REGULATORY INTERRELATIONS BETWEEN GABA AND POLYAMINES. II. EFFECT OF GABA ON ORNITHINE DECARBOXYLASE AND PUTRESCINE LEVELS IN CELL CULTURE

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GABA added to rat hepatoma (HTC) cells in spinner culture at the time of induction of cell proliferation increased levels of ornithine decarboxylase (ODC) up to two- to threefold above that of control cells. The increases in ODC were also reflected by concomitant increases of intracellular putrescine levels, while spermidine and spermine were unchanged. GABA seems to have a direct stabilizing effect on ODC, since the turnover of the enzyme was slowed almost twofold when measured in cells treated with \(10^{-5}\) M GABA. The stabilizing effect is most pronounced for GABA, although some amino acids such as asparagine, glutamine, and lysine as well as some GABA analogues and homologues also tend to increase ODC but to a significantly lesser extent than GABA itself. GABA metabolites had no effect on ODC. S-Adenosylmethionine decarboxylase and tyrosine aminotransferase were not affected by the presence of GABA. The GABA effect on ODC may be important in certain types of cells for the regulation of polyamine biosynthesis.

INTRODUCTION

A number of regulatory mechanisms for ornithine decarboxylase (ODC) have been described in eukaryotic cells (1). One important control of this enzyme appears to be changes in ODC turnover as determined by measurements of its half-life \( (t^{1/2}) \). Significant changes in ODC half-lives in
various cell systems result from such factors as serum concentration (2) and induction of cell proliferation (3). In N18 mouse neuroblastoma cells, large changes in ODC half-life can be obtained by manipulating asparagine and glutamine concentrations in the medium (4). Certain competitive ODC inhibitors such as α-methylornithine (5) and α-hydrazinoornithine (6) stabilize the enzyme, slowing its turnover probably because of some interaction at the active site.

The previous report (7) demonstrated that there is a specific interrelation between increases of GABA in vivo in mouse brain and concomitant increases of putrescine. Using a system of cultured rat hepatoma (HTC) cells which have only trace intracellular levels of GABA, we have been able to demonstrate that high concentrations of exogenously added GABA have a direct effect on cellular ODC activity and consequently increase putrescine levels. We presume that this effect is the result of stabilization of the enzyme by GABA.

METHODS

Materials. HTC (rat hepatoma tumor) cells were grown in spinner culture in Swin's 77 medium supplemented with 10% calf serum as previously described (8). The various GABA analogues and homologues and other amino acids described in the text were purchased from Sigma Chemical Co. (St. Louis). α-Acetylenic GABA (4-amino-hex-5-ynoic acid) (RMI 71645) and gabaculine (5-amino cyclohexa-1,3-dienyl carboxylic acid) were synthesized at the Centre de Recherche Merrell International, Strasbourg, according to published procedures (9,10).

Enzyme Assays. Aliquots of cells were washed by centrifugation twice with phosphate-buffered saline at 4°C, sonicated, and assayed for enzyme activities in the crude cell sonicates. Ornithine decarboxylase activity was assayed as previously described (8). S-Adenosylmethionine decarboxylase was assayed as described by Pegg and Williams-Ashman (11) using a buffer (100 mM sodium phosphate, pH 7.2, 5 mM dithiothreitol, and 0.1 mM EDTA) supplemented with 2.5 mM putrescine, 0.4 mM S-adenosyl-L-methionine, and 1 μCi S-adenosyl[carboxyl-14C]methionine (60 mCi/mmol, Amersham). Tyrosine aminotransferase was assayed as previously described (12). Cell protein was measured by the fluorescamine method (13). The half-life (t1/2) of decrease in ornithine decarboxylase activity was calculated by linear least-squares reduction.

Metabolite Assays. All metabolites were measured in aliquots of cells which had been washed twice with phosphate-buffered saline and then disrupted by sonication in 0.9 ml HCl 0.1 N. Proteins were precipitated by adding 0.1 ml of 2 N perchloric acid. Polyamines were assayed by a modification (14) of the method of Marton and Lee (15) using a Durrum D-500 amino acid analyzer. Ornithine, GABA, and α-acetylenic GABA were also assayed by high-pressure liquid chromatography by the method of Lee (16). o-Phthalaldehyde was used as a detection reagent instead of ninhydrin.

RESULTS

Effect of GABA on ODC and Polyamines. ODC was induced in HTC cells by dilution of confluent cells into fresh medium with 10% calf serum.