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ACCELERATED COMMUNICATION

Regulation of Basic Fibroblast Growth Factor and Nerve Growth Factor mRNA by β-Adrenergic Receptor Activation and Adrenal Steroids in Rat Central Nervous System

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SUMMARY

Basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) are two neurotrophic factors that play a role in neuronal maintenance and repair. The identification and characterization of mechanisms regulating neurotrophic factor availability in the central nervous system are vital to the development of therapeutic tools for prevention of neuronal degeneration. The lipophilic β-adrenergic receptor (BAR) agonist clenbuterol was used to assess whether activation of central BAR changes the levels of NGF and bFGF mRNA. Within 5 hr, clenbuterol (10 mg/kg, intraperitoneally) elicited a 2–3-fold increase in bFGF and NGF mRNA content in rat cerebral cortex. The induction of bFGF and NGF mRNA expression showed anatomical specificity. Among the various brain areas examined, bFGF mRNA levels were increased in the cerebral cortex, hippocampus, and cerebellum, whereas induction of NGF mRNA was observed only in the cerebral cortex. Isoproterenol, a BAR agonist that does not cross the blood-brain barrier, also elicited a 2–3-fold increase in bFGF and NGF mRNA in the cerebral cortex. Propranolol (5 mg/kg, intraperitoneally), a lipophilic BAR antagonist, blocked the induction of NGF and bFGF mRNA mediated by either isoproterenol or clenbuterol, whereas nadolol (5 mg/kg, intraperitoneally), a BAR antagonist that does not cross the blood-brain barrier, blocked only the effect of isoproterenol. Therefore, activation of both central and peripheral BAR play a role in the regulation of bFGF and NGF mRNA expression. Moreover, in adrenalectomized rats, isoproterenol failed to increase bFGF and NGF mRNA, whereas clenbuterol elicited a 2-fold increase in bFGF mRNA in the cortex and hippocampus. Our data suggest that both adrenal steroids and noradrenaline might regulate the availability of selective neurotrophic factors in the adult central nervous system.

Several lines of indirect evidence support a role for NGF and bFGF as trophic factors for cholinergic neurons of the basal forebrain (1, 2). In addition, recent data have shown that bFGF causes a reduction in hippocampal neuronal death caused by glutamate by raising the threshold for glutamate neurotoxicity (3), suggesting that bFGF might play a protective role against excitotoxic damage. Moreover, NGF infused into the brain of a patient with Alzheimer's disease was reported to have some beneficial effects on cholinergic parameters and cognitive functions (4). In light of these findings, the possibility of using NGF and bFGF to delay or reverse the progression of neurodegenerative diseases is becoming more appealing. However, because of their chemical properties, bFGF and NGF fail to cross the blood-brain barrier and are degraded by peptidases when given systemically. Hence, these trophic factors cannot be used therapeutically unless special surgical procedures are used to facilitate their entry into the CNS. Alternative strategies have been proposed, such as the grafting of NGF-synthesizing cells into the brain (5) or the enhancement of endogenous production of neurotrophic factors (6).

Information on the mechanisms regulating NGF and bFGF biosynthesis in the CNS is still fragmentary. Alteration of NGF or bFGF levels has been shown to occur after mechanical injury or ischemia (7, 8). Moreover, it has been reported that NGF and bFGF gene expression is induced in selective rat brain structures in various models of seizures (9–12). These data suggest that alteration of synaptic activity *per se* elicits the induction of these neurotrophic factors, and they show that a variety of stimuli could be used to increase the efficiency of mechanisms controlling NGF and bFGF biosynthesis in the CNS.

The stimulation of BAR in cultured astroglial cells enhances the synthesis and release of NGF (13–16). These results lead

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ABBREVIATIONS: NGF, nerve growth factor; bFGF, basic fibroblast growth factor; CNS, central nervous system; BAR, β-adrenergic receptor(s); PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); ACTH, adrenocorticotropic hormone.

to the hypothesis that noradrenaline might regulate the synthesis and release of trophic factors in the intact CNS. These findings might be of clinical relevance if the injection of BAR agonists promotes an increase of NGF and bFGF biosynthesis in the CNS. Data in this report supply significant new insights into the mechanisms regulating NGF and bFGF expression in the brain. These studies may aid in the design of a novel approach for the treatment of neuronal degeneration.

