CHOLECYSTOKININ MODULATION
OF DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS OF
THE SPONTANEOUSLY HYPERTENSIVE RAT

BY

GILBERT J. KIROUAC

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Department of Anatomy
Faculty of Medicine
University of Manitoba
Winnipeg, Manitoba
The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-612-13254-4
**Neuroscience**

### Subject Categories

#### THE HUMANITIES AND SOCIAL SCIENCES

<table>
<thead>
<tr>
<th>Subject Category</th>
<th>Field</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communications and the Arts</td>
<td>Architecture</td>
<td>0729</td>
</tr>
<tr>
<td></td>
<td>Art History</td>
<td>0707</td>
</tr>
<tr>
<td></td>
<td>Cinema</td>
<td>0900</td>
</tr>
<tr>
<td></td>
<td>Dance</td>
<td>0578</td>
</tr>
<tr>
<td></td>
<td>Fine Arts</td>
<td>0879</td>
</tr>
<tr>
<td></td>
<td>Information Science</td>
<td>0723</td>
</tr>
<tr>
<td></td>
<td>Journalism</td>
<td>0591</td>
</tr>
<tr>
<td></td>
<td>Library Science</td>
<td>0399</td>
</tr>
<tr>
<td></td>
<td>Mass Communications</td>
<td>0708</td>
</tr>
<tr>
<td></td>
<td>Music</td>
<td>0413</td>
</tr>
<tr>
<td></td>
<td>Speech Communication</td>
<td>0459</td>
</tr>
<tr>
<td></td>
<td>Theater</td>
<td>0465</td>
</tr>
<tr>
<td>Education</td>
<td>General</td>
<td>0513</td>
</tr>
<tr>
<td></td>
<td>Administrative</td>
<td>0516</td>
</tr>
<tr>
<td></td>
<td>Adult and Continuing</td>
<td>0516</td>
</tr>
<tr>
<td></td>
<td>Agricultural</td>
<td>0517</td>
</tr>
<tr>
<td></td>
<td>Art</td>
<td>0517</td>
</tr>
<tr>
<td></td>
<td>Bilingual and Multicultural</td>
<td>0282</td>
</tr>
<tr>
<td></td>
<td>Business</td>
<td>0688</td>
</tr>
<tr>
<td></td>
<td>Community College</td>
<td>0275</td>
</tr>
<tr>
<td></td>
<td>Curriculum and Instruction</td>
<td>0727</td>
</tr>
<tr>
<td></td>
<td>Early Childhood</td>
<td>0518</td>
</tr>
<tr>
<td></td>
<td>Elementary</td>
<td>0524</td>
</tr>
<tr>
<td></td>
<td>Finance</td>
<td>0277</td>
</tr>
<tr>
<td></td>
<td>Guidance and Counseling</td>
<td>0277</td>
</tr>
<tr>
<td></td>
<td>Health</td>
<td>0580</td>
</tr>
<tr>
<td></td>
<td>Higher</td>
<td>0745</td>
</tr>
<tr>
<td></td>
<td>History of</td>
<td>0582</td>
</tr>
<tr>
<td></td>
<td>Home Economics</td>
<td>0279</td>
</tr>
<tr>
<td></td>
<td>Industrial</td>
<td>0521</td>
</tr>
<tr>
<td></td>
<td>Language and Literature</td>
<td>0579</td>
</tr>
<tr>
<td></td>
<td>Mathematics</td>
<td>0680</td>
</tr>
<tr>
<td></td>
<td>Music</td>
<td>0522</td>
</tr>
<tr>
<td></td>
<td>Philosophy of</td>
<td>0698</td>
</tr>
<tr>
<td></td>
<td>Physical</td>
<td>0523</td>
</tr>
</tbody>
</table>

#### THE SCIENCES AND ENGINEERING

<table>
<thead>
<tr>
<th>Subject Category</th>
<th>Field</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological Sciences</td>
<td>Agriculture</td>
<td>0473</td>
</tr>
<tr>
<td></td>
<td>Agronomy</td>
<td>0285</td>
</tr>
<tr>
<td></td>
<td>Animal Culture and Nutrition</td>
<td>0475</td>
</tr>
<tr>
<td></td>
<td>Animal Pathology</td>
<td>0476</td>
</tr>
<tr>
<td></td>
<td>Food Science and Technology</td>
<td>0599</td>
</tr>
<tr>
<td></td>
<td>Forestry and Wildlife</td>
<td>0476</td>
</tr>
<tr>
<td></td>
<td>Plant Culture</td>
<td>0479</td>
</tr>
<tr>
<td></td>
<td>Plant Pathology</td>
<td>0480</td>
</tr>
<tr>
<td></td>
<td>Plant Physiology</td>
<td>0481</td>
</tr>
<tr>
<td></td>
<td>Range Management</td>
<td>0777</td>
</tr>
<tr>
<td></td>
<td>Wood Technology</td>
<td>0746</td>
</tr>
<tr>
<td>Biology</td>
<td>General</td>
<td>0306</td>
</tr>
<tr>
<td></td>
<td>Anatomy</td>
<td>0287</td>
</tr>
<tr>
<td></td>
<td>Biochemistry</td>
<td>0308</td>
</tr>
<tr>
<td></td>
<td>Botany</td>
<td>0309</td>
</tr>
<tr>
<td></td>
<td>Cell</td>
<td>0290</td>
</tr>
<tr>
<td></td>
<td>Cell Biology</td>
<td>0291</td>
</tr>
<tr>
<td></td>
<td>Ecology</td>
<td>0302</td>
</tr>
<tr>
<td></td>
<td>Entomology</td>
<td>0353</td>
</tr>
<tr>
<td></td>
<td>Genetics</td>
<td>0669</td>
</tr>
<tr>
<td></td>
<td>Limnology</td>
<td>0793</td>
</tr>
<tr>
<td></td>
<td>Microbiology</td>
<td>0410</td>
</tr>
<tr>
<td></td>
<td>Molecular Neurobiology</td>
<td>0317</td>
</tr>
<tr>
<td></td>
<td>Oceanography</td>
<td>0416</td>
</tr>
<tr>
<td></td>
<td>Physiology</td>
<td>0533</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>0621</td>
</tr>
<tr>
<td></td>
<td>Veterinary Science</td>
<td>0778</td>
</tr>
<tr>
<td></td>
<td>Zoology</td>
<td>0472</td>
</tr>
<tr>
<td>Biophysics</td>
<td>General</td>
<td>0768</td>
</tr>
<tr>
<td>Earth Sciences</td>
<td>Biogeography and Geography</td>
<td>0425</td>
</tr>
<tr>
<td></td>
<td>Geochemistry</td>
<td>0996</td>
</tr>
<tr>
<td></td>
<td>Geology</td>
<td>0370</td>
</tr>
<tr>
<td></td>
<td>Geophysics</td>
<td>0372</td>
</tr>
<tr>
<td></td>
<td>Hydrology</td>
<td>0373</td>
</tr>
<tr>
<td></td>
<td>Meteorology</td>
<td>0411</td>
</tr>
<tr>
<td></td>
<td>Paleobotany</td>
<td>0345</td>
</tr>
<tr>
<td></td>
<td>Paleobotany</td>
<td>0345</td>
</tr>
<tr>
<td></td>
<td>Paleontology</td>
<td>0418</td>
</tr>
<tr>
<td></td>
<td>Palaeontolgy</td>
<td>0395</td>
</tr>
<tr>
<td></td>
<td>Palaeontology</td>
<td>0418</td>
</tr>
<tr>
<td></td>
<td>Physical Geography</td>
<td>0368</td>
</tr>
<tr>
<td></td>
<td>Physical Oceanography</td>
<td>0415</td>
</tr>
<tr>
<td>Health and Environmental Sciences</td>
<td>Environmental Sciences</td>
<td>0768</td>
</tr>
<tr>
<td></td>
<td>Health Sciences</td>
<td>0564</td>
</tr>
<tr>
<td></td>
<td>General</td>
<td>0556</td>
</tr>
<tr>
<td></td>
<td>Audiology</td>
<td>0992</td>
</tr>
<tr>
<td></td>
<td>Education</td>
<td>0567</td>
</tr>
<tr>
<td></td>
<td>Dentistry</td>
<td>0567</td>
</tr>
<tr>
<td></td>
<td>Environmental Health</td>
<td>0769</td>
</tr>
<tr>
<td></td>
<td>Hospital Management</td>
<td>0769</td>
</tr>
<tr>
<td></td>
<td>Human Development</td>
<td>0758</td>
</tr>
<tr>
<td></td>
<td>Immunology</td>
<td>0982</td>
</tr>
<tr>
<td></td>
<td>Medicine and Surgery</td>
<td>0564</td>
</tr>
<tr>
<td></td>
<td>Mental Health</td>
<td>0547</td>
</tr>
<tr>
<td></td>
<td>Nutrition</td>
<td>0570</td>
</tr>
<tr>
<td></td>
<td>Obstetrics and Gynecology</td>
<td>0380</td>
</tr>
<tr>
<td></td>
<td>Occupational Health and Safety</td>
<td>0354</td>
</tr>
<tr>
<td></td>
<td>Ophthalmology</td>
<td>0381</td>
</tr>
<tr>
<td></td>
<td>Otolaryngology</td>
<td>0419</td>
</tr>
<tr>
<td></td>
<td>Pharmacy</td>
<td>0572</td>
</tr>
<tr>
<td></td>
<td>Pharmacology</td>
<td>0573</td>
</tr>
<tr>
<td></td>
<td>Radiology</td>
<td>0574</td>
</tr>
<tr>
<td></td>
<td>Radiology</td>
<td>0575</td>
</tr>
<tr>
<td></td>
<td>Speech Pathology</td>
<td>0600</td>
</tr>
<tr>
<td></td>
<td>Toxicology</td>
<td>0383</td>
</tr>
<tr>
<td></td>
<td>Home Economics</td>
<td>0386</td>
</tr>
<tr>
<td>Physical Sciences</td>
<td>Pure Sciences</td>
<td>0605</td>
</tr>
<tr>
<td></td>
<td>Chemistry</td>
<td>0491</td>
</tr>
<tr>
<td></td>
<td>General</td>
<td>0459</td>
</tr>
<tr>
<td></td>
<td>Acoustics</td>
<td>0986</td>
</tr>
<tr>
<td></td>
<td>Astronomy and Astrophysics</td>
<td>0606</td>
</tr>
<tr>
<td></td>
<td>Atmospheric Science</td>
<td>0608</td>
</tr>
<tr>
<td></td>
<td>Atomic</td>
<td>0748</td>
</tr>
<tr>
<td></td>
<td>Electronics and Electromagnetism</td>
<td>0607</td>
</tr>
<tr>
<td></td>
<td>Elementary Particles and High Energy</td>
<td>0798</td>
</tr>
<tr>
<td></td>
<td>Fluid and Plasma</td>
<td>0759</td>
</tr>
<tr>
<td></td>
<td>Mathematical Physics</td>
<td>0609</td>
</tr>
<tr>
<td></td>
<td>Nuclear</td>
<td>0610</td>
</tr>
<tr>
<td></td>
<td>Optics</td>
<td>0752</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>0756</td>
</tr>
<tr>
<td></td>
<td>Solid State</td>
<td>0611</td>
</tr>
<tr>
<td></td>
<td>Statistics</td>
<td>0463</td>
</tr>
<tr>
<td></td>
<td>Applied Sciences</td>
<td>0346</td>
</tr>
<tr>
<td></td>
<td>Applied Mechanics</td>
<td>0346</td>
</tr>
<tr>
<td></td>
<td>Computer Science</td>
<td>0984</td>
</tr>
<tr>
<td>Psychology</td>
<td>General</td>
<td>0621</td>
</tr>
<tr>
<td></td>
<td>Behavioral</td>
<td>0384</td>
</tr>
<tr>
<td></td>
<td>Clinical</td>
<td>0622</td>
</tr>
<tr>
<td></td>
<td>Developmental</td>
<td>0620</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0621</td>
</tr>
<tr>
<td></td>
<td>Industrial</td>
<td>0624</td>
</tr>
<tr>
<td></td>
<td>Personality</td>
<td>0625</td>
</tr>
<tr>
<td></td>
<td>Physical Education</td>
<td>0588</td>
</tr>
<tr>
<td></td>
<td>Psychobiology</td>
<td>0294</td>
</tr>
<tr>
<td></td>
<td>Psychometrics</td>
<td>0332</td>
</tr>
<tr>
<td></td>
<td>Social</td>
<td>0451</td>
</tr>
</tbody>
</table>
CHOLECYSTOKININ MODULATION OF DOPAMINE RELEASE IN THE
NUCLEUS ACCUMBENS OF THE SPONTANEOUSLY HYPERTENSIVE RAT

BY

GILBERT J. KIROUAC

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

© 1995

Permission has been granted to the LIBRARY OF THE UNIVERSITY OF MANITOBA to lend or sell copies of this thesis, to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film, and LIBRARY MICROFILMS to publish an abstract of this thesis.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or other-wise reproduced without the author's written permission.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS vi
LIST OF TABLES vii
LIST OF FIGURES viii
LIST OF ABBREVIATIONS xi
ABSTRACT xii

## SECTIONS PAGE
1. INTRODUCTION 1
2. REVIEW OF THE LITERATURE 4
   2.1 Hyperkinetic Circulation in Hypertension 4
   2.2 Emotional Arousal and Blood Pressure 5
   2.3 The Mesotelencephalic Dopaminergic System 7
      2.3.1 Anatomy of the Mesotelencephalic Dopaminergic Pathways 7
      2.3.2 Function of the Mesotelencephalic Dopaminergic Pathways 8
   2.4 Dopaminergic Mechanisms in Cardiovascular Regulation 10
      2.4.1 Peripheral Effects of Dopamine 10
      2.4.2 Central Effects of Dopamine 11
   2.5 Role of Central Dopamine in Hypertension 13
   2.6 Possible Role for the Nucleus Accumbens in Hypertension 16
   2.7 Modulation of Dopamine Function by Cholecystokinin 19
3. OBJECTIVES AND HYPOTHESES 21
4. MATERIALS AND METHODS

4.1 Materials

4.2 Systolic Blood Pressure Measurements

4.3 Cholecystokinin Receptor Autoradiography

4.3.1 Preparation of Autoradiograms

4.3.2 Image Analysis System for Quantifying Cholecystokinin Binding Density

4.3.3 Protocol for Quantification of Cholecystokinin Binding and Data Analysis

4.4 In Vitro Microdialysis

4.4.1 Surgical Preparation

4.4.2 Protocol for Stimulation of Cholecystokinin Receptors

4.4.3 High Pressure Liquid Chromatography

4.4.4 Analysis of Data

4.5 Pharmacological Treatment Experiments

4.6 Tract-Tracing Experiments

4.7 Statistical Analyses

5. RESULTS

5.1 Cholecystokinin Receptor Density in the Striatum of SHRs and WKYs

5.2 Cholecystokinin-Induced Release of Dopamine in the Nucleus Accumbens

5.2.1 10 μM Cholecystokinin Experiments
5.2.2 1 μM Sulphated- and 10 μM Unsubphated-
 Cholecystokinin Experiments

5.3 Effects of Cholecystokinin Agonism and Antagonism on Hypertension

5.4 Demonstration of a Possible Relay in the Nucleus Accumbens

6. DISCUSSION

6.1 Cholecystokinin Receptor Density in the Striatum of the SHR

6.2 Cholecystokinin-Induced Release of Dopamine in the
 Nucleus Accumbens of the SHR

6.3 Effects of Cholecystokinin Agonists and Antagonists on Hypertension

6.4 Organization in the Nucleus Accumbens of Afferents from the
 Amygdala and Efferents to the Hypothalamus

6.5 Interpretation of Results

7. SUMMARY

8. REFERENCES
Somebody said that it couldn't be done,
But he with a chuckle replied
That "maybe it couldn't," but he would be one
Who wouldn't say till he'd tried.
So he buckled right in with the trace of a grin
On his face. If he worried he hid it.
He started to sing as he tackled the thing
That couldn't be done, and he did it.

