TETRAHYDROBIOPTERIN ADMINISTRATION TO RHESUS MACAQUES
Its Appearance in CSF and Effect on Neurotransmitter Synthesis*

L. MILLER¹, T. INSEL,², M. SCHEININ³, J. ALOI²,
D. L. MURPHY², M. LINNOILA⁴, AND W. LOVENBERG¹

¹Section on Biochemical Pharmacology
NHLBI

²Clinical Neuropharmacology Branch
NIMH

⁴Clinical Director
DICBR, NIAAA
National Institutes of Health
NHLBI
Bethesda, Maryland 21205

and

³Department of Pharmacology
University of Turku
Turku, Finland

Accepted July 23, 1985

Tetrahydrobiopterin, the hydroxylase cofactor (BH₄) was administered (i.v. 20 mg/kg) to Rhesus monkeys. Within 90 min of its administration CSF cofactor levels increased significantly above baseline levels. Peak CSF levels were attained at 90–180 min time period following cofactor injection and returned to baseline gradually over the next 15 hrs. The increased brain cofactor levels had no apparent effect on synthesis of dopamine, norepinephrine or serotonin as evidenced by a lack of change in the levels of the metabolites homovanillic acid, 3-methoxy-4-hydroxyphenyleneglycol, and 5-hydroxyindoleacetic acid. The present results

Supported by Dystonia Medical Research Foundation, 9615 Brighton Way, Suite 416, Beverly Hills, California 90210

Abbreviations: BH₄, tetrahydrobiopterin; CSF, cerebrospinal fluid; 5-HIAA, 5-hydroxyindoleacetic acid; HAV, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenyleneglycol.

* Address reprint requests to: Dr. Walter Lovenberg, Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215.
using primates suggest no apparent effect of increased cofactor levels on monoame
amine biosynthesis. However, it remains to be explored whether monoamine syn-
thesis could be affected by increased cofactor levels in the pathological situation.

INTRODUCTION

Over the past few years there has been an emergence of interest in a possible role of tetrahydrobiopterin, hydroxylase cofactor (BH₄), in the pathophysiology of a number of neurological disorders such as Parkinson’s disease and dystonia (1, 2). It is well established that BH₄ is the cofactor for a number of monooxygenases, including tyrosine and tryptophan hydroxylases (3–5). These two enzymes catalyze rate-limiting steps in catecholamine and serotonin synthesis, respectively. With respect to dopamine metabolism it has been calculated that synthesis rates in vivo are only 1% of the total enzymatic capacity as examined in vitro under saturating conditions of substrate and cofactor (5). There are a number of factors to explain this apparent modulation of enzymatic activity in vivo. One factor is possibly limiting cofactor concentrations. At present there are both supporting (6, 7) and conflicting reports (8) on the potential limiting role of cofactor in dopamine synthesis. However, if cofactor concentrations are indeed limiting in vivo, then the possibility exists for therapeutic intervention in a number of neurological disorders where cofactor concentrations have been shown to be decreased (9, 10). Therefore, we decided to evaluate the ability of i.v. administered cofactor not only to cross the blood-brain barrier but also to affect synthesis of CNS neurotransmitters (dopamine, norepinephrine and serotonin) in primates.

EXPERIMENTAL PROCEDURE

The present study was performed with 5 chair adapted, male Rhesus monkeys weighing from 5–9 kg. Two days prior to experimentation each of the animals was removed from his home cage under ketamine HCl (10 mg/kg) anesthesia. A cannula (sterile PE-50 tubing) was inserted into the lumbar cerebrospinal canal and the animal was placed in an experimental restraining chair. The CSF cannulae were then connected to a fraction collector kept refrigerated at −20°C allowing continuous CSF sampling at a rate of 1.5 ml/90 min for the duration of the study. To establish baseline levels of monoamine metabolites (HVA, MHPG, and 5-HIAA) a continuous series of CSF samples were collected for 180 min intervals over a 24 hr time period just prior to BH₄ administration. At the time of experimentation BH₄ was administered to each monkey intravenously at a dose of 15–20 mg/kg. Prior to injection the concentration of cofactor was confirmed spectrophotometrically by determining the O.D. at 264 nm. The BH₄ as obtained from the laboratory of Dr. Schricks, Switzerland, was a mixture of 6R and 6S diastereoisomers with a relative proportion of about 2.5:1. The 6R-BH₄ is the naturally occurring form (11). BH₄ in the serial CSF samples were