these two neurotrophic molecules is also indicated by their sites of synthesis. NGF is expressed both in the periphery (Ebendal *et al.*, 1983; Korsching and Thoenen, 1983; Heumann *et al.*, 1984; Shelton and Reichardt, 1984) and in the central nervous system (CNS) (Korsching *et al.*, 1985; Shelton and Reichardt, 1986; Whittemore *et al.*, 1986). The densities of innervation by NGF responsive neurons reflect the levels of NGF in the corresponding target tissues (Korsching and Thoenen, 1983; Heumann *et al.*, 1984; Korsching *et al.*, 1985; Shelton and Reichardt 1984, 1986; Whittemore *et al.*, 1986). In contrast, BDNF is predominantly expressed in the CNS in neurons and the levels of BDNF mRNA are considerably higher than those of NGF mRNA, e.g. ~50-fold in the hippocampus (Hofer *et al.*, 1990). In the peripheral nervous system, NGF is synthesized by various non-neuronal cell types (Bandtlow *et al.*, 1987), whereas in the brain it is mainly localized in neurons, as demonstrated by *in situ* hybridization (Rennert and Heinrich, 1986; Ayer-LeLièvre *et al.*, 1988; Whittemore *et al.*, 1988; Bandtlow *et al.*, 1990). In view of the predominant localization of both BDNF and NGF mRNA in central neurons we asked the question as to whether neuronal activity might be involved in the regulation of the synthesis of these two neurotrophic molecules.

We wish to report that in cultures of hippocampal neurons depolarization with high potassium resulted in an increase in the levels of both BDNF and NGF mRNAs. Of a large number of transmitter substances investigated, kainic acid, a glutamate receptor agonist, proved to be the most effective; NMDA had no effect on the BDNF and NGF mRNA levels. The results obtained in cultures of hippocampal neurons were confirmed by systemic administration of kainic acid *in vivo*.

Results

Regulation of BDNF and NGF mRNA in neuronal cultures of rat hippocampus

A preliminary series of experiments was performed with neuronal cultures of embryonic (E17) rat hippocampus. As shown in Figure 1a, depolarization of the hippocampal neurons with high (50 mM) potassium resulted in an increase in BDNF mRNA. Maximal levels were reached between 3 and 6 h after increasing the potassium concentration. The potassium mediated increase in BDNF mRNA could be inhibited by omitting calcium ions from the medium and it was also reduced by inhibiting calcium influx by the calcium channel blocker, nifedipin (Figure 1b). Moreover, even the basal levels of BDNF mRNA were reduced in the absence of calcium (and presence of 1 mM EGTA) which further emphasizes the importance of calcium in the regulation of BDNF mRNA.

Because the hippocampal cultures used consisted of a mixed population of neurons exhibiting different patterns of transmitter and receptor expression, we studied the effect of various physiological and synthetic receptor agonists on BDNF and NGF mRNA expression. The results presented in Table I show that of all the substances tested, kainic acid, a glutamate receptor agonist (Monaghan *et al.*, 1989), produced by far the largest increase in BDNF mRNA in hippocampal neurons. In contrast, other molecules, such as carbachol (a muscarinic, acetylcholinesterase resistant receptor agonist) and to a lesser extent histamine and

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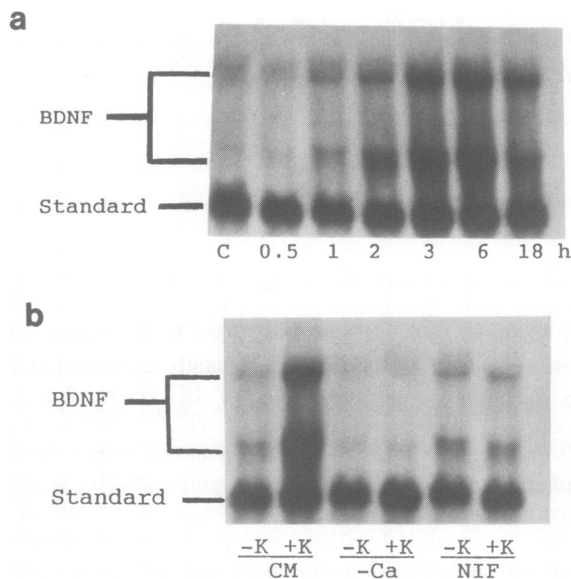