Somebody scoffed: "Oh, you'll never do that;
At least no one ever has done it;"
But he took off his coat and he took off his hat,
And the first thing we knew he'd begun it.
With a lift of his chin and a bit of a grin,
Without any doubting or quiddit,
He started to sing as he tackled the thing
That couldn't be done, and he did it.

There are thousands to tell you it cannot be done,
There are thousands to prophesy failure;
There are thousands to point out to you, one by one
The dangers that wait to assail you.
But just buckle in with a bit of a grin,
Just take off your coat and go to it;
Just start to sing as you tackle the thing
That "cannot be done," and you'll do it.

EDGAR A. GUEST
ACKNOWLEDGMENTS

I am grateful to Dr. Ganguly for giving me the opportunity to pursue research questions that were of interest to myself and for extending to me colleague status.

I would like to express my appreciation to the members of my advisory committee: Dr. H. Bergen, Department of Anatomy; Dr. D. Nance, Department of Pathology; and Dr. D. Smyth, Department of Pharmacology and Therapeutics, for their valuable suggestions and contributions. In addition, I would like to acknowledge my external examiner, Dr. J. Ciriello, Department of Physiology, University of Western Ontario, for his participation in my thesis examination.

I would also like to express my appreciation to the faculty members, technicians, and support staff from the Department of Anatomy for their assistance and encouragement. In addition, I would like to acknowledge Dr. K. Lal, Department of Biochemistry, Dr. R. Nirula, Mr. Richard Frechette, and Mr. K. Banerji for their assistance in the laboratory.

I am extremely grateful to my wife Margrit for her unrelenting belief in my ability to succeed during my academic training. She has been, and continues to be, my strongest source of encouragement and support.

Finally, I dedicate this thesis to my mother and father. They have instilled in me the values of honesty, integrity, hard work, and commitment. These values have guided me through my personal and academic life.
LIST OF TABLES

TABLES

Table I. Basal concentrations of DA and metabolites recovered in the posterior portion of the NA in the SHR and WKY
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1. Location of densitometric measurements</td>
<td>28</td>
</tr>
<tr>
<td>Figure 2. Instrumentation for microdialysis experiments</td>
<td>30</td>
</tr>
<tr>
<td>Figure 3. Example of an autoradiogram</td>
<td>38</td>
</tr>
<tr>
<td>Figure 4. Standard curve for autoradiogram (5-week-old rats)</td>
<td>39</td>
</tr>
<tr>
<td>Figure 5. Standard curve for autoradiogram (15-week-old rats)</td>
<td>40</td>
</tr>
<tr>
<td>Figure 6. CCK8S receptor density in the prefrontal cortex of SHRs and WKYs</td>
<td>41</td>
</tr>
<tr>
<td>Figure 7. CCK8S receptor density in the medial caudate-putamen of SHRs and WKYs</td>
<td>43</td>
</tr>
<tr>
<td>Figure 8. CCK8S receptor density in the lateral caudate-putamen of SHRs and WKYs</td>
<td>44</td>
</tr>
<tr>
<td>Figure 9. CCK8S receptor density in the NA of SHRs and WKYs</td>
<td>45</td>
</tr>
<tr>
<td>Figure 10. HPLC chromatogram</td>
<td>46</td>
</tr>
<tr>
<td>Figure 11. Microdialysis probe placements in the NA</td>
<td>47</td>
</tr>
<tr>
<td>Figure 12. Microdialysis probe placements in the NA of SHRs and WKYs for the 10 µM CCK8S experiments</td>
<td>48</td>
</tr>
<tr>
<td>Figure 13. Effect of 10 µM CCK8S on basal DA in SHRs and WKYs</td>
<td>51</td>
</tr>
<tr>
<td>Figure 14. Effect of 10 µM CCK8S on basal DOPAC in SHRs and WKYs</td>
<td>52</td>
</tr>
<tr>
<td>Figure 15. Effect of 10 µM CCK8S on basal HVA in SHRs and WKYs</td>
<td>53</td>
</tr>
<tr>
<td>Figure 16. Effect of 10 µM CCK8S on basal HIAA in SHRs and WKYs</td>
<td>54</td>
</tr>
</tbody>
</table>
Figure 17. Microdialysis probe placements in the NA of SHRs for the 1 μM CCK8S experiments

Figure 18. Effect of 1 μM CCK8S on basal DA in SHRs

Figure 19. Effect of 1 μM CCK8S on basal DOPAC in SHRs

Figure 20. Effect of 1 μM CCK8S on basal HVA in SHRs

Figure 21. Effect of 1 μM CCK8S on basal HIAA in SHRs

Figure 22. Microdialysis probe placements in the NA of SHRs for the 10 μM CCK8S experiments

Figure 23. Effect of 10 μM CCK8S on basal DA in SHRs

Figure 24. Effect of 10 μM CCK8S on basal DOPAC in SHRs

Figure 25. Effect of 10 μM CCK8S on basal HVA in SHRs

Figure 26. Effect of 10 μM CCK8S on basal HIAA in SHRs

Figure 27. Effect of proglumide and ceruletide on body weight in SHRs

Figure 28. Effect of proglumide and ceruletide on blood pressure in SHRs

Figure 29. Effect of proglumide and ceruletide on heart rate in SHRs

Figure 30. Location of fluorescent tracer injection sites

Figure 31. Photomicrograph of FG and FR-labeled cells and fibers in the NA

Figure 32. Schematic representation of FG and FR-labeled cells and fibers in the NA
Figure 33. Diagram of neural pathways in the NA

Figure 34. Flowchart of DA's and CCK8S's role in hypertension
LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BLA</td>
<td>basolateral nucleus of the amygdala</td>
</tr>
<tr>
<td>CCK8S</td>
<td>cholecystokinin (sulphated-octapetide)</td>
</tr>
<tr>
<td>CCK8US</td>
<td>cholecystokinin (unsulphated-octapetide)</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>FG</td>
<td>Fluoro-Gold</td>
</tr>
<tr>
<td>FR</td>
<td>Fluoro-Ruby</td>
</tr>
<tr>
<td>HIAA</td>
<td>5-hydroxyindolacetic acid</td>
</tr>
<tr>
<td>HVA</td>
<td>homovanallic acid</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>intracerebroventricular</td>
</tr>
<tr>
<td>LHA</td>
<td>lateral hypothalamic area</td>
</tr>
<tr>
<td>NA</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>PHA-L</td>
<td><em>Phaseolus vulgaris</em>-leucoagglutinin</td>
</tr>
<tr>
<td>SHR</td>
<td>spontaneously hypertensive rat</td>
</tr>
<tr>
<td>WKY</td>
<td>Wistar-Kyoto rat</td>
</tr>
</tbody>
</table>
Changes in dopamine (DA) neurotransmission in the nucleus accumbens (NA) of the spontaneously hypertensive rat (SHR) may be involved in the pathogenesis of hypertension in this genetic model of hypertension. This thesis tested the hypothesis that changes in the modulation of DA function by cholecystokinin (CCK8S) may be responsible for the altered DA function in the NA of the SHR. A significant increase in CCK8S receptor density was found using autoradiography in the NA of the prehypertensive and hypertensive SHR when compared to its control, the Wistar-Kyoto rat (WKY). It was also found using in vivo microdialysis that perfusion of the NA with CCK8S increased DA levels in the NA of SHRs to a much greater extent than in WKYs. These results indicate that CCK8S-induced release of DA in the NA is greater in the SHR. This effect is probably due to an upregulation of CCK8S receptors in the NA. It is suggested that changes in CCK8S function may be responsible for the alterations in DA activity in the SHR and the pathogenesis of hypertension.

Tract-tracing experiments were performed to demonstrate an overlap in the NA between the terminal field of neurons originating in the basolateral amygdala and neurons which project to the lateral hypothalamus. In addition, neurons in the basolateral amygdala appear to make synaptic contact with neurons in the NA that project to the lateral hypothalamus. This provides anatomical evidence for a relay of information in the NA from the amygdala to the hypothalamus. This relay may be involved in the autonomic responses that accompany emotional arousal and the development of hypertension.
1. INTRODUCTION

Cardiovascular diseases such as coronary heart disease and cerebrovascular stroke are the most common cause of death in affluent societies like Canada. It is well known that essential or primary hypertension, which is high blood pressure without any definable cause, is a major risk factor in the development of these diseases (McNeil, 1989). This recognition has served as an impetus for researchers to understand the etiology and pathophysiology of this common condition. Despite the enormous efforts dedicated to this subject and the major contributions made by numerous investigators, the causal mechanisms that lead to hypertension remain highly controversial (Freis, 1990; Pickering, 1990). For example, different researchers have emphasized dysfunctions in renal, vascular, hormonal, or neurogenic mechanisms as the important factor that causes or maintains high blood pressure. Indeed, this has guided the development of antihypertensive agents that exert their actions by improving renal function, reducing vascular reactivity, blocking the actions of circulating hormones, or reducing the activity of the sympathetic nervous system. However, these drugs are not always effective and they often have physiological effects that may be detrimental to the cardiovascular system. In addition, the side-effects of antihypertensive drugs presently in use may result in poor compliance over the long periods of treatment that are necessary for life-long control of hypertension (MacMahon, 1989; Freis, 1990). For these reasons, hypertension and the search for more effective antihypertensive agents remain some of the most intensely studied subjects in medicine.
Epidemiological studies have shown that genetic factors play an important role in the development of essential hypertension (Folkow, 1982). This recognition led to the development of strains of genetically hypertensive rats. For example, Okomoto and Aoki (1963) produced the most intensively studied of these genetic models by inbreeding Wistar rats with higher than normal blood pressures. The offsprings of these rats were called the spontaneously hypertensive rat (SHR). An enormous amount of data has accumulated on the physiology of the SHR and its appropriateness to study human hypertension. Despite some controversy, researchers in the field generally agree that the SHR is the most appropriate model to study human essential hypertension (Trippoda & Frohlich, 1981; Folkow, 1982; Julius, 1993). This conclusion is based on the similarities in the hemodynamic pattern of SHRs and humans with essential hypertension. In both cases, the hemodynamic pattern is characterized by an initial increase in cardiac output and heart rate without any noticeable rise in blood pressure (Folkow, 1982; Julius, 1993). The enhanced output from the heart is believed to cause structural and functional changes in the cardiovascular system that shift the hemodynamics from a high output to a high resistance pattern (Folkow, 1982; Julius, 1993). Eventually, an elevation in total peripheral resistance serves to maintain blood pressure at a new and hypertensive level.

The early rise in cardiac output seen in the SHR and hypertension-prone individuals may be due to a hyperreactivity of the cardiovascular system to environmental stimuli (Folkow, 1982). This in fact may represent one of the inherited predisposing factors in genetic forms of hypertension (Folkow, 1982; Pickering & Gerrin, 1990; Julius,
1993). Therefore, it is important that we understand the neural mechanisms that lead to this behavioral and cardiovascular hyperreactivity.

Dopamine (DA), a neurotransmitter well known for its role in regulating an organism's reactivity to the environment may be involved in hypertension (Folkow, 1982). For example, the centrally acting DA agonist bromocriptine was shown to be an effective short-term antihypertensive agent in both humans with essential hypertension and the SHR (Stumpe et al., 1977; Nagahama et al., 1984). This suggests that hypertensive humans and rats have a dysfunction in central DA neurotransmission. While DA is a well known neurotransmitter/neuromodulator that regulates forebrain output and behavioral reactivity, the mechanisms by which DA could initiate or maintain hypertension remain unknown. Consequently, this thesis examines DA neurotransmission in the forebrain of the SHR in an attempt to elucidate some of the mechanisms that may be responsible for behavioral and cardiovascular hyperreactivity in this strain of hypertensive rats.
2. REVIEW OF THE LITERATURE

2.1 Hyperkinetic Circulation in Hypertension

The SHR is considered one of the best models for studying clinical hypertension in humans because of the similarities between the hemodynamic pattern of SHRs and humans with essential hypertension (Trippoda & Frohlich, 1981). An observation that may be meaningful for understanding the pathogenesis of hypertension is that human patients with borderline or labile hypertension and young prehypertensive SHRs have a hyperkinetic circulation (Lundun & Hallback-Norlander, 1980; Folkow, 1982; Conway, 1984). The hyperkinetic circulation during this prehypertensive phase is characterized by an enhanced cardiac output and a normal peripheral vascular resistance. Hypertension-prone individuals respond to their environments with stronger and more frequent pressor responses (Folkow, 1982). In response to these repeated pressor loads, the cardiovascular system adapts by inducing the structural reinforcement of the vasculature. Subsequently, an increase in arteriolar vasoconstriction combined with vascular hypertrophy causes the hemodynamics to shift from a high output to a high resistance pattern. Over time, the cardiac output returns to normal levels while the peripheral resistance gradually rises to a new elevated point. Therefore, Folkow (1982) hypothesizes that hypertension becomes self-sustained through a process of structural reinforcement of precapillary arterioles in response to pressor responses during the early or labile phase of the hypertension.