Materials and Methods

Treatment of rats. Sprague-Dawley male rats (Zivic Miller, Allison, PA) weighing 225–250 g were given intraperitoneal injections of saline, clenbuterol, propranolol, nadolol, or isoproterenol (all purchased from Sigma Chemical Co., St. Louis, MO). Adrenalectomized and sham-operated rats were received from Zivic Miller (adrenalectomy was performed 3 days before the injection of the drugs). Adrenalectomized rats received saline in place of drinking water. During the treatments, all rats were maintained at five per cage in a temperature-controlled room with a 12-hr light-dark cycle. Food and water were provided *ad libitum*. Rats were killed by decapitation and various brain structures were dissected (17), immediately frozen on dry ice, and kept at −70° for analysis at a later time.

Probe preparation. The plasmid RObFGF103 (a gift from Dr. A. Baird, The Whittier Institute, La Jolla, CA) is a derivative of a Bluescript plasmid containing a 1016-base portion of the rat bFGF cDNA (18). *Nco*I-linearized plasmid was used as a template for the *in vitro* transcription assay with T7 RNA polymerase (19). This procedure generates a ^{32}P-labeled 524-base probe that includes the 477 bases of bFGF cRNA and 47 bases of the Bluescript polylinker region.

^{32}P-labeled NGF RNA probe was generated from plasmid BSrNGF (20), which contains a 771-base portion of the rat NGF cDNA (a gift from Dr. S. Whittemore, University of Miami, Miami, FL). This plasmid was used, after linearization with *Nco*I, as a template for the *in vitro* transcription assay with T3 polymerase (19). This procedure generates a ^{32}P-labeled 460-base probe that includes the 415 bases of NGF cRNA and 45 bases of the Bluescript polylinker region.

Plasmid p15GI (19), a derivative of plasmid p1B15, contains a partial sequence of the rat gene for cyclophilin (21), a constitutive protein. The *in vitro* transcription of linearized p15GI with SP6 polymerase generates a cRNA composed of 294 bases complementary to cyclophilin RNA and seven bases of the plasmid polylinkers. Cyclophilin cRNA was used as standard control to monitor for artifacts from extraction of RNA from tissue samples.

RNase protection assay. The RNase protection assay was performed with an RNase protection assay kit (Ambion Inc., Austin, TX) as described previously (19). Briefly, total RNA was extracted from different tissues (22) and dissolved in 20 μl of hybridization solution (80% formamide, 40 mM PIPES, pH 6.4, 400 mM sodium acetate, pH 6.4, 1 mM EDTA) containing 150,000 cpm of a ^{32}P-labeled bFGF or NGF cRNA probe (specific activity, >4–6 × 10^8 cpm/μg of RNA each). To balance the relatively high level of cyclophilin RNA, pIG15 was labeled to a lower specific activity (~1 × 10^6 cpm/μg of RNA). After being heated at 85° for 10 min, the cRNA probes were allowed to hybridize to the endogenous RNA at 50° overnight. At the end of the hybridization, the solution was diluted with RNase digestion buffer (Ambion) containing 5 μg/ml RNase A and 100 units/μl RNase T1 and was incubated for 30 min at 35°. After a proteinase K (0.45 μg/μl) digestion, samples were extracted with phenol/chloroform and precipitated with ethanol. The pellet containing the RNA-RNA hybrids was resuspended in loading buffer (80% formamide, 0.1% xylene cyanol, 0.1% bromphenol blue, 2 mM EDTA), boiled at 95°, and separated on a 5% polyacrylamide/urea sequencing gel. ^{32}P-end-labeled (T4 polynucleotide kinase) *Msp*I-digested pBR322 fragments were used as molecular markers. The gel was dried and the mRNA-protected fragments were visualized by autoradiography on X-ray film, using a Cronex Quanta III intensifying screen.

RNA calculation. Quantification of total RNA was carried out by measuring absorbance at 260 nm. bFGF and NGF mRNA content was calculated by measuring the peak densitometric area of the autoradiograph with a laser densitometer (Hoefer GS 300) and normalizing the area to the peak densitometric area of the cyclophilin autoradiographic band. The exposure time and amount of RNA needed for the quantitation were predetermined by plotting bFGF or NGF autoradiographic density versus increasing amounts of RNA loaded on the gel to obtain a standard curve, to ensure that the autoradiographic bands were in the linear range of intensity. Data are expressed as percentage of control. The mean value of the controls within a single experiment was arbitrarily set equal to 100 and all other values in the same experiment (i.e., all data points obtained from a single assay) were calculated relative to that value, in order to generate values expressed as percentage of control.