Fig. 1. Effect of depolarization on BDNF mRNA levels in hippocampal neurons. **(a)** Time course of BDNF mRNA expression in primary cultures of hippocampal neurons in the presence of 50 mM KCl. Total cellular RNA was extracted from 0.5×10^6 cells analysed by Northern blot as described in Materials and methods. The two upper bands (4 and 1.5 kb) correspond to BDNF mRNA (Hofer *et al.*, 1990) and the lower (700 bp) to 10 pg of a short BDNF cRNA recovery standard which was added to samples before extraction of RNA. **(b)** Calcium dependency. Neurons were incubated for 3 h in normal culture medium (CM), in a modified medium without calcium containing 1 mM EGTA (−Ca) or in culture medium with 10 μM nifedipin (NIF). 50 mM KCl was added where indicated (+K).

Table 1. Effect of various receptor agonists on BDNF mRNA expression in cultured neurons

Addition	Percentage of control
None	100 ± 5
Carbachol (50 μM)	220 ± 25*
Carbachol (50 μM) + atropine 10 (μM)	85 ± 15
Nicotine (100 μM)	110 ± 7
Histamine (50 μM)	150 ± 12*
Serotonin (100 μM)	95 ± 6
Dopamine (100 μM)	115 ± 7
Norepinephrine (25 μM)	75 ± 10
Substance P (1 μM)	85 ± 9
Somatostatin (1 μM)	110 ± 8
Bradykinin (1 μM)	155 ± 15*
Kainic acid (25 μM)	1365 ± 70**
NMDA (25 μM)	105 ± 10

Hippocampal neurons were incubated for 3 h in the presence of the substances indicated. The effect of NMDA was tested in a magnesium-free medium. RNA was extracted and analysed as described in Materials and methods. Values given are the mean ± SEM of three or more experiments. They were compared with the control value by using Student's *t* test: *$p<0.05$; **$p<0.001$.

bradykinin, slightly though significantly elevated BDNF mRNA (Table I). Because the levels of NGF mRNA in the hippocampal neurons were very low, we established a quantitative polymerase chain reaction (PCR) method suitable for determining changes in NGF mRNA. Using this method we found that, like BDNF mRNA, NGF mRNA levels were also increased by potassium and kainic acid in hippocampal neurons (Figure 2).

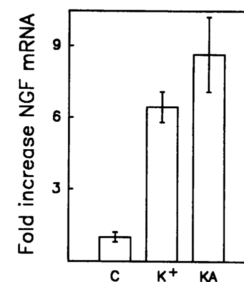

Fig. 2. Increases in NGF mRNA levels in hippocampal neurons by potassium and kainic acid. Neurons were incubated for 3 h in control medium (C) or in the presence of 50 mM KCl (K^+) or 25 μM kainic acid (KA). RNA was extracted and the NGF mRNA levels were determined by the quantitative PCR method as described in Materials and methods. The values are means ± SEM (n = 6).

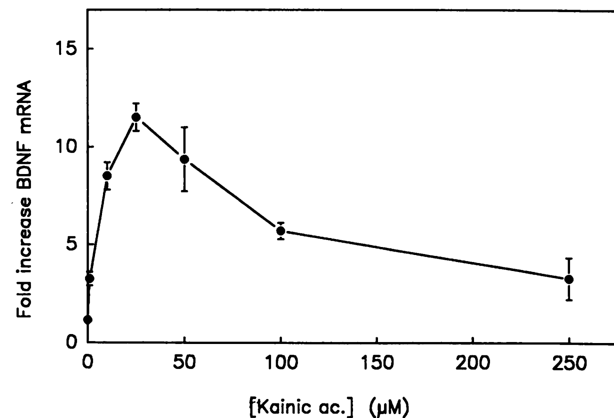

Fig. 3. Dose–response curve for the kainic acid effect. Hippocampal neurons were incubated for 3 h in the presence of various concentrations of kainic acid. RNA was extracted and analysed as described in Materials and methods. Values represent means ± SEM of three experiments.

