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JB Commentary

Low-molecular-weight compounds having neurotrophic activity in cultured PC12 cells and neurons

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Recent reports have indicated that some low-molecular-weight compounds mimic neurotrophic factors inducing neurite outgrowth and neuroprotection. Carnosic acid (CA) promotes neurite outgrowth through the activation of Nrf2 in PC12 cells. CA also protects neurons via the keap/Nrf2 transcriptional pathway from oxidative stress. Forskolin-induced neurite outgrowth is mediated by activation of the PKA signalling pathway and this PKA-mediated neurite outgrowth is achieved by the expression of nur77 in PC12 cells. In addition, forskolin at its low concentration is closely related to the cAMP-induced protective function against L-DOPA-induced cytotoxicity in PC12 cells. A HDAC inhibitor trichostatin A (TSA) increases neurite length via p53 acetylation in rat cultured cerebellar granule neurons and in cerebral cortical neurons, and also protects neurons against glutathione depletion-induced oxidative stress. Recently, it was revealed that Nrf2 and p53 bind to CBP/p300 directly, and Nur77 is acetylated *in vivo* and *in vitro* by CBP/p300. Acetylation of Nrf2, p53 and Nur77 by CBP/p300 may constitute a novel similar regulatory mechanism for low-molecular-weight compounds with neurotrophic activities.

Keywords: carnosic acid/forskolin/HDAC inhibitor/neurite outgrowth/PC12 cell.

Abbreviations: BDNF, brain-derived neurotrophic factor; CA, carnosic acid; CBP/p300, CREB-binding protein/p300; dbcAMP, dibutyryl cyclic AMP; ERK1/2, extracellular signal-regulated kinase1/2; HDAC, histone deacetylase; IEG, immediate early gene; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; Nrf2, NF-E2-related factor 2; NT-3, neurotrophin-3; NT-4/5, neurotrophin-4/5; Nur77, nuclear hormone receptor 77; PB, sodium phenyl butyrate; P/CAF, p300-CBP-associated factor; PI3-K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PLC, phospholipase C; Trk, tropomyosin-receptor-kinase; TSA, trichostatin A; ZIP, PKC-zeta interacting protein.

Knowing the precise mechanisms of neurotrophic processes, including neurite outgrowth and neuronal survival, is important to study brain development and the treatment of various neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Signalling mechanisms controlling neurite outgrowth and neuronal survival have not been well studied and are likely complex processes, requiring the interplay of many signalling events. It has been reported that neurotrophic factors, NGF, BDNF, NT-3 and NT-4/5, have profound effects on neurons, including the promotion of differentiation and survival. The specific receptors of neurotrophic factors NGF, BDNF plus NT-4/5 and NT-3 are TrkA, TrkB and TrkC, respectively. These trk families are membrane-spanning receptors on the cell membrane of neurons (*1*).

Intracellular signalling pathways contain several protein phosphorylation cascades and at least three signalling pathways have been identified downstream of Trk receptors: the Ras/mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway and the PLC-gamma pathway (*2*); however, the delivery of exogenous neurotrophic factors is the greatest obstacle for their therapeutic application since neurotrophic factors are large polypeptide molecules that do not penetrate the blood–brain barrier (BBB) and are easily metabolized by peptidases when administered peripherally. Recently, low-molecular-weight compounds that can mimic the function of neurotrophic factors and substitute for their clinical use as an alternative approach have been identified. In this review, we present recent topics regarding low-molecular-weight compounds, which have neurotrophic activity in PC12 cells and neurons.

