EFFECTS OF SEROTONIN ON TYROSINE HYDROXYLASE AND TAU PROTEIN

IN A HUMAN NEUROBLASTOMA CELL LINE

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ABSTRACT

The direct effects of the neurotransmitter serotonin on the catecholaminergic enzyme, tyrosine hydroxylase and the microtubule-associated tau protein were studied in a human neuroblastoma cell line. Undifferentiated LAN-5 cells, cultured in serum supplemented basal medium, or cells induced to differentiate by 6 day exposure to 10 μM retinoic acid were treated for 48 hr with 50 nM and 50 μM serotonin. In undifferentiated cells, serotonin treatment (50 μM) decreased both tyrosine hydroxylase activity and a 50 kD cytoplasmic fraction tau protein while 50 nM serotonin treatment caused this 50 kD protein to increase in the cytoplasmic fraction but decrease in the membrane fraction. While basal tyrosine hydroxylase activity increased in differentiated vs. undifferentiated cells, serotonin treatment had no effect on the enzyme or tau in differentiated LAN-5. This study shows serotonin to have direct effects on the biochemistry and cytoskeleton of undifferentiated cultured human neuroblastoma.

Key words: serotonin, neuroblastoma, tyrosine hydroxylase, microtubules, tau protein, retinoic acid, differentiation.

INTRODUCTION

The best known cytoskeletal functions in neurons include axoplasmic transport, cell contractility and, during development, nerve fiber outgrowth. Some of these actions

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are related or depend on specific neurotransmitters: for example, dopamine (together with Ca\(^{++}\) and cAMP) regulates elongation and contraction of retinal rods and cones (Burnside and Deary, 1986; Burnside, 1988). Microtubules, major cytoskeletal components, are involved in intracellular transport of cell products and possibly in their secretion and absorption as well (Burgess and Kelly, 1987; Pfeffer and Rothman, 1987). It may be expected, then, that changes in microtubule assembly and disassembly, perhaps promoted by specific proteins such as tau proteins, influence metabolism and transport of neurotransmitters. Reciprocally, neurotransmitters may affect microtubular and associated proteins and, thereby, regulate their own transport and secretion and that of other neurotransmitters. These experiments represent a first attempt to explore some neurotransmitter-microtubule interrelations.

Serotonin [5-hydroxytryptamine (5-HT)] effects have been implicated in both biochemical and cytoskeleton regulation. 5-HT-mediated mechanisms may exert an inhibitory control of noradrenaline metabolism in the locus ceruleus (Renaud et al., 1975; Lewis et al., 1976; Crespi et al., 1980; Devau et al., 1987) and in microtubule-dependent induced stimulation of corticosteroid production (Feuilloley et al., 1988). Moreover, the amine appears associated with microfilaments and microtubules, particularly in developing neural cells (Emanuelsson et al., 1988).

Evidence that 5-HT exerts an inhibitory control on tyrosine hydroxylase (TH) (the rate-limiting enzyme in the catecholamine biosynthesis pathway) activity in noradrenergic neurons of the locus ceruleus (LC) is given by the following biochemical studies: (a) Destruction of specific raphe nuclei which send serotoninergic projections to the LC resulted in an increase in TH activity in the LC region (Lewis et al., 1976). (b) Destruction of serotonergic neurons by 5,6-dihydroxy-tryptamine increased TH activity in rat LC (Renaud et al., 1975; Keane et al., 1978). (c) Inhibition of tryptophan hydroxylase, the rate limiting enzyme of 5-HT synthesis, by parachlorophenylalanine administration, produced a significant increase in TH activity in the rat LC (Crespi et al., 1980). (d) Recently, Devau et al. (1987) have directly demonstrated the inhibitory role of 5-HT on TH activity in in vitro cultured explants of newborn rat LC.

While microtubules have been implicated in the coupling of corticosteroid secretion in adrenal cells in response to 5-HT (Feuilloley et al., 1988), and may play a role in cell-shape changes and morphogenesis in the early chick embryo (Emanuelsson et al., 1988), there are no reports in the literature on the effects of 5-HT on TH or microtubules in neuroblastomas.

In this investigation we used a human neuroblastoma cell line, LAN-5, which has adrenergic properties and can be induced to differentiate (Sidell et al., 1983; 1984; Cole and Timiras, 1987), as a tissue culture model to study the direct effects of 5-HT on TH activity and tau protein, a component of neural microtubules, which acts in vivo chiefly to induce the assembly of tubulin and in vitro to promote microtubule polymerization (Drubin & Kirschner, 1986).