The maximal increase in BDNF mRNA in the hippocampal neurons was obtained with ~25 μM kainic acid (Figure 3). A further increase in the kainic acid concentration resulted in a decrease of the BDNF mRNA levels, which most probably reflects toxic effects of the high concentrations of kainic acid on hippocampal neurons (Figure 3). Glutamate receptor mediated neurotoxicity has previously been reported (Choi *et al.*, 1987; Rothman and Olney, 1987; Choi, 1988) for various central neurons following the application of analogues of glutamate. However, the concentrations of kainic acid necessary to enhance the expression of BDNF and NGF mRNA in the hippocampal neurons could clearly be separated from the concentrations resulting in neurotoxicity.

To investigate the effects of kainic acid in more detail, we studied whether the increase in BDNF mRNA could be blocked by any of the known glutamate receptor antagonists (Monaghan *et al.*, 1989). Kynurenic acid, a broad spectrum glutamate receptor antagonist, as well as the quinoxalinedione compound, CNQX, a competitive inhibitor of non-NMDA receptors (Monaghan *et al.*, 1989), completely blocked the kainic acid mediated increase in BDNF mRNA in hippocampal neurons (Table II). On the other hand, MK 801, which specifically blocks NMDA receptors, was ineffective in blocking the rise in BDNF mRNA in these neurons. Moreover, NMDA itself did not change BDNF

Table II. Effect of the different glutamate receptors antagonists on the kainic acid induced expression of BDNF mRNA

Addition	Percentage of control
None	100 ± 6
Kainic acid (25 μM)	1250 ± 60*
Kynurenic acid (1 mM)	70 ± 6
Kynurenic acid (1 mM) + kainic acid (25 μM)	84 ± 7
CNQX (10 μM)	109 ± 6
CNQX (10 μM) + kainic acid (25 μM)	105 ± 7
MK-801 (5 μM)	96 ± 5
MK-801 (5 μM) + kainic acid (25 μM)	1130 ± 80*

Primary cultures of hippocampal neurons were incubated for 3 h in the presence of the various antagonists added 15 min prior to kainic acid. RNA was extracted and analysed as indicated in Materials and methods. Values given are the mean ± SEM of three experiments. They were compared with the control value by using Student's t test: $*p<0.001$.

mRNA levels (Table I). Thus kainic acid acts directly via its receptors and the observed effects do not result from the release of endogenous glutamate (which also acts on NMDA receptors). It can, therefore, be concluded that kainic acid *in vitro* elevates BDNF mRNA levels via non-NMDA glutamate receptors.

In vivo experiments

To evaluate the physiological significance of our *in vitro* observations, we studied whether similar mechanisms were also operating *in vivo*. Rats were treated with 12 mg/kg of kainic acid and after various time periods, we determined the changes in BDNF and NGF mRNAs in the hippocampus and cortex by Northern blot analysis. Figure 4 shows that kainic acid elicited an increase in the levels of BDNF and NGF mRNAs in both brain regions. The increase in BDNF mRNA was substantially larger than that of NGF mRNA. The larger increase in BDNF mRNA is particularly noteworthy in view of the fact that the basic levels of BDNF mRNA are ~50 times higher than those of NGF mRNA in the hippocampus (Hofer *et al.*, 1990). Moreover, for as long as 24 h after administration of kainic acid, the mRNA levels remained elevated. The time course of BDNF and NGF mRNAs changes showed that the maximal increase in the hippocampus was reached ~3 h after the administration of kainic acid (Figure 4). Moreover, there is a delay of approximately ~2 h in the increase of both BDNF and NGF mRNAs in the cerebral cortex as compared with the increase in the hippocampus suggesting that the signals leading to an enhanced expression of BDNF and NGF are spreading from the hippocampus to the cortex.