Of particular importance for understanding the role that the hyperkinetic state plays in the development of hypertension is the observation that the cardiovascular system of humans with hypertension and SHRs is hyperreactive to a variety of environmental
stimuli (Hallback & Folkow, 1974; McMurtry & Wexler, 1981; Pickering & Gerrin, 1990). For example, young prehypertensive SHRs exposed to a stressful environment respond with a greater increase in cardiac output, heart rate, and mean arterial blood pressure than do their normotensive controls the Wistar-Kyoto rat (WKY). More importantly, this cardiovascular hyperreactivity precedes the onset of hypertension and may represent one of the inherited predisposing factors in genetic hypertension (Folkow, 1982). More direct evidence supporting this hypothesis comes from studies in which social and sensory deprivation of young SHRs attenuated the development of hypertension (Hallback, 1975) while noxious stimulation aggravated the hypertension (Okamoto, 1969). Further support for the hyperreactivity hypothesis comes from an interesting report in which adolescents from hypertensive families responded to forced arithmetic with an accentuated heart rate and blood pressure (Falkner et al., 1979). These studies show some of the similarities between the hemodynamic pattern in SHRs and humans prone to developing essential hypertension. In addition, these studies underscore the role of emotional arousal in the development of hypertension.

2.2 Emotional Arousal and Blood Pressure

One indisputable fact is that emotional arousal, especially of an aversive nature, is accompanied by dramatic adjustments in the autonomic nervous system. Such emotional arousal, often referred to as a defense reaction, can be elicited in humans and animals by natural stimuli (Folkow, 1982, 1987) or by electrical brain stimulation in experimental animals (Smith & DeVito, 1984). The defense reaction induced by electrical brain
stimulation produces escape or flight behavior and a variety of autonomic responses that include profound cardiovascular and respiratory adjustments. Some of the cardiovascular responses that accompany a defense reaction consist of vasodilation in skeletal muscles and in the myocardium with concomitant vasoconstriction in the viscera. The circulatory adjustments result in an increase in cardiac output, heart rate, and blood pressure, which together serves to increase blood flow to the skeletal muscles, brain and heart (Yardley & Hilton, 1986; Smith et al., 1990). This type of cardiovascular reaction can be elicited by the electrical or chemical stimulation of specific brain regions including the lateral hypothalamic area (LHA), mesencephalic central gray matter, and the amygdaloid complex (Yardley & Hilton, 1986; Maskati & Zbrozyna, 1989; Smith et al., 1990). We can conclude from these studies that these brain centers are involved in the integration of autonomic responses that accompany emotional arousal.

Cardiovascular hyperreactivity to environmental stimuli originate from higher levels of the brain where sensory, motor, and autonomic responses are integrated and initiated. Regions of forebrain, which include the limbic system and hypothalamus, are particularly important in integrating and coordinating behavioral and neurohumoral responses to emotional arousal (Smith & DeVito, 1984; Mogenson, 1987). From an anatomical perspective, limbic structures are situated between the sensory cortices and the hypothalamus which monitors and regulates internal body functions. Clearly, if we are to link hypertension to dysfunctions of the central mechanisms controlling an organism's responsiveness to the environment and emotional arousal, we must examine the brain centers and the neural mechanisms that integrate both emotions and autonomic responses.
As will be reviewed in the following sections, neurons using DA as a neurotransmitter have widespread projections to the forebrain. In addition, DA is a well-known regulator of forebrain neural activity and may be an important modulator of cardiovascular hyperreactivity in the SHR (Mogenson, 1987).

### 2.3 The Mesotelencephalic Dopaminergic System

#### 2.3.1 Anatomy of the Mesotelencephalic Dopaminergic Pathways

The anatomy of dopaminergic pathways in the brain has been extensively studied and described by several groups of neuroscientists. The ascending mesotelencephalic dopaminergic system is subdivided into the mesostriatal system and mesolimbocortical systems (Bjorklund & Lindvall, 1984). The mesostriatal system is further subdivided into a dorsal and ventral component. The dorsal component of the mesostriatal dopaminergic system originates from the A9 DA cell group located primarily in the pars compacta of the substantia nigra and partially from the A8 DA cell group in the retrorubral field (Fuxe, 1965; Beckstead et al., 1979). The axons of the nigral dopaminergic cells travel in the medial forebrain bundle to innervate the caudate-putamen. The ventral component of the mesostriatal dopaminergic pathway originates from the A10 DA cell group located in the ventral tegmental area and from the medial part of the substantia nigra (Fallon & Moore, 1978; Lindvall & Stenevi, 1978; Beckstead et al., 1979). The ventral component innervates, via the medial forebrain bundle, the ventral striatum which includes the nucleus accumbens (NA), olfactory tubercle, and the bed nucleus of the stria terminalis. A large proportion of DA containing neurons in the ventral tegmental area also contain the
neuropeptides neurotensin and cholecystokinin (Seroogy et al., 1987; 1988). The functional significance of this co-localization remains to be determined. However, these neuropeptides are coreleased with DA and appear to modulate the release of DA (Ladurelle et al., 1993; Marshall et al., 1991).

The mesolimbocortical dopaminergic system originates mostly from the A10 cell group and partially from the A9 cell group (Lindvall, 1975, Swanson, 1982). The fibers of the mesolimbocortical system ascend in the medial forebrain bundle and innervate the lateral septal nucleus; amygdaloid complex; hippocampus; anterior olfactory nuclei; olfactory bulb; and the prefrontal, cingulate, periform, and entorhinal cortices.

2.3.2 Function of the Mesotelencephalic Dopaminergic Pathways

The projections of the mesotelencephalic system contain both dopaminergic and non-dopaminergic neurons (Swanson, 1982; Loughlin & Fallon, 1983). However, a major portion of neurons in the mesostriatal system are dopaminergic and it has been estimated that less than 5% of A9 and A10 neurons projecting to the dorsal striatum are non-dopaminergic (Van der Kooy et al., 1984). Similarly, the projection to the NA has been estimated to comprise only 10-15% non-dopaminergic neurons (Swanson, 1982). Functionally, the mesencephalic dopaminergic system provides a striking example of how a small number of a single source of divergent neurons can exert global control of forebrain activity. For example, bilateral lesions of this system in rats produce a severe and generalized state of behavioral unresponsiveness which includes such diverse symptoms as akinesia, catalepsy, sensory inattention, aphagia, and adipsia. In explaining
this behavioral unresponsiveness, Mogenson (1987) has provided strong evidence that activation of mesotelencephalic dopaminergic neurons has a modulating or "gating" influence on the relay of information in the NA from the limbic system and neocortex to extrapyramidal motor areas. Evidence for this comes from studies in which activation of NA neurons by stimulation of the amygdala or hippocampus is reduced by the stimulation of dopaminergic cells in the ventral tegmental area (Yim & Mogenson, 1982; Yang & Mogenson, 1984). A similar attenuating effect of excitatory inputs to the NA neurons is produced by the exogenous application of DA to NA neurons (Yim & Mogenson, 1982; Yang & Mogenson, 1984). Nigrostriatal dopaminergic neurons have similar attenuating effects on the excitatory responses of caudate-putamen neurons produced by stimulation of the neocortex (Mogenson, 1987). Indeed, the constellation of motor symptoms characterizing Parkinson's disease which include akinesia, rigidity, and tremor, have been attributed to a dysfunction of a DA-dependent gating mechanism. In this case, DA insufficiency produces an abnormal flow of information from the cerebral cortex via the dorsal striatum to motor systems.

Mogenson (1987) suggested that the electrophysiological responses of NA neurons to DA or ventral tegmental area stimulation reflect important limbic-motor integrative activity that contribute to behavioral response initiation. He also hypothesizes that a DA-dependent gating mechanism may play a role in translating the intent of a behavioral response into such important actions as feeding, drinking, and the defense reaction. In this theoretical framework, the mesotelencephalic dopaminergic system is viewed as a level-setting system which determines the threshold for the production of
behavioral responses in an organism. Therefore, a reduction in dopaminergic activity could result in a disinhibition of forebrain activity and consequently cause a reduction in the threshold for behavioral responding. This could make an organism hyperreactive to its environment. It is also important to note that several forebrain structures which include the amygdala, hippocampus, and parts of the limbic cortex, have been implicated in the defense reaction and the cardiovascular responses that accompany these reactions (Loewy, 1991). Therefore, the emerging role for DA in the modulation of output from the limbic system and the striatum, in addition to the role of forebrain structures in cardiovascular regulation, suggests that the mesotelencephalic dopaminergic system may play an important role in blood pressure regulation and possibly hypertension.

2.4 Dopaminergic Mechanisms in Cardiovascular Regulation

2.4.1 Peripheral Effects of Dopamine

There has been in recent years a growing interest in the use of dopaminergic agents for the regulation of the cardiovascular system. Outside the central nervous system, the D2 DA receptor subtype is located presynaptically on sympathetic nerve terminals in autonomic ganglia whereas the D1 DA receptor subtype is located on vascular smooth muscle and in the kidney (Hilditch & Drew, 1985). Agonists acting at D2 receptors cause a decrease in blood pressure by inhibiting the release of norepinephrine from sympathetic nerve terminals that innervate the heart and vasculature (Goldberg, 1972; Lokhandwala et al., 1987). Conversely, D1 agonists cause a decrease in blood pressure by direct vasodilation (Goldberg, 1972; Lokhandwala et al., 1987). Compared to the well
characterized peripheral effects of DA agonists on the cardiovascular system, the
dopaminergic mechanisms in the central nervous system involved in regulating the blood
pressure are poorly understood.

2.4.2 Central Effects of Dopamine

A number of investigators have described the effects of central administrations of
DA and some of its agonists on cardiovascular variables in several species. For example,
intracerebroventricular (i.c.v.) injections of DA in anesthetized dogs produced a decrease
in blood pressure and heart rate (McCubbin et al., 1960). Whereas, i.c.v. DA caused an
increase in blood pressure in the awake dog (Lang & Woodman, 1979). In conscious
cats, i.c.v. DA produced an increase in blood pressure followed by a depressor response
(Day & Roach, 1976). A decrease in blood pressure and heart rate after i.c.v.
administration of DA has been described in anesthetized and conscious rats (Kondo et al.,
1981; Pazo et al., 1982). In addition, i.c.v. injections of the DA D2 receptor agonist
bromocriptine lowers blood pressure in SHRs and WKYs (Tan & Hutchinson, 1987).
Injections of the more specific D2 agonist quinpirole into the posterior region of the
nucleus of the solitary tract, which is the region of the brain where afferent fibers from the
baroreceptors terminate, produced pressor responses in anesthetized decerebrated rats
while injections of quinpirole into various regions of the hypothalamus had no effect
(Yang et al., 1990). Apomorphine and pergolide, both mixed DA agonists, produced a
mixture of effects on the cardiovascular system that depended on the route of
administration, the presence of anesthesia, and the species studied (Van den Buuse & De
Jong, 1991). However, it is generally agreed that these mixed agonists have centrally mediated depressor effects on blood pressure. In conclusion, by acting at unknown sites in the central nervous system, DA has a predominantly lowering effect on blood pressure in both normotensive and hypertensive animals.

Evidence has also accumulated suggesting that the activity of the mesostriatal system is regulated by changes in blood pressure. For example, denervation of the baroreceptors results in a decrease in striatal DA content and release, and a decrease in the activity of tyrosine hydroxylase, the enzyme that synthesizes DA (Alexander et al., 1984, 1988). Moreover, DA release in the striatum is enhanced after increasing arterial blood pressure with phenylephrine injections or attenuated by decreasing carotid blood pressure by carotid artery occlusion (Yang & Lin, 1993). In turn, electrical stimulation of the origin of mesostriatal dopaminergic pathways in the ventral tegmental area and the substantia nigra pars compacta caused pressor responses in cats and rats (Tan et al., 1983; Angyan, 1989, 1991; Cornish & Van den Buuse, 1994). The pressor effects produced by stimulation of the ventral tegmental area were abolished by the intravenous administration of the DA antagonist haloperidol (Cornish & Van den Buuse, 1994). Therefore, the baroreflex appears to interact with the mesostriatal DA system to modulate DA release in the striatum. Conversely, the mesostriatal dopaminergic pathway appears to exert some control over the circulation in ways that is not yet understood. It is also not known how these two observations are related to one another and if they have any role in the pathogenesis of hypertension.
2.5 Role of Central Dopamine in Hypertension

A consistent effect of dopaminergic drugs is a reduction in blood pressure. For example, clinical trials using the oral administration of bromocriptine to patients with essential hypertension were successful at returning blood pressure to normotensive levels (Stumpe et al., 1977; Kolloch et al., 1981). Similarly, intravenous administrations of bromocriptine to SHRs decreased their blood pressures to levels comparable to their normotensive controls (McMurty et al., 1979; Sowers, 1981; Nagahama et al., 1984). In these studies, bromocriptine was acting centrally because metoclopramide, a DA antagonist that crosses the blood brain barrier, blocked bromocriptine's hypotensive effect, while domperidone, a DA antagonist that does not cross the blood brain barrier had no effect (Nagahama et al., 1984). An interesting and well documented observation is that bromocriptine given to humans with essential hypertension attenuates the cardiovascular hyperreactivity to physical or mental stress (Kolloch et al., 1980. 1981; Sowers et al., 1982a, 1982b). Consistent with these clinical observations, bromocriptine given intravenously to SHRs suppressed the pressor responses to immobilization stress in SHRs (Sowers, 1981). As discussed earlier, both humans with essential hypertension and SHRs have an increase cardiovascular reactivity to environmental stimuli and that this hyperreactivity may play a key role in the pathogenesis of hypertension (Folkow, 1982, 1987). These observations not only link DA to the maintenance of hypertension but also link DA to the enhanced cardiovascular reactivity common in both humans with essential hypertension and spontaneous hypertension in rats. A causal relationship between central
DA activity, cardiovascular reactivity, and hypertension remains to be demonstrated. However, evidence from previous investigations supports this hypothesis.

