Investigation of Catecholamine Inhibition in Tyrosine Hydroxylase

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Statement of Originality

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository**, subject to the provisions of the Copyright Act 1968.

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I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

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## Contents

Abstract ........................................................................................................................................... 7

1. Introduction ..................................................................................................................................... 9
   1.1. The Catecholamines .................................................................................................................. 11
       1.1.1. Catecholamine Systems ...................................................................................................... 11
       1.1.2. Biosynthesis of Catecholamines in Different Systems .................................................... 15
       1.1.3. Control of Catecholamine Synthesis ................................................................................ 18
   1.2. Tyrosine Hydroxylase ............................................................................................................... 19
       1.2.1. Control of Tyrosine Hydroxylase Activity by the Catecholamines ................................. 19
       1.2.2. Other Effectors of Tyrosine Hydroxylase Activity ............................................................ 27
       1.2.2. Catalytic Mechanisms of Tyrosine Hydroxylase ............................................................... 29
       1.2.3. Mechanisms of Catecholamine Inhibition ....................................................................... 32
   1.3. Structural analysis of Tyrosine Hydroxylase .......................................................................... 37
       1.3.1. The Tyrosine Hydroxylase Crystal Structure .................................................................. 37
       1.3.2. Aromatic Amino Acid Hydroxylase Crystal Structures .................................................... 43
       1.3.3. Phenylalanine Hydroxylase: Catechols and N-terminus Structure ................................. 44
   1.4. Aims and Significance ............................................................................................................... 46
   1.5. References ............................................................................................................................... 49

2. General Methods .......................................................................................................................... 63
   2.1. Materials .................................................................................................................................... 64
   2.2. Generation, Expression and Purification of TH Mutants .......................................................... 64
   2.3. TH Phosphorylation by PKA .................................................................................................. 65
   2.4. Inhibition of TH by Dopamine ................................................................................................. 66
   2.5. Isolation of High Affinity Dopamine-Bound TH .................................................................... 66
   2.6. TH Activity Measurements ...................................................................................................... 68
   2.7 References ............................................................................................................................... 69
3. Active Site Catecholamine Binding in Tyrosine Hydroxylase ......................................................... 71
   3.1. Introduction ................................................................................................................................. 72
   3.2. Methods ..................................................................................................................................... 73
      3.2.1. Direct Binding of Dopamine to the High Affinity Site ......................................................... 73
   3.3. Results ........................................................................................................................................ 74
      3.3.1. Substitution of Active Site Residues ..................................................................................... 74
      3.3.2. BH₄ Kinetic Analyses ......................................................................................................... 76
      3.3.3. Inhibition of TH Through the Low Affinity Site ................................................................. 78
      3.3.4. Inhibition of TH Through the High Affinity Site ............................................................... 84
   3.4. Discussion .................................................................................................................................... 93
   3.5 References .................................................................................................................................... 98

4. The Regulatory Unit of Catecholamine Inhibition in Tyrosine Hydroxylase........ 101
   4.1. Introduction ............................................................................................................................... 103
   4.2. Methods .................................................................................................................................... 105
      4.2.1. Generation of Mutant TH, L480A and K170E/L480A ......................................................... 105
      4.2.2. Analysis of Quaternary Structure ....................................................................................... 105
   4.3. Results ....................................................................................................................................... 106
      4.3.1. Quaternary Structure Determination of Wild-type and Mutant TH .................................. 106
      4.3.2. Dopamine Inhibition .......................................................................................................... 108
   4.4. Discussion .................................................................................................................................. 113
   4.5. References .................................................................................................................................. 116

5. Novel Residues Mediating High Affinity Catecholamine Inhibition in Tyrosine Hydroxylase ....................................................................................................................... 119
   5.1. Introduction ............................................................................................................................... 120
   5.2. Methods ..................................................................................................................................... 123
      5.2.1. Identification of Clefts using Sphgen and Showsphere .................................................... 123
5.3. Results ................................................................................................................................. 124
  5.3.1. Identification of a Cleft Extending from the TH Active Site ................................. 124
  5.3.2. Characterisation of TH Mutants .............................................................................. 129
  5.3.3. Dopamine Inhibition Through the High Affinity Binding Site ......................... 130
  5.3.4. Kinetic Analysis of Dopamine-Bound TH .............................................................. 133
5.4. Discussion .......................................................................................................................... 141
5.5. References ....................................................................................................................... 145