After previous studies had provided evidence that NMDA receptors are involved in the regulation of hippocampal mRNAs (Cole *et al.*, 1989), we then investigated whether the NMDA receptor antagonists MK 801 and ketamine could block the increase in BDNF and NGF mRNAs *in vivo*. However, confirming our results *in vitro*, neither MK 801 nor ketamine inhibited the kainic acid mediated increase in hippocampal BDNF mRNA, although both drugs effectively suppressed seizures resulting from NMDA receptor activation (Figure 5). Most probably the NMDA receptor activation observed in our experiments resulted from endogenous glutamate released by kainic acid (Biziere and Coyle, 1978; McGeer *et al.*, 1978). Likewise, the increase in NGF mRNA in hippocampus after kainic acid treatment

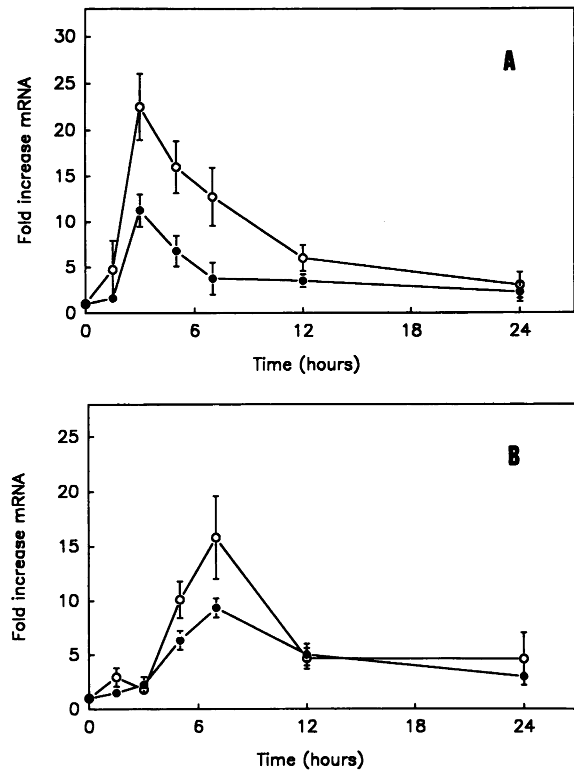

Fig. 4. Time course of increases in BDNF and NGF mRNA levels by kainic acid treatments. Total cellular RNA was extracted and analysed as indicated in Materials and methods from hippocampus (**a**) or cortex (**b**) at the indicated times (in hours) after intraperitoneal injection of kainic acid (12 mg/kg). One single dose of diazepam (10 mg/kg) was injected 90 min after kainic acid. Values corresponding to 1.5 kb BDNF mRNA (○) and 1.3 kb NGF mRNA (●) are shown. Similar increases were observed for the 4 kb BDNF mRNA. Values given represent means ± SEM of three to four experiments.

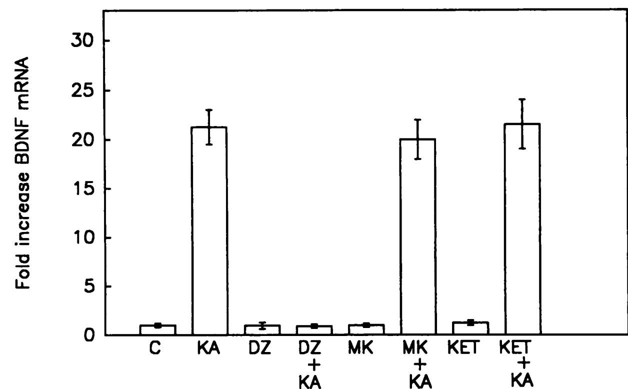

Fig. 5. Effect of anticonvulsants on the kainic acid induced expression of BDNF mRNA. Rats were injected intraperitoneally with diazepam (10 mg/kg) (DZ), MK-801 (2 mg/kg) (MK) or ketamine (40 mg/kg) (KET) 15 min before injection of kainic acid (12 mg/kg) (+KA) or saline (controls). After 3 h hippocampal tissue was used to prepare total RNA as indicated in Materials and methods. Values represent means ± SEM of three experiments.

