THE SYNTHESIS AND BIODISTRIBUTION OF A RADIOIODINATED
G\textsubscript{M1} GANGLIOSIDE DERIVATIVE

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INTRODUCTION

The development of two tracers, N-isopropyl-p-(I\textsuperscript{123})- iodoamphetamine (I\textsuperscript{123}-IMP) (1,2) and N, N, N'-trimethyl-N'--(2-hydroxyl-3-methyl-5-iodobenzyl)-1,3-propanediamine(I\textsuperscript{123})(I\textsuperscript{123}-HIPDM) (3) has resulted in a renewed interest within the realm of nuclear medicine in the measurement of cerebral blood flow. Of particular interest is the use of these radiopharmaceuticals for the early diagnosis of stroke. Using emission tomography with I\textsuperscript{123}-IMP, Hill and co-workers (4) were able to detect regions of cerebral impairment prior to their appearance as abnormalities on CT scans.

It is important to bear in mind that I\textsuperscript{123}-IMP and I\textsuperscript{123}-HIPDM distribute in the brain in proportion to cerebral blood flow and thus delineate regions of vascular insult indirectly; that is, infarcts appear as photon-deficient areas within a "hot" background. Contamination of the image by scattered photons, especially those resulting from the high-energy emissions of I\textsuperscript{124} impurities, and the superimposition of activity from normal brain tissue in adjacent planes, both make it difficult to define the boundaries of a lesion unless tomographic instrumentation is employed.

The development of a radiopharmaceutical which accumulates preferentially in damaged brain tissue might provide a more sensitive and selective method for the early detection of cerebral infarcts. Ganglioside G\textsubscript{M1} is a glycosphingolipid which appears to be involved in the repair process of damaged nerve tissue (5-7). When labelled with an isotope such as I\textsuperscript{123}, G\textsubscript{M1} might be useful as an infarct-avid tracer for the early diagnosis of stroke. The purpose of this study was
first to develop a rapid and efficient technique for labelling 
$G_{M1}$ with $^{125}$I. The stability and neuronal membrane binding 
properties were then studied in vitro. Finally the distribu-
tion of $I^{125}$ following injection of $I^{125}$ labelled $G_{M1}$ was 
studied in the mouse and, in preliminary fashion, in the 
gerbil infarct model. A more detailed description of the in 
vivo aspects of this work will appear elsewhere (8).

METHODS OF PROCEDURE

Labelling $G_{M1}$ with $^{125}$I. Direct iodination. Ganglioside 
$G_{M1}$ isolated from bovine brain by the method of Tettamanti 
and co-workers (9) was obtained from FIDIA Laboratories.
Initial attempts to label $G_{M1}$ with $^{125}$I were performed using 
the iodine monochloride method (10), modified as follows: The 
$I^{125}$ activity (1 mCi, adjusted to pH 7.4) was added to 0.5 ml 
methanol/chloroform (1:1) containing 16 $\mu$mol of $G_{M1}$. Addition 
of 1.6 $\mu$mol each of $I_2$ and $HgCl_2$, both in $CH_3OH/CHCl_3$, generat-
ed the ICI in situ. The reaction was terminated after 12 hr 
by the addition of an excess of sodium metabisulfite. After 
drying under nitrogen, the reaction mixture was resuspended 
in phosphate buffered saline (pH 7.4) and passed through a 
Sephadex G-50 column to separate the $I^{125}$ labelled $G_{M1}$ from 
inorganic $^{125}$I.

Radioiodination of a $G_{M1}$-tyramine conjugate. A method 
alogous to that reported by Klemm and co-workers (11) was 
used to synthesize the $G_{M1}$-tyramine conjugate. Oxidation of 
the terminal galactose residue of $G_{M1}$ was effected by addition 
of 30 $\mu$mol of KIO$_4$ to 20 $\mu$mol of $G_{M1}$ in 0.01 M pH 8.4 borate 
buffer. The reaction was terminated after 4 hr at 37°C by the 
addition of an excess of glycerol. The oxidized $G_{M1}$ micelles 
were purified by dialysis against pH 8.4 borate buffer and 
then reacted for 24 hr at 25°C with a 4-fold molar excess of 
both tyramine and 2 M NaBH$_3$CN in pH 8.4 borate. The reaction 
mixture was then dialyzed against borate buffer for 48 hr.

The $G_{M1}$-tyramine conjugate was labelled with $I^{125}$ using 
iodogen (12). Varying amounts of iodogen in methylene chloride 
were added to 5-dram glass vials and evaporated under nitrogen.