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ACTIVATION OF TUBULINYL-TYROSINE CARBOXYPEPTIDASE BY SPERMINE, SPERMIDINE AND PUTRESCINE

Héctor S. Barra and Carlos E. Argaraña

Departamento de Química Biológica, Facultad de Ciencias Químicas,
Universidad Nacional de Córdoba, Ciudad Universitaria,
5000-Córdoba, R. Argentina

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SUMMARY: Spermine, spermidine and putrescine produce dose dependent stimulation of the in vitro tubulinyl-tyrosine carboxypeptidase. Maximal stimulation was obtained with spermine, spermidine or putrescine at 0.06 mM, 1 mM and 6 mM, respectively. At higher concentrations, the enzyme activity was inhibited. The enzyme was also activated by Mg^{++}; the concentration for maximal effect was 4-6 mM. The stimulation produced by optimal concentration of each amine was unaffected by Mg^{++} up to 2 mM; higher concentration of Mg^{++} showed inhibitory effect. At optimal Mg^{++} concentration, the carboxypeptidase activity was inhibited by increasing amine concentration. The amines at 0.5 or 5 mM did not produce any effect on the incorporation of tyrosine catalyzed by tubulin tyrosine ligase.

INTRODUCTION

Tubulin undergoes a postranslational addition of tyrosine into the COOH-terminal glutamate of the α chain (1-3). The reaction is catalyzed by an ATP-dependent enzyme, tubulin tyrosine ligase (TTL) (4,5). The incorporated tyrosine residue can be removed by another enzyme we named tubulinyl-tyrosine carboxypeptidase (TTCPase). This enzyme was partially purified from rat and chick brain, being some of its properties characterized (6,7). During our studies on the regulation of the activity of these two enzymes we found that the naturally occurring amines, spermine and spermidine, and their precursor putrescine, enhance the TTCPase activity. The results obtained are described in the present communication.

MATERIALS AND METHODS

Chemicals: Spermine tetrahydrochloride, spermidine trihydrochloride and putrescine dihydrochloride were purchased from Sigma Chemical Co., St. Louis, Missouri. L-[U-^{14}C]Tyrosine (specific activity 450 μCi/umol) was obtained from New England Nuclear.

Preparation of tubulinyl-[^{14}C]tyrosine: Brains from 20-30-day-old rats were homogenized in 1 vol of 10 mM sodium phosphate buffer, pH 7, containing 0.24 M sucrose and centrifuged at 100,000 x g for 1 h. The supernatant solution was passed through a column of Sephadex G-25 previously equilibrated with 10 mM sodium phosphate buffer, pH 7. [^{14}C]Tyrosine was incorporated into

Abbreviations used: TTL, tubulin tyrosine ligase; TTCPase, tubulinyl-tyrosine carboxypeptidase.

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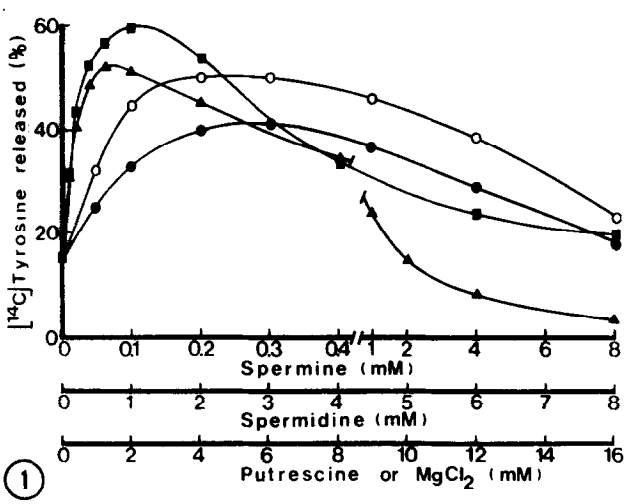
the tubulin present in the Sephadex excluded fraction by incubating, at 37°C for 30 min, a mixture containing 0.9 ml of this fraction, 2.5 µmol of ATP, 150 µmol of KCl, 12.5 µmol of $MgCl_2$ and 0.05 µmol (1 µCi) of [^{14}C]tyrosine, in a total volume of 1 ml. Tubulinyl-[^{14}C]tyrosine was purified by chromatography on DEAE-Sephadex using stepwise elution according to the method described by Murphy and Borisy (8) with slight modifications (9).

Preparation of TTCPase: TTCPase was partially purified from chick brain as previously described (7); the preparation used was that obtained after fractionation with ammonium sulfate.

Determination of TTCPase activity: The incubation mixture contained in a final volume of 0.2 ml: 0.2 mg of protein of the TTCPase preparation, 20 µg (2500 to 3500 cpm) of tubulinyl-[^{14}C]tyrosine and 1 µmol of sodium phosphate buffer, pH 7. The concentrations of $MgCl_2$ and amines, when present, are indicated in each experiment. The pH of the incubation mixture was 7. The radioactivity was measured in the hot trichloroacetic acid-insoluble material (4). The [^{14}C]tyrosine released was calculated as the difference between a blank run without enzyme and the experimental tube.