was neither prevented by MK 801 nor by ketamine (data not shown). Benzodiazepam (valium) treatment of rats prior to kainic acid injections completely blocked the increase in BDNF and NGF mRNAs in the rat hippocampus (Figure 5). Since benzodiazepines are known to enhance the GABA receptor-gated Cl^- currents (Schofield *et al.*, 1987), the

blocking effect of benzodiazepam on the BDNF and NGF mRNA increase most probably resulted from the suppression of neuronal activity via the inhibitory GABAergic system. However, 90 min after the administration of kainic acid (i.e. ~30 min after the start of convulsions) the increase in BDNF and NGF mRNA could no longer be blocked by benzodiazepam, indicating that a relatively short period of augmented activity is sufficient to initiate the cascade of events leading to an increase in the levels of the mRNAs of these two neurotrophic factors.

Discussion

The predominant expression of the mRNAs of BDNF (Hofer *et al.*, 1990) and NGF (Rennert and Heinrich, 1986; Ayer-LeLièvre *et al.*, 1988; Whittemore *et al.*, 1988; Bandtlow *et al.*, 1990) in neurons of the CNS prompted investigations aimed at clarifying whether or not neuronal activity is involved in the regulation of the synthesis of BDNF and NGF. We demonstrated that in neuronal cultures of embryonic rat hippocampus depolarization with high potassium results in an increase of BDNF and NGF mRNA levels. We then analysed the effects of a large number of physiological and synthetic transmitter substances and found that by far the largest increase in both NGF and BDNF mRNA levels was effected by kainic acid. The results obtained *in vitro* were confirmed *in vivo* after systemic administration of kainic acid, a glutamate receptor agonist (Watkins and Olverman, 1987).

Glutamate and structurally related compounds are the main excitatory amino acids (EAA) in the CNS (Cotman *et al.*, 1987; Monaghan *et al.*, 1989). Based upon results of biochemical, pharmacological and electrophysiological studies at least three types of EAA receptors have been identified, namely the kainic acid, the quisqualate acid and the NMDA receptor (Watkins and Olverman, 1987). Whereas specific antagonists for NMDA receptors have been developed, no corresponding compounds are available to distinguish between kainic and quisqualate receptors (Monaghan *et al.*, 1989). This prompted the coining of the term 'non-NMDA receptors'. Non-NMDA receptors mediate fast excitatory responses, whereas NMDA mediates slower responses and appears to play an important role in the mediation of seizure activity, but also in memory processes (Cotman and Iversen, 1987).

Activation of NMDA receptors has previously been shown to increase the expression of some early genes in the hippocampus (Cole *et al.*, 1989). Likewise, limbic seizures were found to elevate NGF mRNA in the rat hippocampus suggesting an involvement of NMDA receptors (Gall and Isackson, 1989). The present results however, provide evidence for a clear dissociation between kainic acid mediated seizures (most probably resulting from the presynaptic release of glutamic acid and subsequent activation of NMDA receptors) and the regulation of NGF and BDNF mRNA. Whereas MK801 completely abolished the kainic acid mediated seizures it did not interfere with the concomitant increase in BDNF and NGF mRNAs.

The increase in BDNF and NGF mRNA by high potassium was dependent on calcium (reduction of the calcium concentration in the culture medium and the calcium channel blocker nifedipin abolished the potassium effect), whereas NMDA, which effectively enhances calcium influx, did not increase BDNF and NGF mRNA levels. This indicates that the effect of high potassium depolarization is not exclusively mediated by calcium influx. We have also observed that the addition of the calcium ionophore, ionomycine or the calcium channel activator, BayK 8644, resulted in a much smaller increase in BDNF mRNA compared with that produced by kainic acid (F.Zafra, D.Lindholm and H.Thoenen, unpublished data). The exact role played by calcium in increasing BDNF and NGF mRNA levels in hippocampal neurons is under investigation. However, the importance of calcium does not reside in the release of glutamate or any other mediator (conventional transmitters, neuropeptides or even macromolecules) released from neurons by depolarization by kainic acid since tetrodotoxin which would block this mechanism of release had no effect on the kainic acid mediated increase in BDNF mRNA (unpublished results). Thus, the observed effects of hippocampal neurons are direct ones mediated via kainic acid receptors.