The antihypertensive effect observed following administration of DA agonists led researchers to propose that hypertensive humans and SHRs suffered from a central dopaminergic insufficiency. Several lines of evidence support this hypothesis. First, hormonal factors under inhibitory dopaminergic control are elevated in individuals with hypertension. For example, the concentration of prolactin in the plasma is elevated in both humans with essential hypertension and SHRs (Stumpe et al., 1977; Sowers et al., 1979). It has also been reported that plasma levels of vasopressin and thyroid-stimulating hormone are increased in SHRs (Kojima et al., 1975; Croften et al., 1978). These peptide abnormalities are consistent with a reduction in dopaminergic activity of the tuberoinfundibular system (Fuxe et al., 1985). A second line of evidence supporting the DA insufficiency hypothesis is provided by studies in which microinjections of DA into the brain of SHRs produce a greater hypotensive effect than in normotensive WKY (Kawebe et al., 1983; Hutchinson & Mok, 1984; Mok & Sim, 1987; Mok et al., 1990). A third line of evidence is provided by studies assessing differences in the content and release of DA in the brain of SHRs. For the most part, investigations of DA concentration in homogenates from dissected brain tissue have been inconsistent (Howes et al., 1984). However, a recent study using the more sensitive technique of microphotometric analysis of DA immunohistochemistry found a large decrease in DA content in the NA and caudate nucleus of the SHR (Sutoo et al., 1993). Moreover, DA release measured using microdialysis, was found to be lower in the caudate nucleus of the awake SHR (Linthorst
et al., 1991). Similarly, in vitro experiments have demonstrated an attenuated release of [3H]DA in tissue slices from the caudate nucleus of the SHR (Linthorst et al., 1990; Tsuda et al., 1991). The investigations which reported a decrease in DA content and release in the striatum of the SHR are consistent with the observations that systemic administrations of the DA agonist bromocriptine and i.c.v. injections of DA have antihypertensive effects in the SHR. In both cases, the endogenous DA insufficiency is corrected by exogenous administrations of DA and its agonist.

As pointed out earlier, changes in systemic blood pressure levels can alter DA release and metabolism in the striatum. An increase in blood pressure in hypertensive rats could induce the well documented changes in dopaminergic neurotransmission in the brain. Furthermore, the random fixation of genes in the SHR that are not related to blood pressure regulation could also account for changes in parameters such as DA levels in the brain of the SHR (Rapp, 1983). However, several studies contradict these alternative hypotheses. First, central administrations of DA in the SHR produced an enhanced depressor response compared to WKY, but this enhanced response was absent in the New Zealand strain of hypertensive rats, renal hypertensive rats, and deoxycorticosterone-salt hypertensive rats (Kawebe et al., 1983; Mok & Sim, 1987). Second, while low levels of DA were found in the extracellular fluid of the dorsal striatum of the SHR, normal levels were found in renal and deoxycorticosterone-salt hypertensive rats (Linthorst et al., 1991). Third, electrically stimulated release of [3H]DA from dorsal striatum slices and an upregulation of DA receptors in the dorsal and ventral striatum was reported in prehypertensive SHRs demonstrating that changes in DA neurotransmission occur before
the onset of hypertension (Linthorst et al., 1990; Kirouac & Ganguly, 1993a). Finally, Tan and Hutchinson (1988) reported that hypertension and the antihypertensive effect of the DA agonist bromocriptine cosegregated in the F2 generation of a SHR/WKY hybrid. This cosegregation establishes a genetic linkage between changes in central DA and hypertension in the SHR. In conclusion, these studies show that the changes in DA activity in the SHR are unique to this model of hypertension and that these changes are probably not secondary to an increase in arterial blood pressure. These studies also support the notion of a genetic link between hypertension and an attenuation in DA neurotransmission in the brain of the SHR. It is important to note that these studies support the premise that the WKY is an appropriate control in the investigation of DA neurotransmission in the SHR.

2.6 Possible Role for the Nucleus Accumbens in Hypertension

The NA, which forms the ventral aspect of the rostral portion of the striatum, has been referred by many as the limbic striatum because of its extensive connections with the remainder of the limbic system and the hypothalamus (Mogenson, 1987; Heimer & Alheid, 1991). As stated earlier, some studies have documented changes in dopaminergic neurotransmission in the NA of the SHR. For example, Sutoo et al. (1993) reported lower tissue levels of DA in the NA of adult hypertensive SHRs. In addition, an upregulation of DA receptors was found in the NA of young prehypertensive and adult hypertensive SHRs (Kirouac & Ganguly; 1993a). The strong link between the limbic system, a well known region of the brain that can influence the cardiovascular system
(Loewy, 1991), and the NA, suggests that changes in dopaminergic neurotransmission in this area of the striatum may have some implications for hypertension.

Recent investigations on the neuroanatomy of the NA have revealed that projection patterns to and from this area of the striatum are highly organized. The caudal part of the NA contains two subterritories with distinct afferent and efferent projections (Zham & Brog, 1992). The core of the NA located immediately around the anterior limb of the anterior commissure is more striatal-like with projections to widespread regions of the ventral pallidum, entopeduncular nucleus, and substantia nigra/ventral tegmental area (Heimer et al., 1991). The shell of the NA, which is located eccentrically on the medial side of the core in the posteromedial portion of the NA, sends numerous projections to the LHA, bed nucleus of the stria terminalis, centromedial amygdala, and the ventral pallidum (Haber et al., 1990; Heimer et al., 1991). The density of afferent projections from limbic structures to the striatum is also organized topographically. The accumbal shell receives a very dense input from the basolateral nucleus of the amygdala (BLA) and the hippocampus (Kitai & Kitai, 1990; McDonald, 1991; Brog et al., 1993), while the dorsal striatum and the accumbal core receive a less dense projection from these limbic structures. Therefore, the projection pattern of the core parallels the rest of the striatum while the shell appears to be a transitional region between the striatum and the limbic system. In addition, the accumbal shell is distinguished from the rest of the striatum by its projections to the rostrocaudal extent of the LHA. Both the BLA and the LHA are involved in the integration and production of emotional behaviors and the autonomic responses that accompany these behaviors. For example, chemical and electrical
stimulation of the BLA and the LHA in the rat produces attack behavior with large increases in blood pressure and heart rate (Yardley & Hilton, 1986; Maskati & Zbrozyna, 1989). Because of their role in emotional arousal and cardiovascular reactivity, the BLA and the LHA have been implicated in the pathogenesis of hypertension. This role is supported by the demonstration that lesions of the BLA in the SHR attenuates the development of hypertension (Galeno et al., 1982). A preliminary investigation has also found support for the existence of a relay from the amygdala to the NA by showing that electrical stimulation of the NA inhibits the cardiovascular effects elicited from stimulation of the BLA (Haifa et al., 1989). Based on the anatomical evidence presented earlier, it is possible that fibers from the BLA may in some way influence neurons in the NA which innervate the LHA. Therefore, an amygdala-NA-hypothalamus pathway could be important for the integration of the behavioral and autonomic responses that comprise the defense reaction.

As presented earlier, activation of mesostriatal dopaminergic neurons has a modulating influence on the relay of information in the NA from the amygdala. For example, activation of accumbal neurons by stimulation of the amygdala is reduced by the stimulation of dopaminergic cells in the ventral tegmental area or by the exogenous application of DA to accumbal neurons (Mogenson, 1987). Consequently, an attenuation in DA neurotransmission in the NA of the SHR may result in alterations in the modulation of the output from the amygdala by way of the NA and the LHA. This could lead to the enhanced defense reaction or behavioral hyperreactivity seen in the SHR.
2.7 Modulation of Dopamine Function by Cholecystokinin

The carboxyl-terminal sulphated cholecystokinin octapeptide (CCK8S) is found in a large concentration in the central nervous system where it is believed to function as a neurotransmitter or neuromodulator (Crawley, 1985; Rehfeld, 1985). Some of the known biological functions of CCK8S include thermoregulation (Morley et al., 1981), antinociception (Jurna & Zetler, 1981), satiation (Morley et al., 1985), and the release of pituitary hormones (Vijuyun et al., 1979). Reports showing that central injections of CCK8S in anesthetized cats (Pagani et al., 1982; Gillis et al., 1983) and in conscious rats (Shido et al., 1989) increased arterial blood pressure, heart rate, and respiratory rate suggest that this neuropeptide may have a role in cardiovascular regulation. In addition, a role for CCK8S in hypertension is suggested by studies reporting decreases in CCK8S content in the cortex, hippocampus, pituitary gland, and spinal cord of the SHR (Jarrot et al., 1987; Shulkes et al., 1989).

The sulphated form of cholecystokinin is found in large concentrations in cortical and amygdaloid neurons that project to the NA (Meyer & Protopapas, 1985; Zaborsky et al., 1985). Moreover, CCK8S has been shown to be co-localized with DA in neurons of the ventral tegmental area and co-localized with tyrosine hydroxylase, the enzyme involved in the synthesis of the DA and norepinephrine, in neurons of the nucleus of the solitary tract (Hökfelt et al., 1980; Seroogy et al., 1987, 1988a, 1988b, 1989a, 1989b; Wang et al., 1992). In addition, a large amount of evidence has accumulated on the functional interaction between CCK8S and DA in the posterior portion of the NA (Crawley, 1991; Crawley & Corwin, 1994).
The studies that have tested the hypothesis that CCK8S modulates the release of DA in the NA have had conflicting conclusions (for review see Marshall et al. 1991). However these studies have largely ignored the anatomical and chemical differences between the anterior and posterior parts of the NA. Indeed, the modulatory effects of CCK8S receptor stimulation appears to be different in subregions of the striatum and dependent on which CCK8S receptor type is being stimulated. Marshall et al. (1991) reported that CCKA receptor subtype mediates CCK8S-stimulation of DA release in the posterior aspect of the NA while CCKB receptor subtype mediates CCK8S-inhibition of DA release in the anterior portion of the NA (Marshall et al. 1991). It is clear from the evidence presented above that presynaptic regulation of DA release in the striatum is complex. It is also important to note that the circumscribed distribution of CCK8S within dopaminergic systems raises the attractive possibility that agents that interact with CCK8S receptors could provide more anatomically specific approaches for correcting the dysfunction in dopaminergic neurotransmission.

The evidence presented above points to the possibility that changes in DA neurotransmission in the ventral striatum of the SHR may be due to changes in CCK8S-induced DA release in this area. This is supported by the observation that the cortex, hippocampus, and brainstem of SHRs contain less CCK8S than in WKYs (Jarrot et al., 1987; Shulkes et al., 1989). However, it is not known if the changes in CCK8S receptor function is related to the changes in DA neurotransmission in the SHR. The objectives listed in the following section were developed to test this hypothesis.
3. OBJECTIVES AND HYPOTHESES

Recent studies suggest that an attenuation in central dopaminergic activity may be a factor in the development and maintenance of primary hypertension in both humans with essential hypertension and the SHR. The NA, which receives a strong innervation from DA containing neurons in the midbrain, is of particular interest for understanding cardiovascular regulatory mechanisms and hypertension. As described in the literature review, several reports have found a decrease in the amount of DA and an upregulation of DA receptors in the NA of SHRs. In terms of its neuroanatomy, the NA receives numerous projections from the BLA and in turn the NA projects to the LHA which innervates and controls many cardiovascular centers in the brainstem and spinal cord. The work in this thesis is based on the hypothesis that hypertension in the SHR develops and is maintained by dysfunctions in normal dopaminergic neurotransmission in the mesoaccumbens dopaminergic pathway. More specifically, it is hypothesized that a change in DA release in the NA of the SHR is related functionally to changes in CCK8S neurotransmission. The objectives and hypotheses are as follows:

Objective #1: To assess CCK8S receptor density in the striatum of the prehypertensive and hypertensive SHR and age-matched control WKY. These experiments were conducted to test the hypothesis that the density of CCK8S receptors in the NA of the SHR is different from the WKY. Tissue sections from SHR and WKY brains were incubated with radiolabeled CCK8S and exposed to radioactivity sensitive film. The binding density of CCK8S in the striatum was determined using microdensitometry. Since CCK8S is involved in the modulation of DA release in the NA,
demonstrating changes in CCK8S receptor density in the SHR could explain the changes in DA neurotransmission in this strain of hypertensive rats.

**Objective #2:** To compare the CCK8S-induced release of DA and its metabolites in the NA of the SHR and WKY. These experiments tested the hypothesis that there is a lower level of DA in the NA of the SHR when compared to the WKY. These experiments also tested the hypothesis that the differential release of DA results from changes in CCK8S receptor numbers or mechanisms. The extracellular fluid concentrations of DA and the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindolacetic acid (HIAA) were measured in the NA using *in vivo* microdialysis before and after stimulation of local receptors with CCK8S. Both DOPAC and HVA are the primary metabolites of DA, while HIAA is formed by the catabolism of serotonin (Commissiong, 1985; Molinoff & Axelrod, 1971). These substances have been used to estimate neuronal activity in dopaminergic and serotonergic systems in the central nervous system. The information will be used to document the DA insufficiency in the NA of the SHR and to determine whether CCK8S plays a role in this DA insufficiency.