RESULTS AND DISCUSSION

The naturally occurring amines, spermine, spermidine and putrescine, commonly referred to as polyamines, produce dose dependent stimulation of the *in vitro* TTCPase activity (Fig. 1). Maximal stimulation of the carboxypeptidase by amines occurred at 0.06 mM spermine, 1 mM spermidine or 6 mM putrescine. At higher amine concentrations the TTCPase activity was inhibited. The effects of amines shown in Fig. 1 were obtained in the absence of Mg^{++}. It has been reported that Mg^{++} has an stimulating effect on the TTCPase activity (6). How-



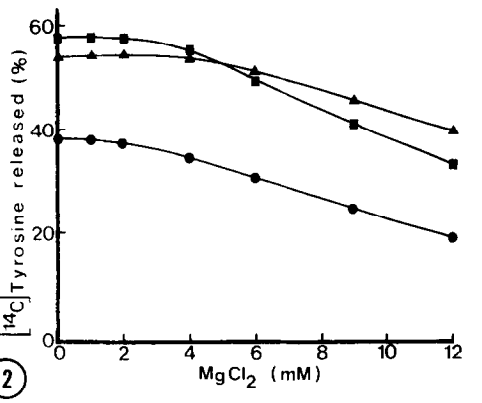


Fig. 1. Effects of putrescine (●), spermine (▲), spermidine (■), or Mg^{++} (O) on the TTCPase activity. The assay conditions were as described under "MATERIALS AND METHODS".

Fig. 2. Effects of increasing Mg^{++} concentrations on the TTCPase activity in the presence of 0.06 mM spermine (▲), 1 mM spermidine (■), or 6 mM putrescine (●). The assay conditions were as described under "MATERIALS AND METHODS".

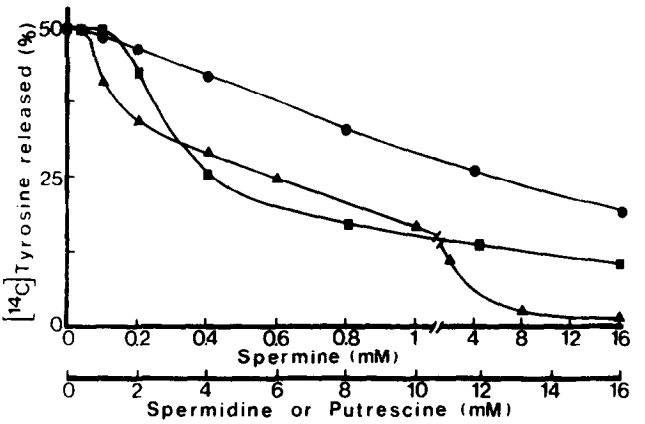


Fig. 3. Effects of increasing putrescine (●), spermine (▲), or spermidine (■) concentrations on the TTCPase activity in the presence of 4 mM Mg^{++}. The assay conditions were as described under "MATERIALS AND METHODS".

ever, the activation of chick brain TTCPase by Mg^{++} shown in Fig. 1 was higher than that previously reported for rat brain TTCPase (6).

Since some of the effects of amines have been attributed to their cationic nature, we tested the combined effects of Mg^{++} and amine. The effect of various Mg^{++} concentrations at optimal concentration of each amine is shown in Fig. 2. At low Mg^{++} concentration the stimulation of TTCPase activity by each amine did not show any significant change. From approximately 2 mM Mg^{++} the enzyme activity decreases by increasing the concentration of Mg^{++}. The effect of increasing amine concentrations at optimal concentration of Mg^{++} was also investigated. Fig. 3 shows that the TTCPase activity was decreased by increasing the amine concentration. An inhibition of 50 % is produced by approximately 0.6 mM spermine or 4 mM spermidine or 13 mM putrescine.

It is known that only a fraction of the brain tubulin molecules is tyrosinated *in vivo* (10-12). The degree of tyrosination of tubulin changes with the development of the animal (13) and it is supposed to depend on the relative activities of TTCPase and TTL. Thus, we investigated whether amines have some effect on the activity of the latter enzyme. The incorporation of [^{14}C]-tyrosine into tubulin catalyzed by TTL requires ATP, Mg^{++} and K^{+} as cofactors (4). We found that 0.5 or 5 mM of each amine had no effect on the rate of the *in vitro* incorporation of [^{14}C]tyrosine carried out under the conditions previously described (4). [^{14}C]Tyrosine incorporation was also carried out in the absence of Mg^{++} or K^{+}. No one of the three amines assayed at 0.5 and 5 mM was able to substitute the requirements for Mg^{++} or K^{+} (data not shown).

The amines, spermine, spermidine and putrescine are present in relatively high concentrations in mammalian nervous tissue. Data for the concentration of these amines in brain have been published for several species. A different

regional distribution for each amine was observed as well as variations of their concentrations during development (14-23).

The activation of TTCPase by spermine and spermidine was produced at concentrations of the amines that may be considered to be within the physiological ranges. This suggests that these two amines may have a regulatory function on the TTCPase activity. Putrescine, on the other hand, showed an activating effect on the TTCPase in a range where the optimal concentration was higher than those reported for this amine in most mammalian nervous tissues.

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