Results

BAR stimulation increases bFGF and NGF mRNA in selected brain regions. In C6 glioma cells, a glial phenotype, BAR stimulation by *l*-isoproterenol has been shown to transiently increase the content of the mRNA encoding for NGF (14, 15) and bFGF.[1] However, in these cells the regulation of neurotrophic factor expression might be altered as a result of the tumoral nature of this cell line. Therefore, we tested whether BAR stimulation could also increase bFGF and NGF mRNA expression in rat CNS.

Isoproterenol crosses the blood-brain barrier poorly; therefore, we administered clenbuterol, a more lipophilic BAR-2 agonist (23–25). We used a dose of clenbuterol of 10 mg/kg (intraperitoneally), based upon its reported potency to stimulate central BAR *in vivo* (24, 25). In order to compare the changes in the content of bFGF mRNA and NGF mRNA, we simultaneously assayed both mRNA species in the identical tissue extracts taken from animals 2 or 5 hr after BAR stimulation. The RNase protection assay with cRNAs for NGF and bFGF revealed that at 5 hr clenbuterol elicited a 2–3-fold increase in bFGF and NGF mRNA in the cerebral cortex (Figs. 1 and 2). Moreover, the BAR agonist increased bFGF but not NGF mRNA in the hippocampus and cerebellum (Fig. 2). No change was observed in the hypothalamus or striatum (Fig. 2). Dose-response studies revealed that the doses of 5 and 1 mg/kg were as active as 10 mg/kg (data not shown). In addition, studies of the temporal patterns of induction showed that 2 hr after clenbuterol administration none of the brain regions investigated displayed a significant change in either bFGF or NGF mRNA content (data not shown).

The increase in bFGF and NGF mRNA is not due to a nonspecific mRNA induction, because the content of the mRNA encoding for cyclophilin, a constitutive protein (21), failed to change as a result of our experimental treatments (Fig. 1). Clonidine (0.5 mg/kg, intraperitoneally), an α-adrenergic agonist, failed to change NGF or bFGF mRNA at 5 hr (data not shown), suggesting that regulation of NGF and bFGF mRNA expression is mediated by BAR activation.

Isoproterenol increases bFGF and NGF mRNA. The stimulation of peripheral BAR can lead to metabolic changes that could indirectly increase both bFGF and NGF mRNA in the CNS. Hence, the clenbuterol-mediated effect could be

[1] I. Mocchetti, unpublished observations.

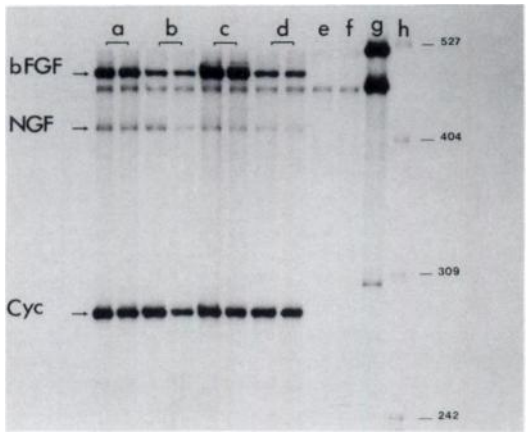

Fig. 1. RNase protection assay of NGF and bFGF mRNAs in the cerebral cortex and hippocampus after clenbuterol administration. Adult rats were treated with clenbuterol (10 mg/kg, intraperitoneally) or saline and were sacrificed 5 hr after the injection. Twenty-five micrograms of total RNA extracted from cerebral cortex and hippocampus were used to evaluate bFGF and NGF mRNA content with the RNase protection assay. *Lanes a*, hippocampus, clenbuterol treated; *lanes b*, hippocampus, saline treated; *lanes c*, cerebral cortex, clenbuterol treated; *lanes d*, cerebral cortex, saline treated; *lane e*, tRNA; *lane f*, digested probe; *lane g*, undigested probe, aliquot (2000 cpm) of the hybridization solution containing the cRNA probe for bFGF, NGF, and cyclophilin; *lane h*, DNA marker. *Arrows*, bFGF, NGF, and cyclophilin (*Cyc*) RNA-protected fragments. The autoradiographic film was exposed overnight at −70°.

