IDENTIFICATION AND PARTIAL CHARACTERIZATION OF RABBIT BRAIN DEOXYURIDINE 5'-TRIPHOSPHATASE

REYNOLD SPECTOR AND BARBARA BOOSE

From the Departments of Medicine and Pharmacology,
University of Iowa College of Medicine
Iowa City, Iowa 52242

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Adult rabbit brain contains the enzymatic machinery to convert deoxyuridine to deoxyuridine triphosphate (dUTP). Although dUTP as dUMP can be readily incorporated into DNA in place of thymidine monophosphate, we detected no (3H)dUMP in newly synthesized (3H)DNA in adult rabbit brain after the intraventricular injection of (3H)deoxyuridine. Only (3H)thymidine was detected. The probable explanation for the lack of incorporation of uracil into adult rabbit brain DNA is the presence of a specific, high affinity dUTPase which converts dUTP to dUMP and PP. After homogenization and ammonium sulfate fractionation of adult rabbit brain (35 to 75% saturation), a high affinity, specific dUTPase was detected in the dialyzed enzyme preparation. The Km and Vmax of the dUTPase were 0.2 μM and 36 pmol/mg protein/min, respectively. No high affinity dUTPase activity was detectable in liver. In brain, another enzyme hydrolyzed dUTP and dTTP (NTPase) to their respective diphosphates. NTPase, unlike dUTPase, was not sensitive to heating at 65°C for five minutes. Thus, brain, like other tissues, contains a high affinity, specific dUTPase presumably to "sanitize" the cells of dUTP and, thus, protect the integrity of newly synthesized DNA.

INTRODUCTION

In our studies of the effects of methotrexate on DNA synthesis in adult rabbit brain in vivo (described in (23) and below), we found that [3H] deoxyuridine was converted to [3H] dTTP1 and subsequently incorporated

1 Abbreviations used: CSF, cerebrospinal fluid; dUMP, dUDP, dUTP, 2′ deoxyuridine 5′ mono-, di-, and triphosphate respectively; dTTP, 2′ deoxythymidine 5′ triphosphate; dGTP, 2′ deoxyguanosine 5′ triphosphate; NDP, NTP, nucleoside or 2′ deoxynucleoside 5′ di- or triphosphate.
into DNA in brain. Presumably, the [3H] deoxyuridine entered brain cells, was phosphorylated by thymidine kinase to [3H] dUMP and then converted by thymidylate synthetase to [3H] dTMP (23). Subsequently, the [3H] dTMP was phosphorylated by thymidine monophosphokinase and nucleoside diphosphokinase to [3H] dTTP (23). Although thymidine monophosphokinase and nucleoside diphosphokinase can phosphorylate [3H] dUMP to [3H] dUTP (12, 18), we found very little [3H] dUDP and [3H] dUTP in the brain after the intraventricular injection of [3H] deoxyuridine (see below). In bacteria, malignant cells and possibly liver, various deoxyuridine 5'-triphosphatases (dUTPase) are responsible for the absence of dUTP in the tissues (2, 9, 11, 13, 20, 26, 27). dUTP is harmful because, in DNA synthesis by both DNA α and β mammalian polymerases (7), dUTP can readily substitute for dTTP and be covalently incorporated into DNA (7). This "error" in DNA synthesis requires removal of the uracil by uracil-DNA glycosylase and subsequent DNA repair (7, 20). Thus, dUTPase presumably "sanitizes" the tissues of dUTP (2, 11, 13, 20, 26, 27).

In this manuscript, we report the presence of a specific high-affinity dUTPase and a nonspecific high-affinity nucleoside triphosphatase (NTPase) in adult rabbit forebrain. The products of the dUTPase and NTPase are dUMP and NDP respectively.

**EXPERIMENTAL PROCEDURES**

*Materials.* [6-3H] deoxyuridine [18 Ci/mmol], [6-3H] dUTP, [14.6 Ci/mmol] and [methyl-3H] dTTP, (20.5 Ci/mmol) were obtained from Amersham-Searle, Arlington Heights, Illinois. Spectrapor I dialysis tubing was obtained from Fisher Scientific, Chicago, Illinois. Methotrexate was obtained from Lederle, Pearl River, New York. Nucleosides, nucleotides, crude calf intestinal alkaline phosphatase (Type I), and crude snake venom phosphodiesterase (Crotolus atrox) were purchased from Sigma Chemical Company, St. Louis, Missouri. All studies were done on 3 to 6 month old New Zealand white rabbits who were fasted for approximately 1 hour before the start of each experiment.

*Intraventricular Injection Studies.* Under intravenous sodium pentothal anesthesia, rabbits were injected in the left lateral ventricle with 0.1 ml artificial CSF containing 23 µCi [3H] deoxyuridine and 0.4 µCi [14C] sucrose by methods previously described in detail (23). The [14C] sucrose was employed as a passive marker to assess the adequacy of the injection (23). These rabbits had been preinjected in the left lateral ventricle under sodium pentothal anesthesia with 0.1 ml artificial CSF containing 1.0 or 0.0 mg methotrexate 30 minutes previously. After 2 hours, the conscious rabbit was overdosed with pentothal and the heart was severed; then, as rapidly as possible, 1 ml cisternal CSF was taken and the whole brain, after removal of the choroid plexus, was weighed and homogenized in appropriate volumes (2.5 ml per gram brain) of chilled 1.2 N perchloric acid. At this point, duplicate aliquots of the CSF, brain homogenates and intraventricular injection solution were assayed for [3H] and [14C] (23). The nature of the [3H] in the perchloric acid-soluble and acid-insoluble pellet