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THE USE OF IRREVERSIBLE INHIBITORS OF HPNMT TO IDENTIFY ACTIVE SITE RESIDUES.

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Phenylethanolamine n-methyltransferase (PNMT) catalyses the terminal step in the biosynthesis of adrenaline, i.e. The conversion of noradrenaline to adrenaline. Although primarily found in the adrenals, PNMT has been found in the CNS, where adrenaline has been proposed to act as a neurotransmitter. The precise role of adrenaline in the CNS has not been established, although it has been implicated in the stress response and in the regulation of cardiovascular function. The development of inhibitors of central PNMT could assist in the elucidation of the role of adrenaline in the CNS.

Recently we have cloned human PNMT, expressed it in *E. coli* and purified it to homogeneity. We have also developed a series of inhibitors of the enzyme, based on a tetrahydroisoquinoline (THIQ) nucleus. One of these, 7-isothiocyanato-THIQ, reacts rapidly and irreversibly with the enzyme and provides us with a probe to identify potential active site residues of hPNMT.

We have used mass spectrometry to determine that a single inhibitor molecule binds to the enzyme. We are currently employing proteolytic cleavage combined with mass spectrometry to identify those residues on the enzyme to which the inhibitor binds.