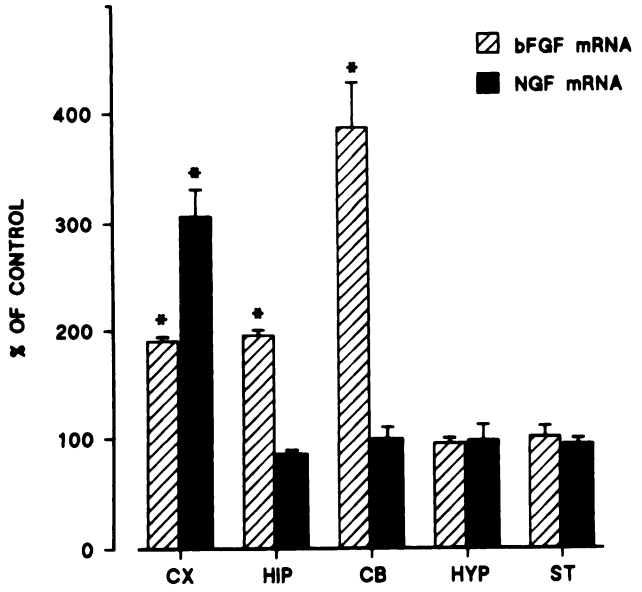

Fig. 2. Clenbuterol increases bFGF and NGF mRNA in specific brain regions. Rats received clenbuterol (10 mg/kg, intraperitoneally) or saline and were sacrificed 5 hr after the injection. RNA was prepared as described in Materials and Methods. bFGF, NGF, and cyclophilin RNA content was determined as described in Materials and Methods. *CX*, cerebral cortex; *HIP*, hippocampus; *CB*, cerebellum; *HYP*, hypothalamus; *ST*, striatum. Data, expressed as percentage of control (saline-treated rats), are the means ± standard errors of three independent experiments ($n = 3$). *, $p < 0.01$, analysis of variance and Dunnett's test.

caused by a mechanism related to the activation of peripheral BAR and not to its central properties. We have tested this hypothesis by injecting isoproterenol, a nonselective BAR agonist that fails to cross the blood-brain barrier efficiently. Surprisingly, isoproterenol (5 or 1 mg/kg, intraperitoneally) induced a 2-3-fold increase in cortical bFGF and NGF mRNA 5 hr after the injection (Fig. 3). In the hippocampus, isoproterenol induced a similar increase in bFGF but not NGF mRNA (Fig. 3). These data suggest that, in the CNS, trophic factor expression can also be triggered indirectly by stimulation of peripheral BAR. To test this hypothesis, we used two BAR antagonists, i.e., liphophilic *l*-propranolol, which crosses the blood-brain barrier, and nadolol, which crosses poorly. *l*-Propranolol (5 mg/kg, intraperitoneally) administered 20 min before clenbuterol (10 mg/kg, intraperitoneally) blocked the ability of both BAR agonists to induce NGF and bFGF mRNA in the cerebral cortex (Fig. 4). Nadolol (5 mg/kg, intraperitoneally) blocked the isoproterenol-mediated induction of cortical NGF and bFGF mRNA (Fig. 4). Moreover, nadolol blocked the clenbuterol-mediated induction of NGF mRNA but failed to block the clenbuterol-mediated induction of bFGF mRNA in both cerebral cortex and hippocampus (Figs. 4 and 5).

In the heart ventricle of the same rats, clenbuterol induced a 16- and 5-fold increase in bFGF and NGF mRNA, respectively. Isoproterenol elicited a similar increase (Fig. 6). Both responses were blocked by nadolol (Fig. 6). Thus, these data demonstrate that nadolol is able to displace both BAR agonists from peripheral BAR, and they suggest that the ability of clenbuterol to induce bFGF mRNA expression in the CNS should be attributed to the activation of central BAR.

Adrenalectomy prevents the isoproterenol-mediated increase in bFGF and NGF mRNA. BAR activation by isoproterenol in the anterior pituitary gland increases the release of ACTH (26). ACTH, in turn, stimulates adrenal steroidogenesis and release of adrenal corticosteroids. Dexamethasone, a synthetic glucocorticoid, has been shown to increase, within 3 hr, the content of NGF mRNA and protein in rat cerebral cortex (27). We have tested the hypothesis that adrenal

