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BRAIN RESEARCH

Research Report

GDNF abates serum deprivation-induced tyrosine hydroxylase Ser19 phosphorylation and activity

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ABSTRACT

High dopamine levels can contribute to neuronal dysfunction, impair plasticity and be toxic to neuronal cells in pathological conditions. The synthesis of dopamine is regulated by phosphorylation of the rate-limiting enzyme tyrosine hydroxylase (TH) under physiological conditions, with the phosphorylation of Ser31 and Ser40 directly increasing TH activity. Although a third phosphorylation site, Ser19, does not appear to directly regulate TH activity in physiological conditions, its role in pathological conditions is poorly understood. In this study, we examined the effects of serum deprivation (to mimic loss of retrogradely/anterogradely transported target-derived neurotrophic factors following axonal injury) and glutamate receptor stimulation (to mimic excitotoxicity) on TH phosphorylation and activity in a cell line and in mesencephalic primary culture cells. In addition, we also tested whether glial cell line-derived neurotrophic factor (GDNF) can alter these changes. We demonstrate that serum-deprivation resulted in a sustained increase in Ser19 phosphorylation beginning at 3 h and lasting up to 10 h without any detectable change in Ser31 or Ser40 phosphorylation within this time frame. This increase in Ser19 phosphorylation was associated with enhanced TH activity and was due, in part, to glutamate-receptor-mediated calcium influx and possibly calcium/calmodulin-dependent protein kinase II (CaMKII) activation. Interestingly in this serum-deprivation model, GDNF blocked the increase in Ser19 phosphorylation and TH activity at the 10-h time point following serum deprivation. Furthermore, GDNF also blocked the glutamate-mediated increase in Ser19 phosphorylation in rat primary mesencephalic neuronal cultures. Taken together, these findings suggest that GDNF may reduce dopamine synthesis in pathological conditions.

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Abbreviations:
CaMKII, calcium/calmodulin-dependent protein kinase II
ERK, extracellular signal-regulated kinase
GDNF, glial cell line-derived neurotrophic factor
PKA, protein kinase A
PKC, protein kinase C
PRAK, p38-regulated/activated kinase
RA, retinoic acid
TH, tyrosine hydroxylase

1. Introduction

The regulation of dopamine signaling is critical for proper neurological function and plasticity (Burke et al., 2004; Goldman-Rakic, 1998; Mani et al., 2000; Zigmond et al., 2002). Dopamine synthesis is dependent on the activity of the rate-limiting enzyme tyrosine hydroxylase (TH). TH activity is directly regulated by phosphorylation at Ser31 and Ser40 (Dunkley et al., 2004; Haycock, 1993; Zigmond et al., 1989). Both in vitro and in situ experiments have shown that phosphorylation of Ser31 by the extracellular signal-regulated kinase (ERK) and Ser40 by protein kinase A (PKA, also by PKC and PKG) increases in response to physiological stimuli (Haycock, 1990; Haycock et al., 1992; Lew et al., 1999; Lindgren et al., 2002; Salvatore et al., 2001; Sutherland et al., 1993; Waymire et al., 1991). These increases in Ser31 and Ser40 phosphorylation are accompanied by enhanced TH activity. Physiological stimuli such as neuronal depolarization that increase intracellular calcium enhance Ser19 phosphorylation, which is thought to result from an increase in calcium/calmodulin-dependent protein kinase activity (CaMKII) (Bunn et al., 1995; Campbell et al., 1986; Isobe et al., 1991; Salvatore et al., 2001). In addition, stressful stimuli have been shown to increase Ser19 phosphorylation via activation of stress-activated protein kinases such as p38MAPK and p38-regulated/activated kinase (PRAK) (Thomas et al., 1997; Toska et al., 2002a). The phosphorylation of Ser19 has been reported to enhance the interaction between TH and the chaperone protein 14-3-3 (Kleppe et al., 2001; Toska et al., 2002a; Yamauchi and Fujisawa, 1981).

Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for dopaminergic neurons. It has been shown that GDNF administration protects neurons under pathological conditions such as serum deprivation, neurotoxin- and axotomy-induced death as well as aging-associated dopaminergic cell loss (Baumgartner and Shine, 1997; Gash et al., 1996; Kearns and Gash, 1995; Keir et al., 2001). This neuroprotection is thought to occur through the upregulation of anti-apoptotic mechanisms (Perrelet et al., 2002). It has been recently demonstrated that GDNF can regulate the activity of TH both in cultured cells as well as in vivo (Kobori et al., 2004; Salvatore et al., 2004). For example, exposure of cultured mesencephalic neurons to GDNF resulted in acute increases in Ser31 and Ser40 phosphorylation that were associated with increased TH activity (Kobori et al., 2004). Furthermore, a single intrastriatal administration of GDNF to normal, aged rats resulted in an increase in basal Ser31 phosphorylation and TH activity which persisted for up to a month following the infusion (Salvatore et al., 2004). In addition to Ser31, Ser19 phosphorylation was also enhanced in these aged rats in response to GDNF. Although Ser19 phosphorylation does not appear to directly regulate TH activity in response to physiological stimuli (Haycock et al., 1998), its role in regulating TH activity under pathological conditions has not been fully explored.

In the present study, we used serum deprivation of a differentiated TH-expressing human neuroblastoma cell line as a model of loss of trophic factor support resulting from axonal injury to examine its effect on TH phosphorylation and activity. Axons of modulatory neurotransmitter systems, such as the dopaminergic system, are long and thus are more vulnerable to damage as a result of head trauma. As neurons depend on target-derived neurotrophic factors such as GDNF for survival, axonal damage will prevent retrograde transport of these factors and can contribute to neuronal death (Tomac et al., 1995; Henderson et al., 1994). Similarly, the death of the target neuron, as can happen in conditions such as ischemia, also results in the reduction of trophic factor support for the presynaptic as well as postsynaptic cells. We present results to show that serum deprivation causes a delayed but sustained increase in Ser19 phosphorylation that is associated with enhanced TH and CaMKII activities. This effect could be blocked by glutamate receptor antagonists, as well as by GDNF treatment. Further, we demonstrate that glutamate receptor activation in primary mesencephalon neurons (to mimic glutamate toxicity) also enhances Ser19 phosphorylation, and this effect could be blocked by GDNF treatment. This suggests that under pathological conditions, Ser19 phosphorylation may increase TH activity, and that GDNF may offer cellular protection by reducing glutamate-mediated calcium influx and TH activity.

2. Results

2.1. *Serum starvation of BE(2)C cells increases Ser19 phosphorylation that is reduced by GDNF*

Retinoic acid (RA) exposure has been shown to cause differentiation of BE(2)-C cells (Bunone et al., 1995; Ross et