Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF)

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Abstract

The serotonin transporter (5-HTT) regulates serotonergic neurotransmission by determining the magnitude and duration of serotonergic responses. We have recently described a polymorphism in the 5-HTT gene promoter (5-HTTLPR) which influences the function of the 5-HTT and is associated with several psychiatric disorders. Immortalized B lymphocytes express the 5-HTT, and a B lymphocyte line has been shown to express the receptor for brain-derived neurotrophic factor, trkB. Since brain-derived neurotrophic factor (BDNF) is a specific growth and differentiation factor for serotonergic neurons, we assessed whether BDNF is able to modulate 5-HTT function in B lymphoblasts. Nerve growth factor (NGF), another neurotrophin which acts via the trkA receptor, was also studied. Eight immortalized B lymphoblast lines were generated and genotyped for the 5-HTTLPR. After treatment with BDNF or NGF, 5-HT uptake and proliferation of the cell lines were assessed. Two of the B cell lines showed a dose-dependent reduction of 5-HT uptake after exposure to BDNF. Both of these cell lines were homozygous for the long allele of the 5-HTTLPR. NGF did not influence 5-HT uptake or cellular proliferation in any of the cell lines. Thus, BDNF but not NGF may influence 5-HT uptake in some B lymphocytes. The fact that regulation of the 5-HTT was observed preferentially in cells of the long/long genotype indicates that presence of a short allele confers reduced regulatory capacity on the 5-HTT. In conclusion, B lymphoblasts represent a practical model for functional regulation of the 5-HTT by neurotrophins in serotonergic neurons. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Serotonergic neurotransmission influences many brain functions such as emotion, cognition, motor function, and pain as well as circadian and neuroendocrine functions including food intake, sleep, and reproductive activity. While there are multiple presynaptic and postsynaptic serotonin (5-HT) receptor subtypes mediating these complex actions of 5-HT, the removal of 5-HT from the synaptic cleft is regulated by a single protein. This protein, the 5-HT transporter (5-HTT), takes up 5-HT into the presynaptic neuron. The 5-HTT determines the magnitude and duration of serotonergic responses and is thus central to the fine-tuning of serotonergic neurotransmission (Lesch and Mössner, 1998).

Attention has recently been focussed on the functional regulation of pivotal proteins such as the 5-HTT. Analysis of the promoter region of the human 5-HTT gene revealed a polymorphism that results in allelic variation in functional 5-HTT expression. This polymorphic repetitive element (5-HT gene-linked polymorphic region, 5-HTTLPR) is located approximately 1 kb upstream of the transcription initiation site and consists primarily of either 14 or 16 repeat elements. The long (l) allele with 16 repeat elements leads to more 5-HTT mRNA, 5-HTT protein, and 5-HTT uptake, than the short (s) allele with 14 repeat elements (Lesch et al., 1996). Furthermore, we and others have shown that this polymorphism may influence the risk of developing affective spectrum disorders such as depression, as well as late-onset Alzheimer’s
2. Experimental procedures

2.1. Cell culture

EBV-transformed B cell lines were cultured in RPMI 1640 medium supplemented with 15% fetal calf serum (FCS), glutamine, and gentamycin at 37°C in a humidified atmosphere at 5% CO₂ and kept in logarithmic growth phase. For treatment with recombinant human BDNF or NGF (R & D Systems, Wiesbaden, Germany), B cell lines were washed twice in serum-free medium and plated at a density of 6 × 10⁴ cells/well.

2.2. 5-HT uptake

Determination of 5-HT uptake was adapted from Jayanthi et al. (1994). B cell lines were washed with Hank’s balanced salt solution (HBSS, Sigma), pH 7.4 and transferred into 12 × 75 mm polypropylene tubes at an original cell concentration of 10⁶ cells/tube. They were then incubated with the indicated concentrations of 5-[1,2-³H(N)]-HT (DuPont, 27.8 Ci/mmol) in HBSS, 200 µl volume, without or with added 0.2 µM imipramine (Sigma), for 10 min at 37°C in a shaking water bath. Cells were then washed twice by centrifugation (1500 rpm, 5 min) in ice-cold HBSS containing imipramine and counted in a liquid scintillation counter. Non-specific, i.e. imipramine-insensitive, uptake was always < 10%. Statistical analysis for effects of BDNF or NGF vs control was performed by the Student’s t-test. All results are expressed as mean ± standard error of the mean (S.E.M.).