**Objective #3:** To compare the effect of CCK8S agonism and antagonism on the development of hypertension in SHRs. These experiments tested the hypothesis that blocking or stimulating CCK8S receptors enhances or attenuates the development of hypertension in the SHR. Young prehypertensive SHRs were treated daily by peripheral injections of the CCK8S antagonist proglumide and the CCK8S agonist ceruletide. Blood pressure, heart rate, and body weight were measured weekly during a 5 week treatment period. The results of these experiments will determine if CCK8S is involved in
the development of hypertension and may offer insights on future strategies to control hypertension in SHRs.

Objective #4: To demonstrate the location of the terminal field from the BLA to the NA and the origin of projection neurons in the NA to the LHA. These preliminary experiments will test the hypothesis that neurons in the BLA relay information to accumbal neurons that project to the LHA. Neurons projecting from the NA to the LHA were labeled by injecting the retrograde tracer Fluoro-Gold (FG) in the LHA. In the same subjects, projection fibers and terminals from the BLA to the NA were labeled by injecting the anterograde tracer Fluoro-Ruby (FR) in the BLA. Tissue sections of the NA were examined to determine if FR-labeled fibers originating in the BLA overlapped with FG-labeled neurons that project to the LHA. The NA was also examined for evidence of the FR-labeled fibers making contact with FG-labeled somas and dendrites in the NA. This will confirm the anatomical substrates for the relay of information in the NA from cardiovascular centers in the amygdala to the hypothalamus. Confirmation of these pathways will suggest a mechanisms by which dysfunctions in DA in the NA could lead to hypertension.
4. MATERIALS AND METHODS

4.1 Materials

The SHR and WKYs were purchased from Charles River (New York, USA) and the Sprague-Dawley rats were obtained from the University of Manitoba Central Animal Care Services. The \(^{125}\text{I}-\text{Bolton-Hunter}\) labeled CCK8S was purchased from NEN Dupont (Boston, USA). The \([^{125}\text{I}]\text{Microscale standards and } [^{3}\text{H}]\text{Hyperfilm were obtained from Amersham (Oakville, Canada). The neuropeptides CCK8S and the unsulphated form of CCK8 (CCK8US), proglumide, and ceruletide were acquired from Sigma (St. Louis, USA). The fluorescent tract-tracers Fluoro-Gold was obtained from Fluorochrome Inc. (Englewood, USA) and Fluoro-Ruby from Molecular Probes Inc. (Eugene, USA). The microdialysis probes were purchased from Bioanalytical Systems (West-Lafayette, USA). All other reagents used in the following experiments were analytical grade.

4.2 Systolic Blood Pressure Measurements

The systolic blood pressure of SHR and WKYs was measured using the tail-cuff method according to previously described methods (Leenen & de Jong, 1971). Rats were placed in an animal restrainer and warmed with a lamp for a few minutes. An inflatable occlusion cuff containing a photoelectric sensor was placed on the animal's tail. The cuff was inflated and the pressure slowly released so that the pulse signal could be detected and amplified with a IITC Model 59 pulse amplifier (Woodland Hills, California, USA). The mean of five readings was recorded as the rat's systolic blood pressure.
4.3 Cholecystokinin Receptor Autoradiography

4.3.1 Preparation of Autoradiograms

The blood pressure of 5 and 15-week-old SHRs and WKYs was measured one day before the experiments were started. The rats were anesthetized using sodium pentobarbital (5 mg/100 g, i.p.) and perfused transcardially with 0.9% ice-cold saline. The brains were removed quickly and blocked in a stereotaxic frame with the incisor bar set at 3 mm below the interaural line. Selected levels of the forebrain (coronal sections of the striatum were selected and matched in fresh tissue according to well demarcated anatomical landmarks such as joining of corpus collosum, shape of lateral ventricles, and location of the anterior commissure) were sectioned at 15 μm in a cryostat and thaw-mounted on glass slides. Cholecystokinin receptor autoradiography was performed according to a previously standardized protocol (Niehoff, 1989). Tissue-mounted glass slides were pre-incubated for 30 min at room temperature in 50 mM Tris HCl (pH 7.4) containing 130 mM NaCl, 4.7 mM KCl, 5 mM MgCl₂, 1 mM EGTA, and 0.5% bovine serum albumin (Tris saline buffer). The tissue sections were incubated for 150 min at room temperature in the Tris saline buffer (pH 6.5) containing 6 μg/ml aprotinin, 0.025% bacitracin, 1 mM dithiothreitol, and 0.1 nM ²⁵I-Bolton-Hunter-CCK8S (²⁵I-BH-CCK8S, 2200 Ci/mmol, NEN Dupont, Boston, USA). The concentration of ²⁵I-BH-CCK8S used for incubation of the tissue equals the dissociation constant (Kₐ) for CCK8S (Niehoff, 1989). The resulting binding would approximate the maximum binding capacity (Bₘₐₓ) for this radioligand (Niehoff, 1989). Non-specific binding was assessed in the presence of 1 μM of unlabeled CCK8S (Sigma, St. Louis, USA). Competition experiments show that
at this concentration, CCK8S displaced the binding of $^{125}$I-BH-CCK8S ($K_s$ for CCK8S is equal to 0.085 nM; Niehoff, 1989). The tissue sections were washed 6 x 15 min at 4°C in preincubation buffer followed by a dip in ice-cold double distilled water. The tissue, along with $[^{125}]$Microscales (Amersham, Oakville, Canada), were exposed for 3 days at 4°C to $[^{3}H]$Hyperfilm (Amersham, Oakville, Canada). The autoradiograms were developed by dipping the films at room temperature in the following solutions: 5 min in Kodak D-19 developer, 5 min in Kodak fixer, and 30 s in distilled water.

4.3.2 Image Analysis System for Quantifying Cholecystokinin Binding Density

The optical density of the autoradiograms were scanned electronically to measure the proportion of constant light that passes through the tissue image. To accomplish this, the image is transmitted from a video camera to a monitor and digitized by a computer. The computer can then be used to create a gray scale for each pixel. Tissue density readings can be taken from anatomically defined areas. The quantification is achieved by comparing the density readings from anatomical areas of the brain and the density of the Microscale standards.

The instrumentation for computer-assisted densitometry consisted of a light-box (Logan Electric, Chicago, USA), a COHU 4810 series CCD video camera (San Diego, USA), an electrohome ECM 1312 U high resolution color monitor (Kitchener, Ontario, Canada), and a microcomputer. The Targa-M8 frame grabber software (Truevision, Indianapolis, USA) stores a single image from the video camera and converts this image to a set of digital values. Interactions with the image displayed on the video monitor was
accomplished using a Numonics Model 2210 digitizing tablet (Montgomeryville, USA). Optical density measurements were taken using the JAVA video analysis software (Jandel Scientific, Corte Madera, USA) which can detect 256 levels of gray.

4.3.3 Protocol for Quantification of Cholecystokinin Binding and Data Analysis

Quantification of ligand binding density in autoradiograms was performed according to previously described methods (Peretti-Renucci et al., 1991). The best statistical fit (polynomial adjustment) for the relationship between the optical density of the standard spots and their radioactivity content was used to generate a standard curve. Using the standard curve, the optical density readings in tissue could be converted to radioactivity content for individual tissue regions. The optical density readings were corrected for background film density and converted to fmol of $^{125}$I-BH-CCK8S bound per mg of tissue according to the reference standard. The matching of sections between the two strains was accomplished by cutting sections according to anatomical landmarks as described above. These matches were verified in thionin-stained sections taken adjacent to the sections used for autoradiography. Optical density measurements were taken from selected brain regions by outlining an anatomical structure using an interactive device. These anatomical structures included the anteromedial prefrontal cortex, the nucleus accumbens in the middle portion of its rostrocaudal aspect, and the medial and lateral portions of the caudate-putamen at the same stereotaxic level as the nucleus accumbens (Figure 1). The optical density readings for each anatomical structure were an average
Figure 1. Sections of the brain adapted from the stereotaxic atlas of Paxinos and Watson (1986) showing the approximate location of densitometry measures used to quantify $[^{125}\text{I}]-\text{BH-CCK8S}$ binding in the receptor autoradiography experiments. Shaded areas represent measures were taken unilaterally in the prefrontal cortex (PFC), nucleus accumbens (NA), and the medial and lateral portions of the caudate-putamen (CPu).
reading for the defined area. Strain differences in systolic blood pressure and CCK8S receptor binding density were compared using analysis of variance (ANOVA). A value of $p < .05$ was taken to be statistically significant.

4.4 In vivo Microdialysis

Microdialysis involves the placement of a small dialysis probe in selected regions of the brain. An infusion pump is connected to the inlet of the dialysis probe to circulate perfusion fluid through the probe. The dialysis membrane allows the free diffusion of water and small solutes between the brain interstitial fluid and the solution used to perfuse the probe. Because the perfusion fluid is constantly circulated through the probe, a concentration gradient is created for substances in the interstitial fluid (Figure 2A). This forms the basis for the diffusion of substances from the brain into the dialysis probe. The dialysate samples are then collected from the outlet of the probe and the neurochemicals of interest are analyzed using an appropriate assay.

4.4.1 Surgical Preparation

Ten-week-old male SHRs and WKYs were housed for one week before experiments were conducted. The rats were housed on a 12/12 hours dark-light cycle in a temperature controlled environment (22 ± 1°C) with free access to food and water. Experiments were conducted during a period between 8:00 and 16:00 hrs. Ten-week-old SHRs and WKYs were anesthetized by an intramuscular injection of Ketamine (4.0 mg/100 g) and Xylazine (0.4 mg/100 g). The anesthetic was given every hour
Figure 2. Diagram showing the instrumentation for the microdialysis experiments. (A) Example of the design of a BAS microdialysis probe. Substances in the interstitial fluid diffuse down their concentration gradient into the probe (inset) where they are collected and analyzed with an appropriate assay. (B) Schematic showing the instruments used for the microdialysis experiments. An anesthetized rat is placed in a stereotaxic frame and the probe is lowered in the desired region of the brain. The inlet port of the probe is connected to a syringe selector which in turn is connected to the syringes in the perfusion pump. The outlet port of the probe is connected to tubing which accesses microvials in the fraction collector. The body temperature of the anesthetized rat is kept at 37°C using a thermal pad.
at the same dose for the remainder of the experiment. A previous report has shown that Ketamine anesthesia does not appear to interfere with normal dopaminergic neurotransmission (Koshikawa et al., 1988). Body temperature was maintained at 37 °C using a homeothermic blanket control unit (Harvard Apparatus, USA). Rats were placed in a Kopf stereotaxic frame with the incisor bar set at 3 mm below the interaural line. A microdialysis probe (CMA/12, Bioanalytical Systems, BAS, West Lafayette, USA) with an exposed dialysis membrane of 1 x 0.5 mm and 20,000 Dalton MW cutoff was lowered in the posteromedial portion of the NA using the following coordinates: AP, + 2.0 mm; ML, + 1.2 mm; DV, - 8.0 mm with bregma and the dura as the reference zero point. The inlet port on the microdialysis probe was connected to a CMA/110 liquid switch (BAS) which in turn was connected to microsyringes placed in a CMA/100 microinjection pump (BAS). The outlet port of the probe was connected to a piece of tubing placed in a CMA/140 microfraction collector (BAS). Figure 2A shows a diagram of the instruments used for the microdialysis experiments. The probe was perfused at a flow rate of 1.6 μl/min with a perfusate containing 147 mM NaCl, 4 mM KCl, 1.2 mM CaCl₂, and 1.1 mM MgCl₂ (pH 6.0). In vitro probe recovery was done prior to each experiments by placing the probe in an eppendorf vial containing 100 ng/ml of DA, and the metabolites DOPAC, HVA, and HIAA dissolved in the perfusate. After a 30 min stabilization period, three 15 min samples were collected at a flow rate of 1.6 μl/min. The mean of the three samples were used to correct the basal values for the microdialysis experiments.
4.4.2 Protocol for Stimulation of Cholecystokinin Receptors

The extracellular fluid concentrations of DA, DOPAC, HVA, and HIAA were measured before and after perfusing the brain with CCK8S. After a 2 hour stabilization period following the implantation of the probe, nine 15 min microdialysis samples were collected in the following order: 3 predrug, 3 drug, and 3 postdrug samples. The CCK agonists were delivered through the microdialysis probe via the perfusate and only one agonist was given to each rat. In the first series of experiments, the caudal portion of the NA of SHRs and WKYs was perfused with a 10 µM concentration of CCK8S ([Tyr(SO3H)27]-cholecystokinin fragment 26-33 amide, Sigma). A second series of experiments was performed in the SHR to determine if perfusion of the NA with a 1 µM concentration of CCK8S would have any effect on the amount of DA and metabolites collected. A third series of experiments was conducted in the SHR to determine if a 10 µM concentration of CCK8US (cholecystokinin fragment 26-33 amide non-sulfated, Sigma, St Louis) had similar effects on DA release in the NA. All drugs were dissolved in perfusate and stored in aliquot at - 80°C until the day of the experiments.

4.4.3 High Pressure Liquid Chromatography

The samples collected were injected directly at a volume of 20 µl in a reverse-phase high pressure liquid chromatography (HPLC) system coupled to an electrochemical detector (Waters, Mississauga, Canada). The HPLC system consisted of Waters Resolve C18 5 µm dimethyloctadecylsilyl bonded silica particle size in a 3.9 mm x 150 mm column, 510 pump, U6K liquid injector, and 460 electrochemical detector. The
glassy carbon working electrode was set at + 0.60 Volts vs. a Ag/AgCl reference electrode. The mobile phase (pH 2.55) contained 50 mM monochloroacetic acid, 50 mM citric acid monohydrate, 44.4 mM sodium hydroxide, 1.4 mM disodium EDTA, 0.69 mM sodium octane sulfonate, 4.5 % acetonitrile, and 0.45 % diethylamine. All reagents were analytical or HPLC grade and the mobile phase was filtered and degassed. Dopamine and the metabolites eluted within 10 min of the injection when the flow rate on the HPLC pump was set at 0.9 ml/min. The chromatographic data was collected on a microcomputer using Baseline 810 chromatographic workstation software. Standard samples containing 10 ng/ml of DA and 100 ng/ml of DOPAC, HVA, and HIAA were used to quantify the samples.