Plants produce a variety of hydrophilic compounds, and some compounds have neurotrophic activity. Kosaka *et al.* (*3*) recently revealed that a hydrophilic antioxidant, carnosic acid (CA), originating from rosemary, promotes neurite outgrowth in PC12 cells. They also revealed that CA-activated Nrf2-induced p62/ZIP expression is essential for neuronal differentiation of PC12h cells. They reported that CA-activated ERK1/2 and PI3-K independent of Nrf2 activation and activation of these kinases leads to the enhancement of Nrf2 accumulation in CA-mediated neuronal differentiation in PC12h cells (*3*). They concluded that Nrf2 contributes to CA-induced neuronal differentiation via the induction of p62/ZIP expression. In addition to the

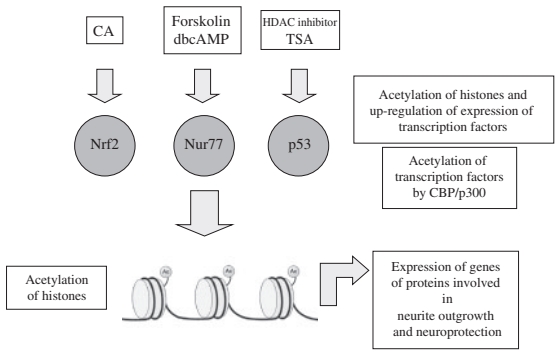

Fig. 1 Schematic representation of signalling events induced by neurotrophic low-molecular-weight compounds. Carnosic acid (CA) and dibutyryl-cAMP (dbcAMP) up-regulate the expression of Nrf2 and Nur77, respectively. Trichostatin A (TSA) induces the acetylation of p53. It is also known that Nrf2, Nur77 and p53 are acetylated by CBP/p300. The detailed relationship between transcriptional activities of Nrf2, Nur77 and p53 and epigenetic regulation of the expression of genes of proteins involved in neurotrophic activities has yet to be revealed.

effect on neurite outgrowth, CA showed neuroprotective activity both *in vitro* and *in vivo* from glutamate/oxidative stress and cerebral ischemia. Satoh *et al.* (*4*) found that CA activates the keap/Nrf2 transcriptional pathway by binding to specific keap1 cysteine residues, thus protecting neurons from oxidative stress and excitotoxicity.

Luteolin, another ingredient of rosemary, also has neurotrophic activity. Lin *et al.* (*5*) revealed that luteolin promotes neurite outgrowth in PC12 cells and protects PC12 cells from serum withdrawal-induced oxidative stress through Nrf2-mediated transcriptional activation of HO-1.

Forskolin is a labdane diterpene that is produced by the Indian Coleus plant. It is an adenylate cyclase activator and is used to raise the level of cyclic AMP (cAMP). In addition, it is known to be a BBB permeant and has effects on neurite outgrowth and neuronal promotion. Hansen *et al.* (*6*) reported that forskolin-induced neurite outgrowth of PC12 cells is mediated by activation of the cAMP-dependent protein kinase (PKA) signalling pathway and synergistic activation of the MAPK (ERK) signalling pathway. Jin *et al.* (*7*) suggested that forskolin at its low concentration is closely related with the cAMP-induced protective function against L-DOPA-induced cytotoxicity and that forskolin at its high concentration induces the cAMP-mediated apoptotic process, which enhances L-DOPA-induced cytotoxicity in PC12 cells.

Hormones and neurotransmitters that increase the intracellular level of cyclic AMP have been shown to induce neuronal differentiation. This process is mediated by PKA activation, which leads to neurite extension or creates new synaptic connections. Thus, some hydrophobic derivatives of cAMP, such as dibutyryl-cAMP (dbcAMP) and the adenylate cyclase activator forskolin, have been described as neurotrophic low-molecular-weight compounds in several neuronal types and other cellular systems. It has also been reported that dbcAMP promotes neurite outgrowth in human neuroblastoma SH-SY5Y cells and PC12 cells (*8*, *9*). In addition, intracellular cAMP protects against oxidative stress when used alone and in association with neurotrophic factors, NGF and EGF in PC12 cells (*10*).