2.3. PCR analyses

DNA was obtained from EBV-transformed B cell lines. Oligonucleotide primers flanking the 5-HTTLPR and corresponding to the nucleotide positions −1416 to −1397 (5'GGCGTTGCGTCTGATGC) and from −910 to −888 (5'GAGGGACTGAGCTGGACAAAACCAC) of the 5-HTT gene 5’-flanking regulatory region were used to generate 484 or 528-basepair fragments. PCR amplification was carried out in a final volume of 30 µl consisting of 50 ng of genomic DNA, 2.5 mM deoxynucleotides, 0.1 µg of sense and antisense primers, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 1 U of Taq DNA polymerase. Annealing was carried out at 61°C for 30 s, extension at 72°C for 1 min, and denaturation at 95°C for 30 s for 35 cycles. For determining the presence of trkA and trkB mRNA by reverse-transcriptase PCR, total RNA was isolated from B lymphoblasts and neuroblastoma cell lines employing RNA Isolator (Genosys, Cambridge, England), a modification of the guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). First-strand cDNA was synthesized using Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (Stratagene, Heidelberg, Germany), RNAse inhibitor (Promega, Heidelberg) and random hexamer primer (Boehringer Mannheim, Germany). PCR for trkA and trkB was performed as described by Schenone and coworkers (1996), using the primers 5'-GCATCTGGAGCTCCGATCT and 5'-CTCTGCCCAGCAGCTCAAGT for amplification of trkA and 5'-GCACACTCAGGATTGTATG GCC and 5'-TCCGTGATTGACTGACGTGATTT for amplification of trkB. PCR reactions were heated to 95°C for 5 min. For trkA, 28 amplification cycles each consisting of denaturation
(95°C, 1 min) and annealing/extension (55°C, 1 min) were performed. For trkB, 30 cycles consisting of denaturation (95°C, 1 min), annealing (54°C, 2 min), and extension (72°C, 3 min), were performed. A final extension time of 7 min at 72°C for one cycle was used for both trkA and trkB.

3. Results

A panel of eight immortalized human B lymphoblast lines was established and genotyped for allelic variation in the 5-HTT gene promoter. Four cell lines were homozygous for the long allele, yielding the l/l genotype. Two cell lines were homozygous for the short allele (s/s), while two cell lines were heterozygous (l/s).

We then assessed the effect of BDNF on the 5-HTT in these B lymphoblasts. All cell lines were incubated with BDNF for two days. Fig. 1 shows that in two of the B cell lines there was a marked and dose-dependent decrease of 5-HT uptake after incubation with BDNF. In the other six B cell lines, 5-HT uptake was only slightly, but not significantly decreased (not shown). To ascertain that the inhibitory effect of BDNF on 5-HT uptake is not caused by an inhibition of proliferation of the lymphoblasts, the effect of BDNF on thymidine incorporation as a measure of cellular proliferation was determined. Fig. 2 shows that while BDNF led to decreased 5-HT uptake in lymphoblast line A, cellular proliferation determined in parallel was not influenced.

When these results were stratified according to the genotype of the lymphoblast lines, both cell lines exhibiting an effect of BDNF were of the l/l 5-HTT promoter genotype (Table 1). On the other hand, none of the cell lines of the l/s or s/s genotypes showed a significant modulating effect of BDNF. This is in accord with previous studies showing that the presence of an s
allele confers reduced regulatory capacity on the 5-HTT.