4.4.4 Analysis of Data

Rats were perfused transcardially with 4.0% paraformaldehyde at the end of each experiment and the brains were removed and stored in 20% sucrose phosphate buffer. Sections of the rostrocaudal extent of the NA were taken using a freezing microtome and stained for Nissl bodies. Brain sections were examined for probe placements and sketched on plates from the stereotaxic atlas of Paxinos and Watson (1986). Data from rats in which the probe placement was outside the caudal portion of the NA were excluded from the analysis.

The predrug baseline data was corrected for differences in probe recovery and the mean of the three samples collected was used as the baseline level of monoamine collected. The baseline data is expressed as the concentration in moles per 20 μl of
collected dialysate and was analyzed using ANOVA. The results from the 9 samples collected from each rat was then converted to percent change from the baseline mean. The microdialysis time-course data was analyzed using ANOVA with repeated measures for time. Post-hoc analysis using Tukey's multiple comparison test was used to compare specific means when the overall ANOVA was found to be significant. A value of p < .05 was taken to be statistically significant.

4.5 Pharmacological Treatment Experiments

Three different groups of young prehypertensive SHRs (4-weeks-old) were treated daily for 4 weeks with subcutaneous injections of vehicle (saline, 0.5 ml/kg), the CCK8S antagonist proglumide (1 mg/kg/0.5 ml; Sigma), and the CCK8S agonist ceruletide (10 μg/kg/0.5 ml; Sigma). Proglumide and ceruletide were reconstituted in a small amount of saline, aliquoted in vials and stored at -80°C until the time of treatment when the pharmacological agent were further diluted in vehicle and injected subcutaneously. Body weight was monitored daily because of the possible effects of stimulation or blockade of CCK8S receptor on satiety and feeding (Crawley & Corwin, 1994). Systolic blood pressure and heart rate were measured weekly using the tail-cuff method as previously described. Body weight, systolic blood pressure and heart rate were analyzed using ANOVA with post-hoc tests when the overall ANOVA was found to be significant. A value of p < .05 was taken to be statistically significant.
4.6 Tract-Tracing Experiments

The experiments were performed on male Sprague-Dawley rats weighing 350 to 450 g. The rats were anesthetized by an intramuscular injection of Ketamine (60 mg/kg) and Xylazine (9 mg/kg). The rats were placed in a stereotaxic frame with the incisor bar set at 5 mm above the interaural line and microinjections of fluorescent dyes were made according to previously described methods (Nance & Burns, 1990). The dyes were dissolved in distilled water (2% to 5% solutions) and pressure injected in the brain using glass micropipettes (30 to 40 μm tip) attached to a 1 μl microsyringe backfilled with mineral oil. The anterograde tracer FR (MW = 10,000; Molecular Probes Inc., Eugene, USA) was microinjected in the BLA. In the same rats, the retrograde tracer FG (Fluorochrome Inc., Englewood, USA) was microinjected in the LHA. The coordinates for the LHA (AP +0.65 mm; ML +0.16 mm; DV - 0.49 mm) and the BLA (AP + 0.52 mm; ML + 0.50 mm; DV - 0.53 mm) were derived from the stereotaxic atlas of Peligrino et al. (1979). The volumes injected ranged from 0.05 to 0.1 μl and were infused at a rate of 0.005 μl/min using a microinjection device. The micropipette remained in the brain for 20 min following the injection. The incision was closed with wound clips and the rats were given 0.3 mg of buprenorphine hydrochloride subcutaneously (Reckitt & Colman, Richmond, USA) every 12 hrs for 3 days to control post-surgical pain.

Following a survival period of 7 to 14 days, the rats were anesthetized and perfused transcardially with a 4.0% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.2). The tissue was postfixed for 4 hrs and then placed in 20% sucrose phosphate buffer for 48 hrs prior to sectioning. The forebrain was sectioned at 30 μm using a
freezing microtome and collected in 0.1 M phosphate buffer. The sections were mounted on chrome-alum/gelatin coated slides and air dried at room temperature. The dried tissue sections were dipped in 50% ethanol for 60 s, 100% ethanol for 30 s, xylene for 3 x 60 s and coverslipped with DPX plastic mounting media (Fluka, Buchs, Switzerland). The slides were air-dried in the dark and then stored in slide boxes at 4°C. Alternate sections of brain regions containing the injection sites were stained for Nissl bodies. The brain sections were studied using an Leitz Dialux 22 microscope equipped with fluorescent filters (Leitz 3-Ploemopak). The FR and FG injection sites were studied and the corresponding locations were mapped on templates taken from the stereotaxic atlas of Pelegrino et al. (1979). Sections of the NA were examined for both FR-labeled fiber terminals and FG-labeled cells using a low-magnification objective (10X) and a high-magnification oil-immersion objective (40X). Once FG-labeled cells were located using a wide band ultraviolet filter, the green filter was substituted on the fluorescent microscope to see if FR-labeled terminals make contact with the FG-labeled cells. Double exposures were taken using Kodak Ektachrome 100HC color film.

4.7 Statistical Analyses

All data are expressed as the mean ± SEM. Group differences were analyzed using ANOVA and specific means were compared using Tukey's multiple comparison test. A value of p < .05 was accepted as statistically different. Detail descriptions of the methods used to process and analyze the data for each experiment are presented in previous sections.
5. RESULTS

5.1 Cholecystokinin Receptor Density in the Striatum of SHRs and WKYs

The systolic blood pressure of 5-week-old SHRs and WKYs was similar (79 ± 1 mmHg and 82 ± 1 mmHg, respectively) while the systolic blood pressure of 15-week-old SHRs was significantly higher than that of WKYs (209.2 ± 17.7 vs 118.2 ± 0.9, \( F_{1,11} = 11.5, p < .001 \)). Figure 3 shows examples of the autoradiograms of a \(^{125}\text{I}\) Microscale standard strip (Figure 3A), and CCK8S specific (Figure 3B) and nonspecific (Figure 3C) binding at the level of the striatum in one rat. Note that application of unlabeled CCK8S in the incubation media almost completely blocked the binding of \(^{125}\text{I}\)-BH-CCK8 to brain tissue. This shows that the \(^{125}\text{I}\)-BH-CCK8S binding in these experiments was specific. The best statistical fit (polynomial adjustment) for the relationship between optical density and radioactivity content of the standard spots was used to generate the standard curves (Figures 4 and 5). The equation that describes this relationship for CCK8S binding in 5-week-old rats is expressed as \( Y = 2.22 - 21.2x + 64.56x^2 - 26.11x^3 \) and the equation that describes this relationship for 15-week-old rats is expressed as \( Y = -1.1 + 13.51x - 30.47x^2 + 43.85x^3 \). Accordingly, the correspondence between the optical density values measured on film for the images of brain tissue and the radioactivity content in this brain tissue could be deduced from these calibration curve. The amount of tissue radioactivity was then converted to fmol of ligand bound to 1 mg of tissue.

As shown in figure 6, the binding density of CCK8S was similar in the prefrontal cortex of SHRs and WKYs at the ages of 5 weeks (\( F_{1,11} = 0.05, p < .827 \)) and 15 weeks
Figure 3. Example of an autoradiogram showing $[^{125}\text{I}]-\text{BH-CCK8S}$ binding in the striatum of a 15-week-old SHR.  
A. Autoradiogram of $[^{125}\text{I}]$Microscale standard strip showing that the grain density increases with the amount of radioactivity in the standard spots.  
B. Autoradiogram showing the grain distribution representing total specific binding of $[^{125}\text{I}]-\text{BH-CCK8S}$ in the caudate-putamen (CPu) and the nucleus accumbens (NA).  
C. Autoradiogram showing the effect of blocking $[^{125}\text{I}]-\text{BH-CCK8S}$ binding by adding unlabeled CCK8S to the incubation medium.
Figure 4. Standard curve showing the best statistical fit for the relationship between optical density (OD) and radioactivity content (nCi/mg) in the $[^{125}I]$Microscale standard for the $[^{125}I]$-BH-CCK8S binding experiments in 5-week-old SHRs and WKYs experiments.
Figure 5. Standard curve showing the best statistical fit for the relationship between optical density (OD) and radioactivity content (nCi/mg) in the $^{[125}I]$Microscale standard for the $^{[125}I]$-BH-CCK8S binding experiments in 15-week-old SHRs and WKYs experiments.
Figure 6. Histogram showing the binding density of $^{125}$I-BH-CCK8S in the prefrontal cortex of 5 and 15-week-old SHRs and WKYs. Data is expressed as the mean ± SEM (n = 6 for all groups).
(F_{1,11} = 0.15, p < .706). In the medial portion of the caudate-putamen, the binding density of CCK8S was not significantly different between 5-week-old (F_{1,11} = 0.07, p < .802) and 15-week-old (F_{1,11} = 2.79, p < .123) SHRs and WKYs (Figure 7). No statistical difference in CCK8S binding was found in the lateral portion of the caudate-putamen of 5-week-old rats (F_{1,11} = 0.83, p < .384) but a significantly greater number of CCK8S receptors was found in the lateral caudate putamen of 15-week-old (F_{1,11} = 4.9, p < .045) SHRs (Figure 8). As shown in Figure 9, the binding density of CCK8S was found to be greater in the posterior portion of the NA in SHRs at both the ages of 5 weeks (F_{1,11} = 11.55, p < .0068) and 15 weeks (F_{1,11} = 5.34, p < .041).

5.2 Cholecystokinin-Induced Release of Dopamine in the Nucleus Accumbens

The systolic blood pressure of SHRs was significantly greater than that of WKYs (197 ± 5 mmHg vs 129 ± 3 mmHg; F_{1,11} = 119.7, p < .0001). Figure 10 shows a typical chromatogram produced by injecting a microdialysis sample from the NA into the HPLC system. The peaks for DA and the metabolites eluted within 10 min of injecting the sample into the HPLC system. The retention time for these substances were different from one another so that each substance could be quantified easily.

5.21 10 μM Cholecystokinin Experiments

Figure 11 shows an example of a microdialysis probe placement in the posteromedial portion of the NA. The probe placements in SHRs and WKYs used in the 10 μM CCK8S experiments are shown diagramatically in Figure 12. The probes were
Figure 7. Histogram showing the binding density of $^{125}$I-BH-CCK8S in the medial portion of the caudate-putamen of 5 and 15-week-old SHRs and WKYs. Data is expressed as the mean ± SEM (n = 6 for all groups).
Figure 8. Histogram showing the binding density of $^{125}\text{I}$-BH-CCK8S in the lateral portion of the caudate-putamen of 5 and 15-week-old SHRs and WKYs. Data is expressed as the mean $\pm$ SEM (n = 6 for all groups). *, p < .05 significantly different from WKYs as determined by ANOVA.
Figure 9. Histogram showing the binding density of $^{125}$I-BH-CCK8S in the NA of 5 and 15-week-old SHRs and WKYs. Data is expressed as the mean ± SEM ($n = 6$ for all groups). * $p < .05$ significantly different from WKYs as determined by ANOVA.
Figure 10. Typical HPLC chromatogram produced by the injection of a microdialysis sample collected in the NA.
Figure 12. Location of probe placements in the posterior portion of NA of SHRs and WKYs used for the 10 μM CCK8S experiments. The position and relative size of the microdialysis probe is shown by the filled rectangles drawn on plates adapted from the stereotaxic atlas of Paxinos and Watson (1986). Note that the position of the probes were similar in SHRs and WKYs. Abbreviations: NA, nucleus accumbens; CPu, caudate-putamen.
located in the posterior portion of the NA on the medial side of the anterior commissure and extended to the ventral portion of the NA above the olfactory tubercle.

Two hours after placing the microdialysis probe in the NA, the levels of DA, DOPAC, HVA, and HIAA had reached stable levels (Figures 13-16, at times 15, 30, and 45 min). The basal level (the mean of the 3 baseline values) of DA in the NA was lower in the SHR but not at a statistically significant level (Table I). The levels of the metabolites were similar in the SHR and WKY (Table I). Perfusing the NA with 10 μM of CCK8S through the microdialysis probe produced a large increase in the amount of DA collected in the NA of both SHRs and WKYs ($F_{8,72} = 33.7$, $p < .0001$, Figure 13). The ANOVA showed a significant strain difference in the CCK8S-induced release of DA ($F_{1,72} = 17.0$, $p < .0002$, Figure 13). The DA collected during the first sample (time 60 min) of the CCK8S perfusion was not different between SHRs and WKYs but the DA collected during the second (at time 75 min: SHR, $577 \pm 44\%$; WKY, $326 \pm 37\%$; $p < .05$) and third samples (at time 90 min: SHR, $289 \pm 59\%$; WKY, $187 \pm 22\%$; $p < .05$) were significantly greater in the SHR. Dopamine returned to levels lower than the predrug baseline after the termination of CCK8S perfusion. An increase in the amount of DOPAC collected during perfusion with 10 μM CCK8S was also found in the SHR and WKY ($F_{8,72} = 9.3$, $p < .0001$, Figure 14), with a small difference observed between these two groups in the post-perfusion samples (time 105 and 150 min). The ANOVA also revealed a time effect for the amount of HVA ($F_{8,72} = 2.3$, $p < .05$, Figure 15) and HIAA collected ($F_{8,72} = 8.2$, $p < .0001$, Figure 16) in response to perfusion with 10 μM CCK8S.
TABLE I. Basal concentrations of DA and metabolites recovered from the posterior portion of the NA in the SHR and WKY.

