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Final Technical Report

The long-term objective is to develop blood-brain barrier (BBB) permeable m2-selective (relative to m1, m3, and m4) receptor-binding radiotracers and utilize these radiotracers for quantifying receptor concentrations obtained from PET or SPECT images of human brain. In initial studies, we concluded that the lipophilicity and high affinity prevented (R,S)-I-QNB from reaching a flow-independent and receptor-dependent state in a reasonable time. Thus, it was clear that (R,S)-I-QNB should be modified. Therefore, during the last portion of this funded research, we proposed that more polar heterocycles should help accomplish that. Since reports of others concluded that radiobromination and radiofluorination of the unactivated phenyl ring is not feasible (Newkome et al.,1982), we, therefore, explored during this grant period a series of analogues of (R)-QNB in which one or both of the six-membered phenyl rings is replaced by a five-membered thienyl (Boulay et al., 1995), or furyl ring.

The chemistry specific aims were to synthesize novel compounds designed to be m2-selective mAChR ligands capable of penetrating into the CNS, and develop methods for efficient radiolabeling of promising m2-selective muscarinic ligands. The pharmacology specific aims were to determine the affinity and subtype-selectivity of the novel compounds using competition binding studies with membranes from cells that express each of the five muscarinic receptor subtypes, to determine the ability of the promising non-radioactive compounds and radiolabeled novel compounds to cross the BBB, to determine the biodistribution, in-vivo pharmacokinetics, and in-vitro kinetics of promising m2-selective radioligands and to determine the distribution of receptors for the novel m2-selective radioligands using quantitative autoradiography of rat brain, and compare this distribution to the distribution of known m2-selective compounds.

THE FUNDAMENTAL ASPECT OF THE RESEARCH HAS BEEN THE CHEMISTRY PORTION. THEREFORE, SOME DETAILS OF THE CHEMISTRY REPORT ARE PROVIDED (January 1996-March 1999).

During this last funded research period, we have prepared the following:

a. 11 heterocyclic analogues of QNB

b. The enantiomers of 3-quinuclidinols were prepared.

c. Ethyl 2-(5-bromo-, 5-iodo- or 5-methylthienyl) glyoxalates were prepared from ethyl oxalyl chloride and aluminum chloride in carbon disulfide and 2-bromo-, 2-iodo- or 2-methylthiophene.

d. Ethyl (R,S)-alpha-hydroxy-alpha-2-(5-bromo-, 5-iodo- or 5-methylthienyl)-alpha-2-thienyl-acetates were prepared from ethyl heteroarylglycolates and 2-thienylmagnesium bromide.

e. Resolution of (R,S)-alpha-hydroxy-alpha-2-(5-bromo- or 5-iodothienyl)-alpha-2-thienylacetic acid. The racemic acids were obtained from the hydrolysis of their ethyl esters by treatment with excess sodium carbonate in water-ethanol. The racemic bromo- or iodo-acid derivative was added to quinine dissolved in boiling ethyl acetate, and maintained at room temperature overnight. The salt which crystallized was filtered and, after 5 recrystallizations from n-butanol, had a constant melting point. The bromo-derivative salt rotation is -53.50 and its acid(acid was liberated with 5 N hydrochloric acid) rotation is -13.40.The iodo-substituted salt rotation is -51.66 and its acid rotation is -12.84. The mother liquor from the first crystallization of the iodo-derivative-quinine salt was evaporated to dryness under reduced pressure and residue was

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recrystallized five times from ethyl actate-hexane. The iodo-salt rotation is -46.34 and its acid +12.84.

This overall project, Development of Gamma-Emitting Receptor Binding Radiotracers for Imaging the Brain has been supported to a great extent during a 17 year period by the DOE OER(DOE ER-61588; DOE ER-60649; DOE ER-60039, 1982-1999). Approximately 90 full publications have resulted from this work. We believe our small research group was remarkably productive. The full details of the chemistry work and the pharmacologic and pharmacokinetic characterization are presenteed in our publications. The publications listed below acknowledge support from DOE.

RESULTS INDICATING PROGRESS TOWARDS THE SPECIFIC AIMS.
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