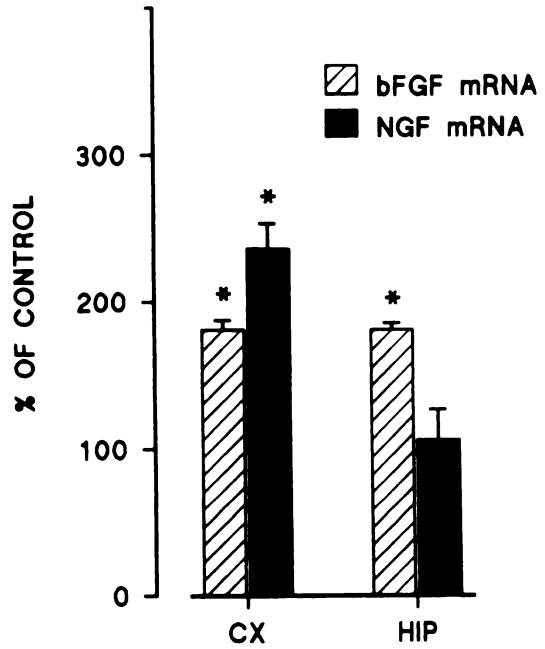

Fig. 3. Isoproterenol increases bFGF and NGF mRNA in cerebral cortex. Rats were treated with isoproterenol (5 mg/kg, intraperitoneally) or saline and were sacrificed 5 hr after the injection. bFGF and NGF mRNA levels were determined in cerebral cortex (*CX*) and hippocampus (*HIP*). Data, expressed as percentage of control (saline-treated rats), are the means ± standard errors of three separate experiments ($n = 3$). *, $p < 0.01$, analysis of variance and Dunnett's test.

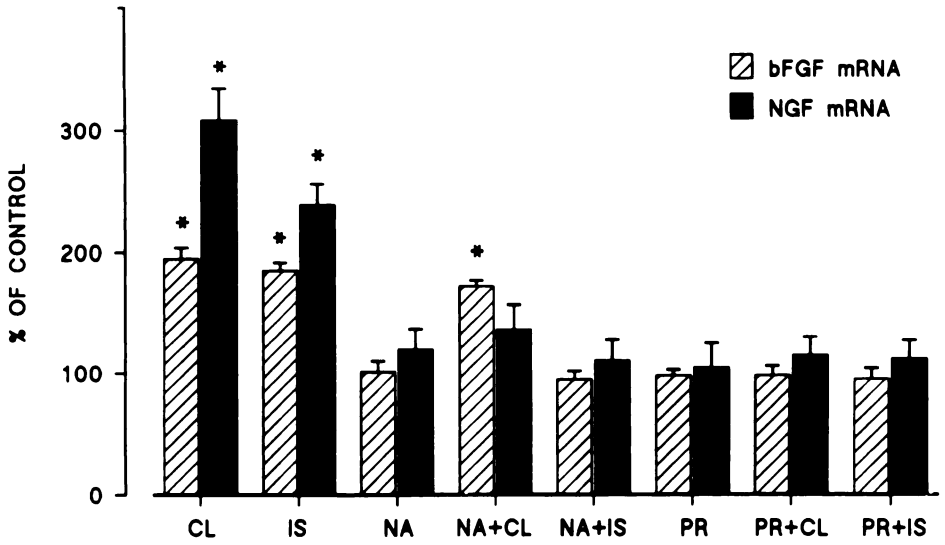

Fig. 4. Propranolol but not nadolol blocks the clenbuterol-mediated increase in cortical bFGF and NGF mRNA. Rats were treated with clenbuterol (*CL*) or isoproterenol (*IS*). Nadolol (*NA*) or propranolol (*PR*) was administered alone or in combination with clenbuterol (*NA+CL*, *PR+CL*) or isoproterenol (*NA+IS*, *PR+IS*). All drugs were administered at a dose of 5 mg/kg (intraperitoneally). BAR antagonists were administered 20 min before BAR agonists. Animals were sacrificed 5 hr after the last injection, and cerebral cortex was dissected. Data, expressed as percentage of control (saline-treated animals), are the means ± standard errors of three separate experiments ($n = 3$). *, $p < 0.01$, analysis of variance and Dunnett's test.

