Vol. 86, No. 4, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1979 Pages 1058-1062

THE RECEPTOR-MEDIATED, SHORT-TERM ACTIVATION OF TYROSINE HYDROXYLASE IN ORGAN CULTURES OF RAT SUPERIOR CERVICAL GANGLIA

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Received January 9,1979

Summary

Tyrosine hydroxylation, measured *in situ* in superior cervical ganglia from neonatal rats, is increased two-fold by the addition of carbachol. This increase occurs within one hour and is not due to the synthesis of new enzyme protein, nor to a direct effect of carbachol on the enzyme. The stimulation is blocked by atropine, but not by hexamethonium. This stimulation represents the first report of a receptor-mediated short-term action on this rate-limiting enzyme.

Introduction

Tyrosine hydroxylase, the enzyme which catalyzes the rate-limiting step in the biosynthesis of catecholamines, appears to be regulated by both long-term and short-term mechanisms. As might be expected, the long-term regulation occurs via changes in the rates of synthesis and degradation of the same enzyme species (1-4). Short-term regulation seems to be more complicated. A large number of studies have been aimed at identifying the factors responsible for the minute-to-minute regulation of this pivotal enzyme. A variety of experimental systems have been used to gain information on this subject; these range from intact nerve-muscle preparations to highly purified enzyme (5-13).

To date, a number of agents have been shown to stimulate tyrosine hydroxylase activity in cell-free systems. It has been substantially more difficult to demonstrate rapid stiumlation of this enzyme *in vivo*, or in experimental systems where cellular integrity has been preserved, although a few such reports have now appeared. Recently,

0006-291X/79/041058-05$01.00/0

it has been shown that stimulation of tyrosine hydroxylase activity within the guinea pig hypogastric nerve occurs in response to electrical stimulation (5). Also, others have demonstrated that *in situ* tyrosine hydroxylase activity in cloned pheochromocytoma cells can be stimulated by high K^+ depolarization or by exogenously administered cyclic AMP (6).

The present report extends our knowledge of the manner in which tyrosine hydroxylase may be rapidly regulated by describing how the *in situ* activity of this enzyme in cultured rat superior cervical ganglia may be stimulated via a receptor-mediated event.

Materials and Methods

Five day old Sprague-Dawley rats (Zivic-Miller, Allison Park, Pa.) were the source of the superior cervical ganglia used in this study. The ganglia were removed, cleaned, decapsulated, and placed in culture as previously described (14). For the *in situ* experiments five ganglia were incubated together in 250 µl portions of BGJ_b medium (Fitton-Jackson modification), containing 10µM L-tyrosine and approximately 350,000 cpm of L-(3,5-^{3}H)tyrosine in an atmosphere of 95% O_2 and 5% CO_2. The incubations were carried out in a humidified incubator at 37° for 60 min after which the tissues and the media were homogenized using all glass homogenizers. Then 100µl of 25% trichloroacetic acid were added and the mixture transferred to Dowex-50 columns (0.6 x 5 cm) for collection of ^3H_2O as a measure of tyrosine hydroxylation. The columns were rinsed with 1 ml portions of water and the combined eluates counted with 15 ml of Aquasol. The results are expressed as total activity (nmol ^3H_2O formed/hr/five ganglia). The tyrosine hydroxylase activity in cell-free extracts of the ganglia was measured by the method of Nagatsu *et al*. (15). For all experiments tritiated tyrosine was re-purified over a Dowex-50 column (15).

Results

The *in situ* activity of tyrosine hydroxylase in cultured rat superior cervical ganglia can be stimulated by the ganglionic agonist carbachol to a value of twice that of the control activity. A representative experiment is shown in Table 1. The fact that this stimulation is totally blocked by atropine, but is unaffected by hexamethonium indicates that the stimulation occurs through activation of the muscarinic class of cholinergic receptors. To verify that it is, in fact, tyrosine hydroxylation that is being measured it was shown in separate experiments that the addition of 0.1 mM 3-iodotyrosine, a specific inhibitor of tyrosine hydroxylase, blocked the release of ^3H_2O completely.