We then tested whether NGF, a neurotrophin which acts via the trkA receptor, is able to modulate 5-HTT function in B lymphoblasts. Cell lines treated with various concentrations of NGF did not show any change in 5-HT uptake or cellular proliferation. Fig. 3 shows the lack of effect of NGF on 5-HT uptake in the two cell lines that were responsive to modulation by BDNF. Finally, to assess whether the lack of influence of NGF can be explained by the presence or absence of its receptor, trkA, on the B lymphoblasts, we performed RT-PCR for trkA and trkB on the B lymphoblasts. In comparison, three human neuroblastoma cell lines (IMR-32, SK-N-SH, and SH-SY-5Y) were analysed, which are known to express both trkA and trkB. As Fig. 4 shows, none of the B lymphoblast lines expressed trkA. Thus, the lack of effect of NGF may be explained by the absence of this receptor. Moreover, all of the cell lines expressed trkB, showing that the presence of this growth factor receptor is characteristic of B lymphoblasts.

4. Discussion

Our results show that B lymphoblasts express mRNA for trkB, the receptor for BDNF. This is mirrored by investigations of the central nervous system showing that BDNF displaceable binding sites (Altar et al., 1993) as well as trkB mRNA (Merlio et al., 1992) are present in the dorsal raphe nucleus. Moreover, BDNF is transported by retrograde axonal transport from 5-HT terminal fields in the cortex to cell bodies in the raphe nuclei (Anderson et al., 1995).
TrkB mRNA, on the other hand, was found to be absent in B lymphoblasts. This is consistent with functional findings in the CNS that NGF fails to induce serotonergic neurite outgrowth in embryonic raphe cultures (Azmitia et al., 1990) or in the adult striatum (Kawaja and Gage, 1991) and is not neurotrophic for cortical serotonergic projections (Mamounas et al., 1995). Conversely, mice lacking trkB have a loss of cholinergic neurons of the basal forebrain as well as of sensory and sympathetic neurons, indicating that trkB plays an important role in mediating trophic effects for these neurons (Smye et al., 1994).

We have shown that BDNF decreases 5-HT uptake in some B lymphoblast lines. This decreased uptake would functionally lead to increased extracellular 5-HT levels. In studies in the rat CNS, it has indeed been found that BDNF increases 5-HT levels in raphe neurons (Siuciak et al., 1994). In a subsequent study, this group showed that BDNF increases tryptophan hydroxylase mRNA levels in rat brain (Siuciak et al., 1998). This effect and the effect we have shown in human B lymphoblasts both work in the same direction to increase extracellular 5-HT levels, which are also thought to play an important part in the action of antidepressants. In this regard it is interesting that BDNF has indeed been shown to have an antidepressant-like effect in rats (Siuciak et al., 1997).

The BDNF effect on 5-HT uptake was preferentially present in lymphoblasts of the I/l genotype of the 5-HTTLPR. The absence of a significant effect in cells of the I/s or s/s genotypes argues for a decreased regulatory capacity of the 5-HT in these genotypes. This has indeed been shown previously for regulation of 5-HTT expression by the adenylate cyclase activator forskolin (Lesch et al., 1996), and by glucocorticoids (Lesch et al., manuscript in preparation). There is also a growing body of evidence from clinical studies that the presence of an s allele of the 5-HTTLPR has important functional consequences on the serotonergic system. For example, people of the I/l or s/s genotypes are at greater risk of developing depression (for review see Lesch and Mössner, 1998). The functional role of the 5-HTT in lymphoblasts is currently unknown. However, the recent generation of mice lacking the 5-HTT may reveal the role of the 5-HTT in these cells (reviewed in Mössner and Lesch, 1998).

In summary, the B lymphoblast model we have established displays several molecular and functional similarities to serotonergic neurons. Our B lymphoblasts may thus serve as a model for serotonergic neurons with respect to regulation of the 5-HTT by neurotrophins. A further similarity between B lymphoblasts and neurons is the finding that the I/l genotype of the 5-HTTLPR leads to more 5-HTT mRNA not only in B lymphoblasts (Lesch et al., 1996) but also in postmortem human midbrain (Little et al., 1998).

Thus, regulation of the variable region of the 5-HTT promoter appears similar in neurons and B lymphoblasts. Finally, allelic variation of the 5-HTTLPR can be modelled in the B lymphoblast system, which is difficult to achieve in conventional animal models, given that the 5-HTTLPR is only present in humans and non-human primates (Lesch et al., 1997).

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