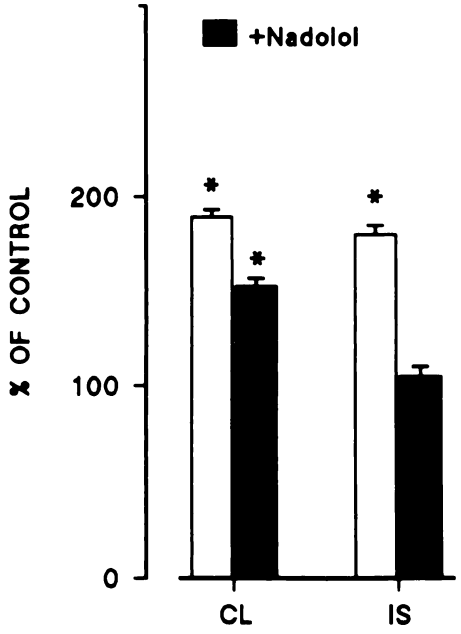

Fig. 5. Nadolol fails to block the clenbuterol-mediated increase in hippocampal bFGF mRNA. Rats received clenbuterol (*CL*) or isoproterenol (*IS*) alone or in combination with nadolol. Animals were sacrificed 5 hr after the last injection, and hippocampus was dissected. bFGF mRNA was determined as described in Materials and Methods. Data are the means ± standard errors of three separate experiments ($n = 3$). *, $p < 0.01$, analysis of variance and Dunnett's test.

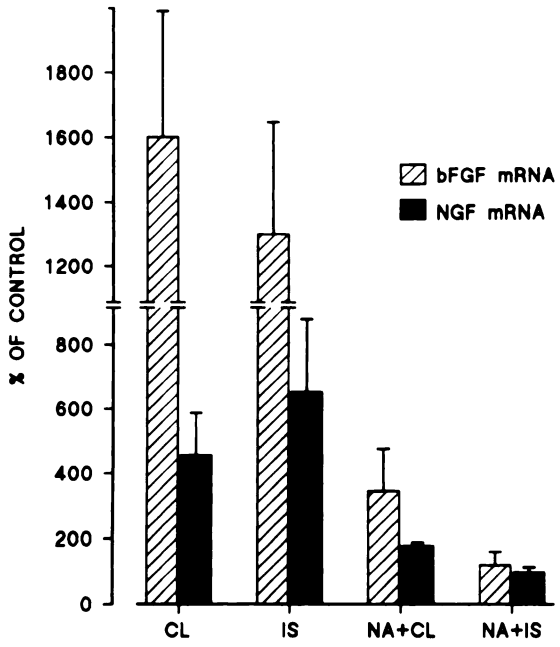

Fig. 6. bFGF and NGF mRNA content increases in heart ventricle after BAR stimulation. Rats were treated with clenbuterol (*CL*), isoproterenol (*IS*), nadolol plus clenbuterol (*NA+CL*), or nadolol plus isoproterenol (*NA+IS*) and were sacrificed 5 hr after the injection. All drugs were used at a dose of 5 mg/kg (intraperitoneally). Data are the means ± standard errors of two separate experiments ($n = 3$).

steroids could mediate the induction of bFGF and NGF mRNA after BAR activation by injecting both isoproterenol and clenbuterol in sham-operated and adrenalectomized rats.

Adrenalectomy *per se* reduced the levels of bFGF and NGF mRNA in the cerebral cortex (Fig. 7) and hippocampus (data not shown). The ability of clenbuterol to increase NGF but not bFGF mRNA was blocked in adrenalectomized rats (Fig. 7). In contrast, in these animals the isoproterenol-mediated induction of both bFGF and NGF mRNA was abolished (Fig. 7). These data suggest that, whereas bFGF mRNA might be induced by activation of central as well as peripheral BAR, the expression of cortical NGF might be mainly regulated by adrenal steroids.

Discussion

NGF and bFGF are synthesized in the CNS, where they are believed to play a role in neuronal maintenance and repair.