In contrast to the results obtained with NMDA receptor antagonists, benzodiazepam, which enhances the GABAergic inhibitory functions, blocked the inducing effect of kainic acid *in vivo*. However, if valium was administered 30 min after the beginning of seizures, it no longer interfered with the increase in BDNF and NGF mRNAs which indicates that a relatively short period of augmented activity is sufficient to initiate the cascade of events leading to the initial increase in the hippocampus and the delayed increase in the cerebral cortex. Interestingly, the increases in BDNF and NGF mRNAs in the hippocampus are preceded by an increase in c-*fos* mRNA (data not shown), but we do not know if the two phenomenons are causally related, as has recently been demonstrated, for the increases in c-*fos* and NGF mRNAs in the rat sciatic nerve after lesion and in cultured fibroblasts (Hengerer *et al.*, 1990).

The demonstration that the synthesis of the neurotrophic molecules BDNF and NGF is regulated by neuronal activity via kainic acid receptors represents a conceptually distinct difference to the regulation of NGF synthesis in the periphery. In the periphery, NGF is exclusively synthesized by non-neuronal cells (Bandtlow *et al.*, 1987), and the regulation of NGF synthesis occurs independently of the neuronal input (Barth *et al.*, 1984; Hellweg *et al.*, 1988; Rohrer *et al.*, 1988; Clegg *et al.*, 1989). The increased levels of NGF protein found in the denervated peripheral target tissues results from the interference with the removal of NGF by responsive neurons and is not due to changes in NGF synthesis (Ebendal *et al.*, 1983; Korsching and Thoenen, 1985). Although there is a very precise correlation between the arrival of fibres of NGF responsive neurons in the target areas and the initiation of NGF synthesis, there is no causal relationship (Davies *et al.*, 1987; Rohrer *et al.*, 1988). It has been demonstrated that the NGF levels in intact and aneural chick limbs are identical which supports the concept that NGF synthesis in the periphery is under a precise neuron independent, autonomous control (Rohrer *et al.*, 1988). This is further supported by recent observations that whisker pads of mouse embryos exhibit a similar time course in expressing NGF mRNA in culture (devoid of neurons) and *in situ* (M.Schörnig, H.Rohrer, R.Heumann and H.Thoenen, unpublished data). In contrast to neurons, NGF synthesis in astrocyte culture, which produce substantial quantities of NGF protein (Lindsay, 1979;

Furukawa *et al.*, 1987; Spranger *et al.*, 1990), is not regulated by excitatory neurotransmitters. The same is true for BDNF (unpublished data). The available information is not sufficient to determine the relative contribution of neurons and astrocytes to NGF synthesize in brain under physiological conditions. However, NGF produced by astrocytes could be of importance under pathophysiological conditions where cytokines, like IL-1 and TGFβ, are coming into play and are known to up-regulate astrocyte NGF synthesis (Lindholm *et al.*, 1990; Spranger *et al.*, 1990).

The regional distribution of BDNF and NGF mRNA in the CNS is, with few exceptions, very similar (Bandtlow *et al.*, 1990; Hofer *et al.*, 1990). Both the distribution of BDNF and NGF mRNA at least partially overlap with the central pathways which use glutamate as a neurotransmitter (Cotman *et al.*, 1987; Monaghan *et al.*, 1989). In particular, there are striking similarities between the distribution of BDNF and NGF mRNA as demonstrated by *in situ* hybridization (Bandtlow *et al*, 1990; Hofer *et al.*, 1990) and kainic acid receptors visualized by autoradiography in hippocampus, cerebral cortex and cerebellum (Monaghan *et al.*, 1989). These observations are compatible with the interpretation that glutamate represents the physiological transmitter regulating BDNF and NGF mRNA in the CNS. The importance of these regulator mechanisms for developmental, physiological and pathophysiological regulation still remains to be proven, though the data presented here strongly suggests such a regulatory function.

