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DISTRIBUTION OF PHYSOSTIGMINE AND METABOLITES IN BRAIN SUBCELLULAR FRACTIONS OF THE RAT*

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Summary

The distribution of ^{3}H-physostigmine (Phy) has been studied in the rat brain subcellular fractions at various time intervals following i.v. injection.

^{3}H-Phy or its metabolites rapidly accumulate into the cytoplasm of cells and penetrates the intracellular compartments. Kinetic studies of the subcellular distribution of radioactivity (RA) per gm of rat brain following i.v. injection of ^{3}H-Phy show peak concentrations at 30 min in all subcellular fractions with the exception of mitochondria. In the mitochondrial fraction the RA levels continue to rise from 4682 ± 875 DPM/gm at 5 min to 27474 ± 2825 DPM/gm at 60 min ($P<.05$). The cytosol contains the highest RA: 223341 ± 21044 DPM/gm at 30 min which declined to 53475 ± 3756 DPM/gm at 60 min. RA in synaptosome, microsomes and myelin increases from 5 to 30 min, and declines at 60 min. *In vitro* studies did not show a greater uptake of RA by the mitochondrial or synaptosomal fractions.

The finding of relatively high concentrations of RA in the mitochondrial fraction at 60 min increases the likelihood that Phy or its metabolites could interfere with the physiological function of this organelle.

Introduction

Physostigmine (Phy), a carbamate cholinesterase inhibitor, has been shown to be useful in the treatment of glaucoma, tricyclic antidepressant overdosage, and organophosphate poisoning (1,2). More recent studies have shown that Phy may improve memory function in Alzheimer's patients (3,4,5,6). The efficacy of Phy may be limited, however, by its pronounced side effects (7).

It is presumed that Phy benefits Alzheimer patients by enhancing muscarinic transmission in the cortical and subcortical cholinergic nuclei and terminals (8,9). The concentration of radioactivity in six areas of brain was

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reported after three different dosages of [^{3}H]-Phy (100, 500, and 650 µg/kg) (10). The rate of accumulation of RA in the hippocampus and cortex was significantly greater than in other brain areas at higher dosages. Phy could enter these brain regions and theoretically exert its action of cholinesterase (ChE) inhibition at the nerve endings (synaptosomes) where the enzyme has been localized (11). Physostigmine could also affect neurotransmission in Alzheimer's patients by serving as an acetylcholine (ACh) receptor agonist. In a recent study, Phy was also shown to function as a nicotinic receptor agonist with the ability to open membrane ion channels (12). This study also concluded that the site of action of the drug was on the extracellular segment of the ACh receptor complex. Recently, Scremin and Scremin have reported that Phy enhanced cerebral blood flow in ischemic cortex of rat brain and decrease in oxygen consumption (13).

Somani and Khalique (14), in a study elaborating the pharmacokinetics of Phy, have shown that the drug is rapidly concentrated in the rat brain: however, no information regarding the subcellular distribution is available. The objective of the present study is to determine the subcellular location of Phy in the rat brain. The knowledge of the accumulation of Phy into specific subcellular organelles would aid in the understanding of the mechanism of action and toxicity of the drug.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing approximately 160-200 g were used in the present study.

Materials

Physostigmine (Phy) (free base) was purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]-Phy was custom synthesized by Amersham Corporation (Chicago, IL). Sucrose was purchased from Fisher Scientific Co. (Fair Lawn, NJ). Ready-Solv EP was obtained from Beckman Instruments, Inc. (Fullerton, CA) as was Beckman BTS-450 tissue solubilizer. Methanol (HPLC grade) was obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI) and, Monophase-40 plus was obtained from Packard Instruments (Downers Grove, IL). Other chemicals used in experiments were purchased from the usual commercial sources.

Preparation of the [^{3}H]-Phy solution

Physostigmine is labelled with tritium on both ortho positions to the carbamate side chain on the aromatic ring of the physostigmine. [^{3}H]-Phy was diluted with unlabeled Phy to two different concentrations (189.3 µCi/200 µg/ml for *in vitro* studies and 3.8 µCi/µg/5 µl for *in vivo* studies). The solution was prepared using physiological saline (0.9% wt/vol) in which 10 µl of hydrochloric acid was included to assure that the solution was in an acidic pH range. The purity of Phy was assessed using high performance liquid chromatography (HPLC) using an ultra-violet detector and by also monitoring the [^{3}H]-Phy in the eluant. The solution used in all experiments was greater than 95% pure.