In another experiment it was shown that the inclusion of the protein synthesis inhibitor, cycloheximide, at 10 µg/ml, a concentration known to block protein synthesis

TABLE 1: Stimulation of tyrosine hydroxylation in rat superior cervical ganglia by carbachol

Condition	Tyrosine hydroxylation	Per cent activity
	pmol ^3H_2O/hr/five ganglia	%
Control	1.6	(100)
Carbachol (5 mM)	3.2	200
Carbachol (5 mM) + atropine (0.1 mM)	1.7	106
Carbachol (5 mM) + hexamethonium (0.1 mM)	3.8	237

Comparable incubations without ganglia or in the presence of 0.1 mM 3-iodotyrosine gave blank values amounting to 1.9 pmoles ^3H_2O/hr. Values represent the average of duplicate wells. The carbachol stimulation has been observed in 11 experiments and the inhibition by atropine in 4.

in the ganglia (16), did not affect control tyrosine hydroxylation (1.2 pmoles/hr) and allowed an 83% stimulation by carbachol. Thus, it appears that this receptor-mediated activation of tyrosine hydroxylase is not dependent upon the synthesis of new protein. Finally, it should be noted that when tyrosine hydroxylase activity was measured in cell-free extracts from five ganglia, the activity observed was somewhat higher than that seen *in situ* (3.6 pmoles/hr). Addition of 5 mM carbachol to the assay mixture had no effect.

Discussion

A wide variety of agents and conditions have been shown to stimulate tyrosine hydroxylase activity in cell-free preparations. These include mucopolysaccharides (8) and anionic phospholipids (9), partial proteolytic digestion (10), and exposure to cAMP-dependent phosphorylating conditions (11,12). In each of the above cases, the stimulation observed appears to be largely due to a decrease in the K_m of the enzyme for its pterin cofactor. Since the concentration of endogenous pterins in highly adrenergic tissues is low (17) and may, in fact, limit tyrosine hydroxylase activity (18,19), mechanisms which improve the K_m of this enzyme for its cofactor are likely to be of considerable physiological importance.

In order to determine whether these cell-free actions have any physiological meaning it is necessary to know their effects on tyrosine hydroxylation in the cell. Only very recently has the rapid stimulation of *in situ* tyrosine hydroxylation been demonstrated. Weiner and coworkers observed that electrical stimulation of the vas deferens-hypogastric nerve preparation is accompanied by a 2-3 fold increase in *in situ* tyrosine hydroxylase activity (5). This activation could be blocked by removal of Ca^{++} from the incubation medium and could be mimicked by the addition of a stable analog of cAMP. After stimulation, inspection of crude tyrosine hydroxylase preparations made from the tissue showed that the activation was due to a lowering of the K_m of the enzyme for its pterin cofactor.

Using a neoplastic tissue, PC-12 cells derived from a transplantable rat adrenal medullary pheochromocytoma, Greene and Rein have shown that *in situ* tyrosine hydroxylation in these cells can be rapidly increased 2-3 fold by addition to the culture medium of either high K^{+} (52 mM) or 1.0-2.0 mM dibutyryl cAMP (6). These stimulatory effects also required the presence of Ca^{++} in the extracellular medium and were not affected by the presence of an inhibitor of DOPA decarboxylase.

The significance of the present report is the observation that a rapid activation of tyrosine hydroxylase can occur via a receptor-mediated event. It will be of interest to determine whether this stimulation is due also to an increased affinity of the enzyme for its cofactor, and whether it is acheived through a receptor-mediated activation of a cyclic nucleotide pathway. In the present study we observed that the tyrosine hydroxylating capacity of a cell-free extract of ganglia is at least twice that seen when hydroxylation is measured *in situ*. It should be noted that the cell-free system was fortified with 6,7-dimethyltetrahydropterin rather than the more active 6-substituted derivatives (20), so the hydroxylation capacity with saturating cofactor is probably even higher than that seen here. This observation is at least consistent with the suggestion that the activation occurs through an increase in the affinity of the enzyme for its cofactors. Elucidation of the mechanism involved in this stimulation is underway.

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