6. Novel Allosteric Modulators of TH Activity ................................................................. 147
  6.1. Introduction ...................................................................................................................... 148
  6.2. Methods ............................................................................................................................ 151
    6.2.1. Docking of Compound Structures into the TH Crystal Structure .................. 151
    6.2.2. Preparation of Compounds ....................................................................................... 153
    6.2.3. Incubation of Compounds with TH ......................................................................... 153
    6.2.4. Dopamine Inhibition Time Course Experiments ............................................... 153
  6.3. Results .............................................................................................................................. 155
    6.3.1. Docking Results ....................................................................................................... 155
    6.3.2. In Vitro Analyses ..................................................................................................... 161
    6.3.3. Further Docking ....................................................................................................... 170
    6.3.4. In Vitro Analyses 2 ................................................................................................. 172
  6.4. Discussion ....................................................................................................................... 176
  6.5. References ....................................................................................................................... 179

7. Salsolinol Inhibition of Tyrosine Hydroxylase ............................................................. 183
  7.1. Introduction ...................................................................................................................... 184
  7.2. Methods ............................................................................................................................ 187
    7.2.1. Competition Experiment ......................................................................................... 187
  7.3. Results .............................................................................................................................. 188
7.3.1. Docking of Salsolinol to TH .......................................................... 188

7.3.2. Salsolinol Binds to the Dopamine Binding Sites ............................... 189

7.3.3. Salsolinol Inhibits TH Activity in the non-phosphorylated and phosphorylated forms...................................................................................... 190

7.3.4. Salsolinol Inhibits Phosphorylated TH More Strongly than Dopamine .... 193

7.3.5. The Effect of Salsolinol on TH Kinetic Parameters ............................. 196

7.3.6. Reactivation of Salsolinol-Bound TH by Ser40 Phosphorylation ............ 200

7.4. Discussion ............................................................................................ 202

7.5. References .......................................................................................... 204

8. Conclusions ............................................................................................ 207

References ................................................................................................... 216
Abstract

Tyrosine Hydroxylase (TH) performs the first and rate-limiting step in the synthesis of the catecholamines, dopamine, adrenaline and noradrenaline by converting tyrosine to DOPA. The catecholamines produce end-point feedback inhibition of TH, allowing for the control of cytosolic catecholamines in neurons and adrenal chromaffin cells. Catecholamines inhibit TH by binding to two sites; they bind irreversibly to the active site and will dissociate when the TH regulatory domain is phosphorylated at Ser40, and they bind to another site reversibly, regardless of Ser40 phosphorylation status. The location of this site had not been identified until now, and was shown to be located in the active site of TH, relying on E332 and Y371, which position catecholamine within the cofactor binding site, where it exerts its inhibition. These residues were found to play the same role in high affinity catecholamine binding, indicating that these two sites are colocalised. Since high and low affinity catecholamine binding occur simultaneously and produce stoichiometries of 1mol catecholamine per TH dimer for the high affinity site, and 1mol catecholamine per TH dimer for the low affinity site, it was hypothesised that these sites could not exist in the same active site, and may be spread across the tetramer of TH. Substitution of a residue at the tetramerisation interface (L480A), combined with a substitution of a residue involved in the dimerisation salt bridge (K170E) produced a pure dimeric species of TH that possessed both high and low affinity catecholamine binding sites. This indicated that the dimer is the core regulatory unit of TH for catecholamine inhibition. The high and low affinity catecholamine binding sites on TH were also explored by using another physiologically relevant catechol, salsolinol, which was shown to produce a more potent inhibition of phosphorylated TH than dopamine by producing a greater effect on cofactor binding.
High affinity catecholamine binding was further explored by examining possible regulatory domain-interacting regions on the catalytic domain that are crucial for the irreversible binding of catecholamine. A297, D361 E362 and E365 were shown to mediate high affinity catecholamine binding, and, due to their location outside the active site, are likely to interact with the regulatory domain rather than catecholamine itself. This region of the crystal structure was then used for \textit{in silico} screening of compounds, 11 of which were tested \textit{in vitro}. Three compounds were found to be allosterically competitive with dopamine. One of the three activated TH moderately at high micromolar concentrations in the absence of dopamine, while the other two compounds markedly reduced activity in the absence of dopamine low micromolar range.