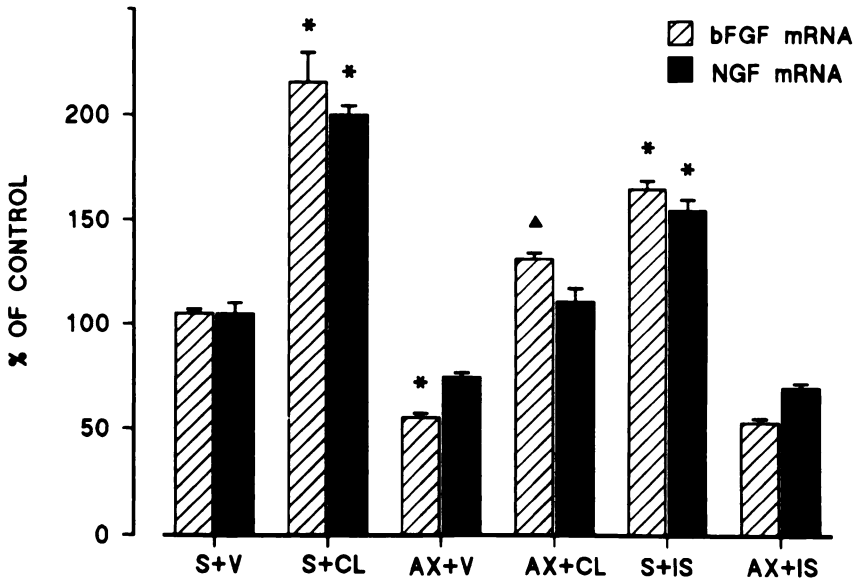

Fig. 7. Isoproterenol but not clenbuterol fails to increase cortical bFGF mRNA in adrenalectomized rats. Adrenalectomized or sham-operated rats were given injections of isoproterenol, clenbuterol, or saline 3 days after the operation. Rats were sacrificed 5 hr after the injection and the amount of cortical bFGF and NGF mRNA was determined. *S+V*, sham plus saline; *S+CL*, sham plus clenbuterol; *AX+V*, adrenalectomized plus saline; *AX+CL*, adrenalectomized plus clenbuterol; *S+IS*, sham plus isoproterenol; *AX+IS*, adrenalectomized plus isoproterenol. Results are expressed as a percentage of the control values (sham-operated animals) and represent the means ± standard errors of three separate experiments ($n = 3$). *, $p < 0.01$ versus control; ▲, $p < 0.01$ versus adrenalectomized rats, analysis of variance, Dunnett's and Scheffe's tests.

The physiological importance of bFGF and NGF in neuronal trophism suggests that a pharmacologically induced increase in the biosynthesis and availability of these neurotrophic factors may have therapeutic significance in the treatment of neurodegenerative diseases characterized by a decrease of neuronal plasticity. Because the chemical structure of bFGF and NGF prevents their use by parenteral administration, we studied whether bFGF and NGF mRNA expression in the CNS could be enhanced pharmacologically.

In C6 rat glioma cells, synthesis and release of NGF are modulated by stimulation of specific neurotransmitter receptors coupled to adenylate cyclase, such as BAR and prostaglandin receptors (13–15). Moreover, nuclear run-on studies have shown that the BAR-mediated increase in NGF mRNA content is the result of increased gene transcription (28). Hence, NGF gene expression in cells derived from the neural plaque can be regulated by activation of molecular mechanisms that lead to an increase in intracellular cAMP. These findings lead to the speculation that BAR stimulation might modulate neurotrophic factor availability in the CNS. To test whether BAR stimulation can increase NGF and bFGF *in vivo*, we used clenbuterol, a lipophilic BAR-2 agonist that crosses the blood-brain barrier. Clenbuterol elicited a rapid increase in cortical bFGF and NGF mRNA levels. The increase in bFGF mRNA was not confined to the cortex but was observed also in hippocampus and cerebellum, two other brain areas that possess BAR. The rapid change in NGF and bFGF mRNA after BAR but not α-adrenergic receptor stimulation allowed us to speculate that noradrenaline could tonically regulate NGF and bFGF biosynthesis via the activation of central BAR. However, this suggestion is in contrast to the finding that isoproterenol, which crosses the blood-brain barrier poorly, also increases the mRNA for both neurotrophic factors.

We have investigated whether the peripheral effects of isoproterenol could account for the induction of trophic factor mRNA by using BAR antagonists that cross or do not cross the blood-brain barrier. Propranolol, a lipophilic BAR antagonist, blocked the effect of both clenbuterol and isoproterenol, whereas nadolol, a BAR antagonist that like isoproterenol fails to cross the blood-brain barrier, antagonized the effect of isoproterenol but not that of clenbuterol. In heart ventricle, the clenbuterol-mediated induction of bFGF and NGF mRNA was blocked by nadolol, demonstrating that nadolol is capable of displacing clenbuterol from peripheral BAR. However, in spite of the blockade of its peripheral effect, clenbuterol induced bFGF mRNA, suggesting that the stimulation of central BAR most likely plays a role in the regulation of this neurotrophic factor. Moreover, the results obtained support the hypothesis that availability of trophic factors in the brain is regulated by mechanisms that can be related or unrelated to the stimulation of central BAR. Among various biological responses, BAR agonists increase the release of ACTH by interacting directly with the BAR-2 subtype localized in the anterior pituitary (26). The increased secretion of ACTH from the pituitary stimulates adrenal mitochondrial steroidogenesis and release of adrenal steroids into the blood stream. Previous studies have shown that dexamethasone, a synthetic glucocorticoid, increases NGF mRNA and protein in rat cerebral cortex (27). Consequently, the BAR-mediated increase in NGF and bFGF mRNA could be initiated by adrenal steroids. In order to test this hypothesis and, therefore, to clarify the role of central versus peripheral BAR, we injected isoproterenol or clenbuterol into adrenalectomized rats. Isoproterenol failed to change NGF and bFGF mRNA content in these rats; in contrast, clenbuterol was still able to increase bFGF but not NGF mRNA in both cerebral cortex and hippocampus. Thus, our data suggest that the isoproterenol-mediated increase in NGF and bFGF expression depends upon intact pituitary-adrenalcortical function and that adrenal steroids play a major role in the induction of NGF mRNA in the cerebral cortex. Clenbuterol instead appears to