<table>
<thead>
<tr>
<th></th>
<th>DA fmol/20µl</th>
<th>DOPAC pmol/20µl</th>
<th>HVA pmol/µl</th>
<th>HIAA pmol/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>135 ± 39</td>
<td>6.88 ± 1.61</td>
<td>2.55 ± 0.23</td>
<td>1.14 ± 0.28</td>
</tr>
<tr>
<td>SHR</td>
<td>93 ± 15</td>
<td>6.79 ± 0.88</td>
<td>2.18 ± 0.26</td>
<td>1.50 ± 0.17</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 5).
Figure 13. Time-course effect of a 45 min local perfusion of 10 µM CCK8S (dark bar) on the extracellular level of DA in the posterior portion of the NA in WKYs (open squares) and SHRs (filled squares). The 100% value represents the mean of three baseline samples taken before the administration of CCK8S in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5). *, p < .05; SHRs compared to WKYs (Tukey's multiple comparison test).
Figure 14. Time-course effect of a 45 min local perfusion of 10 μM CCK8S (dark bar) on the extracellular level of DOPAC in the posterior portion of the NA in WKYs (open squares) and SHRs (filled squares). The 100% value represents the mean of three baseline samples taken before the administration of CCK8S in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5). *, p < .05; SHRs compared to WKYs (Tukey's multiple comparison test).
Figure 15. Time-course effect of a 45 min local perfusion of 10 μM CCK8S (dark bar) on the extracellular level of HVA in the posterior portion of the NA in WKYs (open squares) and SHRs (filled squares). The 100% value represents the mean of three baseline samples taken before the administration of CCK8S in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5).
Figure 16. Time-course effect of a 45 min local perfusion of 10 μM CCK8S (dark bar) on the extracellular level of HIAA in the posterior portion of the nucleus accumbens in WKYs (open squares) and SHRs (filled squares). The 100% value represents the mean of three baseline samples taken before the administration of CCK8S in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5).
No strain differences were found in either the HVA ($F_{1,72} = 1.76, p < .189, \text{Figure 15}$) and HIAA ($F_{1,72} = 2.28, p < .136, \text{Figure 16}$) collected during or post CCK8S perfusion. Perfusion of brain tissue with 10 μM CCK8S in regions posterior to the NA in the bed nucleus of the stria terminalis and the preoptic area in some rats produced no changes in DA release (data not shown).

5.2 1 μM Sulphated and 10 μM Unsulphated-Cholecystokinin Experiments

The probe placements for the 1 μM vs 10 μM CCK8S experiments in the SHR are shown in Figure 17. Perfusion of the NA with a 1 μM concentration of CCK8S produced no increase in the amount of DA, DOPAC, HVA, and HIAA collected in the NA (Figures 18-21). The probe placements for the 10 μM CCK8US vs 10 μM CCK8S experiments are shown in Figure 22. CCK8US at a 10 μM concentration had no effect on the amount of DA, HVA, HIAA collected in the NA of SHRs (Figures 23, 25, 26), but produced a large significant increase in the amount of DOPAC when compared to 10 μM CCK8S ($F_{8,72} = 25.4, p < .0001, \text{Figure 24}$).

5.3 Effects of Cholecystokinin Agonism and Antagonism on Hypertension

Daily subcutaneous administrations of proglumide (1 mg/kg) or ceruletide (1 μg/kg) in SHRs from the prehypertensive age of 5 weeks to hypertensive age of 8 weeks produced no drug effect on the SHRs' final body weights when compared to saline treated rats ($F_{2,51} = .12, p < .23; \text{Figure 27}$). These rats showed a gradual increase in
Figure 17. Location of probe placements in the posterior portion of NA of SHRs used for
the 1 vs 10 μM CCK8S experiments. The position and relative size of the microdialysis
probe is shown by the filled rectangles drawn on plates adapted from the stereotaxic atlas
of Paxinos and Watson (1986). Abbreviations: NA, nucleus accumbens; CPu,
caudate-putamen.
Figure 18. Time-course effect of a 45 min local perfusion (dark bar) of 1 µM CCK8S (open squares) and 10 µM CCK8S (filled squares) on the extracellular level of DA in the posterior portion of the NA in SHR's. The 100% value represents the mean of three baseline samples taken before the administration of CCK8S in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5). *, p < .05; 10 µM compared to 1 µM CCK8S (Tukey's multiple comparison test).
Figure 19. Time-course effect of a 45 min local perfusion (dark bar) of 1 µM CCK8S (open squares) and 10 µM CCK8S (filled squares) on the extracellular level of DOPAC in the posterior portion of the NA in SHRs. The 100% value represents the mean of three baseline samples taken before the administration of CCK8S in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5). *, p < .05; 10 µM compared to 1 µM CCK8S (Tukey's multiple comparison test).
Figure 20. Time-course effect of a 45 min local perfusion (dark bar) of 1 μM CCK8S (open squares) and 10 μM CCK8S (filled squares) on the extracellular level of HVA in the posterior portion of the NA in SHRs. The 100% value represents the mean of three baseline samples taken before the administration of CCK8S in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5)
Figure 21. Time-course effect of a 45 min local perfusion (dark bar) of 1 μM CCK8S (open squares) and 10 μM CCK8S (filled squares) on the extracellular level of HIAA in the posterior portion of the NA in SHRs. The 100% value represents the mean of three baseline samples taken before the administration of CCK8S in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5)
Figure 22. Location of probe placements in the posterior portion of NA of SHRs used for the 10 µM CCK8US vs CCK8S experiments. The position and relative size of the microdialysis probe is shown by the filled rectangles drawn on plates adapted from the stereotaxic atlas of Paxinos and Watson (1986). Abbreviations: NA, nucleus accumbens; CPu, caudate-putamen.
Figure 23. Time-course effect of a 45 min local perfusion (dark bar) of 10 µM CCK8US (open squares) and 10 µM CCK8S (filled squares) on the extracellular level of DA in the posterior portion of the NA in SHRs. The 100% value represents the mean of three baseline samples taken before the administration of CCK agonists in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5). *, p < .05; 10 µM CCK8S compared to 10 µM CCK8US (Tukey's multiple comparison test).
Figure 24. Time-course effect of a 45 min local perfusion (dark bar) of 10 μM CCK8US (open squares) and 10 μM CCK8S (filled squares) on the extracellular level of DOPAC in the posterior portion of the NA in SHRs. The 100% value represents the mean of three baseline samples taken before the administration of CCK agonists in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5). *, p < .05; 10 μM CCK8US compared to 10 μM CCK8S (Tukey's multiple comparison test).
Figure 25. Time-course effect of a 45 min local perfusion (dark bar) of 10 μM CCK8US (open squares) and 10 μM CCK8S (filled squares) on the extracellular level of HVA in the posterior portion of the NA in SHRs. The 100% value represents the mean of three baseline samples taken before the administration of CCK agonists in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5).
Figure 26. Time-course effect of a 45 min local perfusion (dark bar) of 10 μM CCK8US (open squares) and 10 μM CCK8S (filled squares) on the extracellular level of HIAA in the posterior portion of the NA in SHRs. The 100% value represents the mean of three baseline samples taken before the administration of CCK agonists in the perfusate. The results are expressed as the percentage of the baseline mean ± SEM (n = 5).
Figure 27. Effect of daily subcutaneous injections of saline (triangles), proglumide (1 mg/kg, diamonds), and ceruletide (1 μg/kg, squares) on body weight in SHRs from the age of 5 weeks to 8 weeks. Data is expressed as the mean ± SEM, n = 7 for each group.
blood pressure ($F_{3,51} = 44.36, p < .0001$; Figure 28) and a decrease in heart rate ($F_{3,51} = 12.2, p < .001$; Figure 29). When compared to saline and ceruletide treated SHRs, proglumide treated rats showed a significantly increased blood pressure at 7 and 8 weeks of age ($F_{2,51} = 9.5, p < .0004$; Figure 28). However, the ANOVA revealed no drug effect for heart rate ($F_{2,51} = .35, p < .71$).

5.4 Demonstration of a Possible Relay in the Nucleus Accumbens

In four rats, the FR injections were confined to the region of the BLA and the FG injections were localized in the LHA. The location and size of the FG and the FR injections in these four cases are shown schematically in Figure 30. The FG injections were limited to the posterior half of the LHA and were concentrated in the periforcal region. The FG injections were limited laterally by the cerebral peduncles and the optic tract without involving the entopeduncular nucleus. In some cases, the FG injection extended in the medial hypothalamus involving the lateral parts of the dorsomedial, ventromedial, and posterior hypothalamic nuclei. The FR injections were located mostly in the posterior region of the BLA. In some cases, the FR injection diffused dorsally involving portions of the lateral nucleus of the amygdala or diffused ventrally involving the basomedial nucleus of the amygdala. However, the most concentrated portion of the FR injections were confined to the BLA.

The fluorescent tracers labeled fibers and cells on the ipsilateral side of the injection sites. Injections of FG in the LHA resulted in the dense labeling of the soma
Figure 28. Effect of daily subcutaneous injections of saline (triangles), proglumide (1 mg/kg, diamonds), and ceruletide (1 µg/kg, squares) on systolic blood pressure in SHRs from the age of 5 weeks to 8 weeks. Data is expressed as the mean ± SEM, n = 7 for each group. *, p < .05; proglumide compared to saline and ceruletide (Tukey's multiple comparison test).
Figure 29. Effect of daily subcutaneous injections of saline (triangles), proglumide (1 mg/kg, diamonds), and ceruletide (1 μg/kg, squares) on heart rate in SHRs from the age of 5 weeks to 8 weeks. Data is expressed as the mean ± SEM, n = 7 for each group.
Figure 30. Series of drawings adapted from the stereotaxic atlas of Pelligrino et al. (1979) showing transverse sections taken through the caudal half of the hypothalamus extending from 4.6 to 5.4 mm rostral to bregma. The location of FG injections in the lateral hypothalamus and FR injections in the amygdala are shown in four cases (Cases 1 to 4). Abbreviations are as follows: BLA, basolateral nucleus of the amygdala; BMA, basomedial nucleus of the amygdala; CNA, central nucleus of the amygdala; CoA, cortical nucleus of the amygdala; EP, entopeduncular nucleus; FX, fornix; LHA, lateral hypothalamic area; LNA, lateral nucleus of the amygdala; MNA, medial nucleus of the amygdala; MT, mamillothalamic tract.
and dendrites of many neurons in the posteromedial portion of the NA (Figure 31, plate A and Figure 32). This region corresponds to the shell region of the NA as defined by Heimer et al. (1991). Fluoro-Gold completely filled the soma and dendrites of these neurons resulting in very brightly labeled cells. FG-labeled cells were not found dorsally in the caudate-putamen or in the area surrounding the anterior commissure which corresponds to the core region of the NA. Numerous FG-labeled neurons were located in the medial portion of the olfactory tubercle and in the lateral septal nucleus (Figure 32). A few labeled neurons were found in the ventral portion of the olfactory tubercle and the rostral extension of the ventral pallidum.

Injections of FR in the BLA resulted in labeling of fibers and terminals in the core and shell region of the NA and in the dorsal striatum. The highest density of FR terminals was found in the shell region of the NA while less dense labeling was found in the core (Figure 31, plate B and Figure 32). A distinct overlap of FR-labelled terminals with FG-labeled cells was found in the shell region of the NA (Figure 31, plate C and Figure 32). The area of overlap corresponded to the region where the highest density of FR-labelled fibers and FG-labeled neurons were located. Fluoro-Ruby labeled fibers were classified as axon-like if they appeared as long structures of relatively uniform diameter that often exhibit branching processes. Fluoro-Ruby labeled fibers were classified as terminals if they showed varicose swellings at the terminal end of axons, swelling at the ends of thin fibers or swellings along the axon-like processes (Figure 31, plate D). When a region of overlapping fiber terminals and cells was examined under high magnification, FR-labeled terminals appeared to make contact on FG-labeled cells (Figure 31, plates E
and F). In these cases, the shape of the FG-labeled cells could easily be distinguished from labeled terminals making contact on the cell. The FR-labeled terminal buttons were seen in proximity to the dendrites and the soma of FG-labeled cells. In some cases, the same axon terminals appeared to make axodendritic and axosomatic contacts on some FG-labeled neurons. In one case, the axon and its varicose swellings could be seen outlining the contour of the soma of a FG-labeled cell (Figure 31, plate E).
Figure 31. Photomicrographs showing FG-labeled cells and FR-labeled fibers and terminals in the NA. (A) FG-labeled cells in the posteromedial NA (arrow) following an injection of FG in the lateral hypothalamic area (300X). Labeled cells are also located in the medial portion of the olfactory tubercle. (B) FR-labeled fibers and terminals in the posteromedial region of the NA (arrow) following an injection of FR in the basolateral nucleus of the amygdala (300X). Note that FR-labeled fibers form a dense plexus over most regions of the posteromedial NA. (C) Double-exposure of the posteromedial NA showing overlapping of FR-labeled fibers and FG-labeled cells (300X). (D) FR-labeled axon at high magnification (1200X). The axon is of uniform diameter with enlarged portions that corresponds to the fiber terminals (arrows). (E-F) Double-exposure of a region of the posteromedial NA taken at high-magnification (1200X) showing labeled cells and fiber terminals. FR-labeled terminals can be seen in proximity to the soma and dendrites of FG-labeled cells (arrows).
Fig. 32. Schematic representation showing FG (dots) and FR (lines) labeling in the rostrocaudal extent of the NA following FG and FR injections into the LHA and BLA of the one rat, respectively. Note the dense overlap of FG-labeled cells and FR-labeled fibers in the shell region of the caudal portion of the NA (B) and the lack of labeling in the region around the anterior commissure. The rostral (A) and the extreme caudal portion (C) of the NA contains few labeled cells and fibers. Abbreviations are as follows: NA, nucleus accumbens; CPu, caudate-putamen.
6. DISCUSSION

The experiments presented in this thesis provide new information regarding the role of central dopaminergic neurotransmission in the development of hypertension in a rat model of genetic hypertension. More specifically, these experiments provide insights on how changes in CCK8S-induced release of DA may be responsible for some of the documented changes in DA activity in the ventral striatum of the SHR. In addition, neuroanatomical evidence presented in the thesis indicates a possible mechanism by which changes in CCK8S and DA activity in the ventral striatum could lead to hypertension.

