IN VITRO AND EX VIVO EVALUATION OF N-METHYL BENZILATES AS POTENTIAL PET AGENTS FOR MUSCARINIC RECEPTORS

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The apparent association between muscarinic receptors (mAChR) and cognitive functions together with the strong association of damage to muscarinic areas of the brain and onset of certain dementias such as Alzheimers has prompted an effort to develop new mAChR imaging agents. Potent antagonists of mAChR, particularly piperidyl and tropanyl esters of benzilic acid (1,2) were used to generate a list of ligands amenable to straight-forward labeling with high specific activity carbon-11. These ligands were selected for further evaluation: 4-N-methylpiperidyl benzilate (NMPB), (+)-tropanyl benzilate (TRB), scopolamine and quinuclidinyl benzilate (QNB). The "nor" derivatives of NMPB, TRB and scopolamine (PB, nor-TRB, and nor-scopolamine) were also included to assess possible metabolites.

In order to avoid radiosynthesis of each prospective ligand, a potentially time consuming and expensive process, a screening procedure which would provide information on both binding affinity and *in vivo* distribution of the ligands was sought. The procedure employed consisted of two parts: 1) a traditional *in vitro* competitive binding assay using ^{3}H-QNB and mouse brain homogenates and 2) an *ex vivo* assay in which the availability of receptors was measured after *in vivo* injection of the ligand, sacrifice and *in vitro* binding of ^{3}H-QNB.

Table 1 contains the IC_{50} values obtained for each compound. These values indicated that QNB, scopolamine, NMPB, TRB and nor-TRB had potential as imaging agents. Demethylation reduces the affinity for scopolamine, TRB and NMPB but the greatest effect was observed for NMPB.

To validate the use of the *ex vivo* assay several studies were completed. First, it was determined that different ligands did affect the degree of receptor occupancy when equimolar solutions were administered. Table 2 summarizes the results for four of the ligands: TRB and nor-TRB were about twice as effective at blocking mAChR than the other ligands. Second, the effect of variation of moles ligand injected was determined. TRB occupies more receptors than either scopolamine or NMPB from 1×10^{-6} to 1×10^{-5} M (moles ligand/kg body weight). At 1×10^{-7} M, no differences between control (saline) and experimental tissues could be detected for any of the ligands. Third, the effect of time on receptor occupancy was examined. Receptor occupancy for QNB and TRB was determined in both brain and heart. TRB, at 2 min post injection, occupied about the same number of receptors as QNB, however the availability of receptors for ^{3}H-QNB binding in the brain increased almost twice as fast for TRB compared to QNB. In the heart, the availability increased almost four times faster for TRB compared to QNB.

The combination of *in vitro* competitive binding assays and *ex vivo* binding assays suggests that TRB has significant potential for mAChR imaging: 1) the binding affinity of TRB is comparable to that of QNB, 2) TRB is more amenable to labeling with carbon-11 than QNB, 3) the increased rate of washout from the brain may avoid the problems of long retention associated with QNB. Based on these studies, carbon-11 TRB has been synthesized and evaluated. Details of the radiosynthesis and evaluation of TRB will be reported elsewhere (3).

Table 1. IC_{50} values for potential mAChR ligands from competitive binding assays using ^3H-QNB and mouse brain homogenates.

Ligand	IC_{50}, nM
QNB	0.8 ± .2
Scopolamine	1.3 ± .3
Nor-scopolamine	6.9 ± .9
NMPB	1.8 ± .1
PB	17.3 ± 2.7
TRB	0.7 ± .3
Nor-TRB	1.3 ± .4

Table 2. Percentage of ^3H-QNB binding relative to controls after *in vivo* drug administration. Ligand concentration was 1 x 10^{-5} moles/kg mouse; sacrifice at t = 45 min.

Ligand	Percent of control
Saline	100
Scopolamine	65
TRB	35
Nor-TRB	37
NMPB	74

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