Materials and methods

Compounds used

DMEM, fetal calf serum and laminin were obtained from Gibco; MK-801 from Research Biochemical Inc.; CNQX from Tocris Neuramin; RNasin was purchased from Promega; Taq polymerase from Genofit; AMV-reverse transcriptase from Life Science and NuSieve/agarose from FMC Bioproducts. All other reagents were obtained from Sigma.

Cell culture

Hippocampi were prepared from 17 day old rat embryos (E17), dissected and incubated for 20 min at 37°C in phosphate buffered saline (PBS) without calcium or magnesium ions, but containing 10 mM glucose, 1 mg/ml albumin, 6 μg/ml DNase and 12 U/ml papain. After washing with papain-free solution, the hippocampal cells were carefully dissociated with a fire polished Pasteur pipette. Cells were collected by centrifugation at low speed (900 *g*, 5 min), resuspended in DMEM, supplemented with 10% fetal calf serum and plated on plastic culture dishes (0.5×10^6 cells per 35 mm) which were precoated with poly-DL-ornithine (0.5 mg/ml) and laminin (5 μg/ml). Three hours after plating, the medium was changed to a serum-free one, which contained the supplements as described by Brewer and Cotman (1989), but without glutamate. Neurons remained viable up to 3 weeks in culture and were usually used 7 days after plating. At this time, the number of astrocytes present in the cultures was $<5\%$ as evaluated by GFAP immunostaining. After 2 weeks the number of astrocytes increased but the increased number of astrocytes did not influence the changes in BDNF mRNA levels observed under our experimental conditions.

RNA analysis

Total cellular RNA was extracted from 0.5×10^6 cells after the addition of a shortened BDNF cRNA (700 bp) recovery standard (10 pg). RNA was glyoxylated and analysed by electrophoresis on a 1.3% agarose gel (Heumann and Thoenen, 1986; Lindholm *et al.*, 1988). RNA transferred to Hybond N filters was hybridized with a ^{32}P-labelled cRNA probe (specific activity, 10^9 c.p.m./μg) specific for mouse BDNF and produced by run-off transcription *in vitro*.

For the quantitative PCR method RNA was extracted as described by Chomzcynski and Sacchi (1987) from 0.5×10^6 cells after the addition of a shortened NGF cRNA recovery standard (30 fg). NGF mRNA and cRNA were co-amplified in a combined one tube reverse transcription/polymerase chain reaction (RT/PCR) containing 1/5 of the extracted RNA, 1 × RT/PCR buffer (10 mM Tris−HCl ph 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 0.1% Triton X-100), 0.25 mM dNTP, 0.1 μM each 5′ and 3′ primer, 5 U RNasin, 3.2 U AMV-reverse transcriptase and 2 U Taq polymerase in a total volume of 25 μl. The mixture was overlaid with mineral oil, incubated at 41°C for 30 min, heated to 92°C for 10 min followed by 17 amplification cycles (denaturation 92°C for 60 s, primer annealing at 55°C for 60 s and primer extension at 72°C for 60 s). The amplification products (203 bp for NGF mRNA and 153 bp for recovery standard) were separated on a 3% NuSieve/agarose 3:1 gel, alkali-blotted to a Hybond N plus membrane and hybridized as described (Heumann and Thoenen, 1986; Lindholm *et al.*, 1988). For absolute quantification, known amounts of *in vitro* transcribed NGF mRNA and recovery standard were co-amplified in parallel reactions. Recently a similar method was described by Wang *et al.* (1989).

Treatment of animals

Adult Wistar rats of both sexes weighing 180−200 g were injected intraperitoneally with 12 mg/kg of kainic acid or with equivalent volumes of physiological saline. 90 min later rats usually received benzodiazepam (10 mg/kg) to suppress extensive seizure activity. At the indicated times rats were decapitated and their brains quickly removed from the skull. Hippocampus and cortex were dissected, frozen on dry ice and stored at −70°C. Valium given after kainic acid did not interfere with the further increase in BDNF and NGF mRNA levels. Rats which did not received valium showed similar increases of BDNF and NGF mRNA levels in hippocampus 3 h after kainic acid, compared with animals given kainic acid followed by valium.

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