We have reported that treatment with dbcAMP leads to the expression of immediate early genes (IEGs), including c-fos and nur77, as does treatment with NGF in PC12 cells. We have also observed that their expressions are regulated via the PKA pathway and the acetylation of histone H3 (*9*). It is thought that detailed studies on low-molecular-weight compounds with neurotrophic activity will be necessary for advances in this field.

It is known that histone deacetylase (HDAC) inhibitors, which are known as anticancer agents, also have the ability to induce neurite outgrowth and neuroprotection. In addition, it has been reported that HDAC inhibitors. such as trichostatin A (TSA), vorinostat, sodium phenyl butyrate (PB), MS275 and valproic acid, penetrate the BBB (*11*, *12*). We consider that HDAC inhibitors are also included in neurotrophic low-molecular-weight compounds. Gaub *et al.* (*13*) revealed that HDAC inhibitors, TSA and PB, induced a significant increase in total average neurite length in rat cultured cerebellar granule neurons and in cerebral cortical neurons. They also showed that TSA induced the acetylation of p53 at K373 and K320 residues, and TSA-dependent acetylation of p53 led to the expression of GAP-43 and Coronin 1b, which are known as the target genes of p53 (*13*). Ryu *et al.* (*14*) showed that treatment with HDAC inhibitors, TSA, PB or vorinostat, protected against glutathione depletion-induced oxidative stress.

Post-translational modification, such as acetylation of transcription factors, is an important process in the regulation of gene expression (*15*). Control of transcription by epigenetic modifications has proven to be important for neurite outgrowth and neuroprotection during neuronal development in the peripheral nervous system. Increased acetylation of histone and transcription factors in neurons has been shown to promote neural differentiation, whose molecular mechanisms are partially shared during neurite outgrowth. It is thought that neurotrophic activities of these low-molecular-weight HDAC inhibitors are also mediated by epigenetic regulation of histone proteins as well as non-histone proteins.

Recent findings have shown that many non-histone proteins, particularly transcription factors, are substrates for CBP/p300, greatly expanding the possible mechanisms of CBP/p300 in transcriptional activation (*16*). Tedeschi *et al.* (*17*) and Giovanni *et al.* (*18*) showed that p53 forms transcriptional complexes with CBP/p300 and C/CAF on the promoters of genes, such as GAP-43 and Coronin 1b. Gaub *et al.* (*13*) also showed that TSA induced neurite outgrowth as both permissive and inhibitory substrates for the induction of gene expression via histone H3 K9-14 acetylation on the promoters of CBP/p300 and P/CAF, which in turn promote neurite outgrowth and mediate H3K9-14 as well as p53 acetylation.

As well as p53, Sun *et al.* (*19*) revealed that CBP/p300 directly bound to and acetylated Nrf2 in response to arsenite-induced oxidative stress. Acetylation of

Nrf2 by CBP/p300 showed the possibility to constitute a novel regulatory mechanism for Nrf2-dependent neurotrophic activity (*19*). Nur77 is acetylated *in vivo* and *in vitro* by CBP/p300 detected using acetylation-specific antibodies, i.e., anti-Pan-acetyl and anti-acetylated Lys antibodies (*20*). We reported that Nur77 was involved in dbcAMP-induced neurite outgrowth in PC12 cells. Acetylation of Nur77 by CBP/p300 may also constitute a novel regulatory mechanism for Nur77-dependent neurotrophic activity. As shown in Fig. 1, we propose a novel similar regulatory mechanism by which low-molecular-weight compounds induce neurite outgrowth and neuroprotection. Low-molecular-weight compounds may lead to acetylation of histones and thus induce the expression of transcription factors. And the increased transcription factors may be acetylated by CBP/p300. Both acetylated transcription factors and acetylated histones may lead to the increased expression of genes of proteins, which are involved in neurite outgrowth and neuroprotection (Fig. 1).

It is expected that the detailed relations between neurotrophic activities of low-molecular-weight compounds and specific epigenetic regulation of gene expression will be revealed in the future.

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Conflict of interest

None declared.

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