induce bFGF mRNA by its direct interaction with the central BAR. However, clenbuterol failed to restore a full response in adrenalectomized rats. Thus, we cannot rule out the possibility that adrenal steroids may also play a role in the induction of bFGF mRNA.

Our data indicate an anatomical specificity in the induction of NGF and bFGF and suggest that noradrenaline and adrenal steroids might act directly in the CNS by way of BAR or steroid receptors to stimulate induction of bFGF and NGF mRNA. Dexamethasone also increases bFGF mRNA in the cerebral cortex.[1] Therefore, in the cerebral cortex, where both NGF and bFGF mRNA levels were increased by BAR stimulation, we cannot determine whether NGF and bFGF mRNA content increases via similar or different transduction mechanisms. In the cerebral cortex, hippocampus, and cerebellum, but not in the hypothalamus and striatum, bFGF mRNA was significantly increased after BAR stimulation. These data suggest that regulation of bFGF mRNA levels depends upon stimulation of specific transsynaptic mechanisms linked to BAR activation and not a widespread induction of gene expression caused by increased cAMP. It is possible that expression of neurotrophic factors might be regulated by activation of selected brain circuitry. This consideration implies that bFGF and NGF expression might not be induced in all cells possessing BAR or steroid receptors, because these cells may be under the influence of different extracellular signals. On the other hand, it has been shown that transcription selectivity in a given cell population is conferred by different transcriptional factors and by the activation and interaction of different DNA responsive elements. Therefore, it is likely that the enhancement of bFGF and NGF mRNA in specific brain areas is a complex phenomenon occurring in particular cell types.

The regional specificity of bFGF and NGF induction is still unclear. It is tempting, though, to speculate that the increase in NGF and bFGF mRNA levels occurs as part of an adaptive response to excessive neural stimulation elicited by either BAR agonists or adrenal steroids. Indeed, the rapid increase in NGF and bFGF mRNA content is consistent with it being an acute response to alteration of synaptic activity. This response might be important for protecting against cell damage and thus maintaining the integrity of the neuronal network. This has been suggested to be the case in the increased NGF and bFGF mRNA expression after seizure activity (9, 12). Therefore, it appears that the induction of these trophic factors is an adaptive change developed by the CNS to integrate the altered metabolic and energetic balance due to excessive synaptic activity or to minimize subsequent cell damage. This speculation is further encouraged by the observation that bFGF and NGF can protect hippocampal and cortical neurons in culture from cell death induced by hypoglycemia (29). Because long term treatment with glucocorticoids has been shown to cause neuronal damage (30, 31), it will be important to establish whether NGF and bFGF expression can still be enhanced after chronic exposure to BAR agonists or glucocorticoids.

In conclusion, bFGF and NGF expression might be under a tonic physiological inhibition that controls their neurotrophic activity. The levels of bFGF and NGF mRNA were reduced in adrenalectomized rats, suggesting that adrenal steroids tonically regulate trophic factor expression in specific brain areas. Moreover, threshold levels of glucocorticoids, such as those obtained during stress (27) or after excessive BAR stimulation, induce NGF and bFGF availability in an anatomically specific manner. The role of noradrenaline in the regulation of neurotrophic factors is still under investigation. It is tempting to speculate that noradrenaline might also tonically regulate production of bFGF by activating specific signal transduction mechanisms in selective cell populations. All these data suggest that the increased production of trophic factors in the CNS might play an important role in maintaining neuronal viability and homeostasis to overcome intense physiological activity. It is possible that in neurodegenerative disorders there might be insufficient trophism because of limited neurotrophic factor availability. If that is so, then pharmacological stimulation of neurotrophic factor biosynthesis may lead to a valid therapeutic approach to prevent or overcome, in part, neuronal degeneration.

Acknowledgments

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