6.1 Cholecystokinin Receptor Density in the Striatum of the SHR

The autoradiography experiments have demonstrated an increase number of CCK8S receptors in the NA of young prehypertensive and adult hypertensive SHRs when compared to the same age normotensive WKYs. In contrast, CCK8S receptor density in the prefrontal cortex and the medial portion of the caudate-putamen in both the prehypertensive and hypertensive SHR was not significantly different from the WKY. The binding density of CCK8S was greater in the lateral caudate-putamen of hypertensive SHRs but not different between young prehypertensive SHRs and WKYs. Several conclusions can be made from the finding of an upregulation of CCK8S receptors in the NA of the SHR. First, the increase number of CCK8S receptors is not the result of high blood pressure because the increase was observed in young prehypertensive SHRs. Second, alterations in CCK8S receptor density is probably not secondary to an attenuation in DA neurotransmission because changes in CCK8S receptors were not found in the
dorsal striatum where DA neurotransmission have been demonstrated to be suppressed. Finally, a role for CCK8S in the pathogenesis of hypertension is suggested because an upregulation of CCK8S receptors was detected in the NA of prehypertensive rats. An increase number of CCK8S receptors in the lateral portion of the caudate-putamen of adult hypertensive SHRs may be secondary to a rise in blood pressure or DA activity in that area of the striatum.

This is the first report concerning the status of CCK8S receptors in the brain of hypertensive rats. It may provide some information on the mechanisms producing alterations in dopaminergic neurotransmission in the striatum of the SHR. Indeed, an extensive amount of literature has accumulated on the interaction between CCK8S and DA in the ventral striatum. First, in vitro receptor binding studies have detected possible interactions between CCK8S and DA receptors. For example, chronic treatment with the D$_2$ antagonist haloperidol increased CCK8S binding in the NA while treatment with CCK8S enhanced D$_2$ receptor binding (Chang et al., 1983; Dumbrille-Ross & Seeman, 1984). Secondly, CCK8S has been shown to modulate DA-induced behaviors such as locomotion, exploration, and self-stimulation in rodents (Crawley & Corwin, 1994). Finally, CCK8S has been shown to affect the release of DA in the NA (Marshall et al., 1991; Ladurelle et al., 1993). Therefore, changes in CCK8S receptor density may be responsible for alterations in DA activity in the ventral striatum of the SHR. Furthermore, alterations in the CCK8S mediated release of DA in the NA may cause changes in dopaminergic neurotransmission in the NA of SHRs. This hypothesis was tested by
stimulating CCK8S receptors in the NA and measuring the concentration of DA in the extracellular fluid of the NA using microdialysis.

6.2 Cholecystokinin-Induced Release of Dopamine in the Nucleus Accumbens of the SHR

The *in vivo* microdialysis experiments assessed the effect of CCK8S on the level of DA and its metabolites in the NA of SHRs and WKYs. The results show that stimulation of local CCK8S receptors using a 10 μM concentration of CCK8S significantly increased the release of DA and its major metabolite DOPAC in the posterior portion of the NA in both the SHR and WKY. More importantly, the CCK8S-induced release of DA was found to be two-fold greater in the SHR than in the WKY. A 1 μM concentration of CCK8S or a 10 μM concentration of the unsulphated form of CCK (CCK8US) produced no significant change in the amount of DA recovered from the NA of the SHR.

The difference in the amount of DA recovered in the microdialysis samples from the NA of the SHR and WKY did not reach statistical significance. In contrast, Sutoo et al. (1992) by quantifying the immunohistochemical labeling of DA in the brain, found a lower tissue level of DA in the NA of the SHR. The failure to detect a significant difference in the amount of DA recovered in the present experiments could be due to the source of DA recovered in the brain using microdialysis. As proposed by Grace (1991), DA release occurs by two different mechanisms. First, phasic DA release occurs rapidly and the DA that is released is removed quickly by fast re-uptake mechanisms. Secondly, tonic DA release occurs more slowly and the DA released accumulates in the extracellular
fluid for some time before it is removed or metabolized. As suggested by Grace (1991),
DA assayed by microdialysis is probably derived from the tonic release of DA. The source
of DA sampled using microdialysis may account for the difficulty in using technique to
demonstrate lower levels of DA in the NA of the SHR.

The observation that stimulation of local CCK8S receptors increased basal levels
of DA and DOPAC in the posterior portion of the NA is in agreement with previous
importantly, this is the first investigation showing that CCK8S-induced release of DA in
the NA is greater in the SHR than in the normotensive WKY. The demonstration of an
increase number of CCK8S receptors in the NA of the SHR provides a mechanism for
the enhanced CCK8S-induced release of DA. Indeed, the enhanced CCK8S-induced
release of DA in SHRs could result from an increase in the number of CCK8S receptors
that mediate DA release in the posterior NA.

Cholecystokinin is believed to act via CCKA and CCKB receptor subtypes found
within various regions of the brain (Woodruff et al., 1991). Stimulation of DA release in
the posterior part of the NA has been shown using in vitro superfusion techniques and in
vivo microdialysis to be mediated by CCKA receptors while stimulation of CCKB
receptors has no effect on DA release in this part of the NA (Rehfeld, 1985; Ruggeri et al.,
1987). In the present investigation, we found that perfusion of the posterior NA with
10 μM CCK8S increased DA release in the NA. However, perfusion of the posterior NA
with a 10 μM concentration of CCK8US, an agonist with a thousand-fold lower affinity to
CCKA but similar affinity to CCKB receptors as does CCK8S (Woodruff et al., 1991),
had no effect on DA release. This leads to the conclusion that the enhanced CCK8S-induced release of DA in the NA of SHRs in this study was mediated by CCKA receptors. CCKA receptors have not been demonstrated in the NA of the rodent brain (Woodruff et al., 1991). However, low level of CCKA receptors have been detected in the NA of the bovine brain (Barett et al., 1989). In addition, behavioral and pharmacological studies support the existence of CCKA receptors in the posterior part of the NA (Crawley, 1991; Crawley & Corwin, 1994). It is likely that a much higher concentration of CCKB receptors interferes with attempts at demonstrating CCKA receptors using binding assays. Strategically placed CCKA receptors on dopaminergic terminals in the NA could be responsible for CCK8S effect on DA release. Therefore, the increase in DA collected during perfusion of the NA with CCK8S could be due to an enhancement of DA release or/and inhibition of reuptake of DA at dopaminergic nerve terminals. It was also found that CCK8US, preferentially a CCKB agonist (Woodruff et al., 1991), caused a large increase in the amount of DOPAC recovered without affecting DA release. This is the first report using in vivo techniques to test the effect of CCK8US on DA and DOPAC release in the NA. The unsulphated form of cholecystokinin (CCK8US) is present in concentrations of less than 1% of that of the sulfated form (CCK8S) in the brain (Rehfeld, 1985). As described above, the posterior portion of the NA contains a large number of CCKB receptors and possibly some CCKA receptors located on dopaminergic terminals. Perfusion of the NA with CCK analogs with different affinities to CCKA and CCKB receptors will act on a combination of these subreceptors resulting in perhaps opposite effects on DA release and metabolism. Moreover, the
extracellular levels of DOPAC measured by microdialysis is derived from newly synthesized DA and not from the metabolism of recently released DA form the nerve terminals (Zetterström et al., 1988). Therefore, CCK8US could be acting on receptors involved in the intraneuronal metabolism of DA without causing an effect on the actual release of DA from dopaminergic terminals.

Microdialysis is a powerful tool for the in vivo investigation of the neurochemical environment of the brain that allows one to sample the extracellular fluid of small regions of the brain while stimulating local receptors with pharmacological agents (Benveniste, 1984; Benveniste & Huttermeier, 1990). Another advantage of delivering a pharmacological agent through the microdialysis probe is that the agent diffuses slowly in the extracellular fluid and stimulates only tissue immediately surrounding the probe. For example, Ruggeri et al. (1987) using a 2 x 0.5 mm size probe and a 2 μl/min flow rate reported that perfusion of the brain with radiolabeled neurotensin, a neuropeptide with a similar molecular weight as CCK8S, reached only 0.05 mm³ of tissue. This suggests that in the present study, only CCK receptors within the posterior portion of the NA would have been stimulated by the CCK8S. Since the recovery rate for CCK8S of a probe of the dimension used in this study is less than 5% (Záhorszky et al., 1985) a 10 μM concentration in the perfusate would probably result in less than 500 nM in the brain tissue immediately surrounding the probe.

As described in the literature review, dopaminergic neurotransmission appears to be suppressed in the striatum of the SHR and possibly in humans with essential hypertension. However, the role in which an interaction between CCK8S and DA could
play in the pathology of hypertension is speculative. Upregulation of CCK8S receptors in the NA of the SHR suggests that CCK8S neurotransmission in this part of the striatum could be suppressed in SHRs. Indeed, tissue levels of CCK8S have been reported to be lower in the cortex, hippocampus, and brainstem of the SHR (Jarrot et al., 1987; Shulkes et al., 1989). CCK8S is found in cortical, hippocampal, and amygdaloid neurons that project to the NA (Myers & Protopapas, 1985; Záborszky et al., 1985) and is co-localized with DA in neurons of the ventral tegmental area which also projects to the posterior part of the NA (Hökfelt et al., 1980; Seroogy et al., 1987, 1988a, 1988b, 1989a, 1989b). Depletion of the CCK8S in projection neurons to the NA in the SHR may lead to the upregulation of CCK8S receptors that are involved in the regulation of DA release in the NA. As is the case in the present investigation, exogenous administrations of CCK8S in the NA of the SHR would be expected to produce a greater increase in the release of DA. Consequently, changes in CCK8S neurotransmission and/or receptors could be responsible for the altered dopaminergic activity in the striatum of the SHR.

In summary, the present experiments demonstrate that CCK8S-induced DA release in the posterior portion of the NA is greater in the SHR than in the WKY. The experiments also show that this response was mediated by the CCKA receptor subtype. Therefore, alterations in the CCK8S mediated release of DA may be responsible for the changes in dopaminergic neurotransmission in the NA of SHRs and may play a causal role in the development of hypertension. This hypothesis was tested by assessing the development of hypertension during the chronic treatment of young SHRs with a CCK8S agonist and antagonist.
6.3 Effects of Cholecystokinin Agonists and Antagonists on Hypertension

As explained above, there is an attenuation in DA neurotransmission in the NA of the SHR. This attenuation may be due to a decrease in CCK8S-induced release of DA in this area of the striatum. The observations that SHRs have lower levels of CCK8S in brain regions that project to the NA along with the demonstration of an increase density of CCK8S receptors in the NA of SHRs support this hypothesis. Consequently, upregulation of CCK8S receptors could be an adaptive response to a decrease in CCK8S available at the synapse where CCK8S modulates DA release. Because of these observations, it was expected that daily subcutaneous injections of the CCK8S agonist ceruletide would correct the lower levels of CCK8S in the brain and subsequently attenuate the development of hypertension in the SHR. Conversely, treatment with the CCK8S antagonist proglumide was expected to block normal CCK8S neurotransmission which would be expected to further attenuate DA neurotransmission and possibly accelerate the development of hypertension in the SHR. The results of the experiments showed that a 10 μg/kg dose of ceruletide had no effect on the development of hypertension. However, proglumide at a dose of 1 mg/kg resulted in more hypertensive SHRs when compared to both the ceruletide and saline treated rats. No significant difference was found in the heart rate of saline, ceruletide, and proglumide treated SHRs. The doses selected for these experiments were based on previous studies examining the effect of CCK8S agents on exploratory behaviors. For example, a peripheral administration of a 1 mg/kg dose of proglumide was effective in blocking the decrease in exploratory behavior produced by CCK8S injections while a 1 μg/kg dose of ceruletide decreased exploratory behavior in
rodents (Harro et al., 1990; Harro & Vasar, 1991). The effect of ceruletide and proglumide on exploratory behavior is believed to be mediated by its interaction with DA in the NA (Crawley & Corwin, 1994). Consequently, the 1 mg/kg dose of proglumide used in the experiments presented in this thesis may have been successful at blocking the effect of endogenous CCK8S on DA release in the ventral striatum of the SHR. This may have attenuated the already low levels DA in the NA, and possibly other regions of the brain, resulting in an enhancement of the neural mechanism responsible for the development of hypertension. The reason why chronic administration of ceruletide did not have any antihypertensive effect could be due to a variety of factors. First, the dose selected for the present experiments was based on the previous investigation of the effect of a single administration of ceruletide and its immediate effect on exploratory behavior in rodents (Harro et al., 1990). It is not known if at a 10 µg/kg dose of ceruletide could maintain its inhibitory effect on exploratory behavior. Therefore, the treatment dose selected to test ceruletide's antihypertensive effect may be too low. Second, it is possible that ceruletide acts for a very short period of time before it is metabolized and/or excreted from an organism's body. In this case, ceruletide may have short-term effects that would be insufficient to affect blood pressure regulatory mechanisms over a 24 hr period. Third, despite the fact that a correlation between hypertension, exploratory behavior, and DA neurotransmission in the striatum exists in the SHR, the role in which exploratory behavior may have in the pathogenesis of hypertension is controversial (Van den Buuse et al., 1986). Ceruletide's effect on exploratory behavior may be unrelated to the development of hypertension in the SHR. Therefore, the dose selected may be sufficient to affect
exploratory behavior in acute experiments (Harro et al., 1990; Harro & Vasar, 1991), but the same dose may be insufficient to affect the development of hypertension in SHRs. Finally, the ability of peripheral injections of ceruletide to cross the blood brain barrier and to affect DA release has been questioned by a report showing that the release of DA in the prefrontal cortex induced by peripheral injections of ceruletide were abolished by bilateral vagotomy (Hagino & Moroji, 1994). This may be taken to suggest that the peripheral administrations of ceruletide may affect DA release in the prefrontal cortex by acting peripherally and not by interacting with CCK8S receptors in the cortex. Consequently, ceruletide may not cross the blood brain barrier sufficiently to enhanced dopaminergic neurotransmission in the NA.

In summary, the CCK8S antagonist proglumide, which would be expected to further attenuate the release of DA in the NA, enhanced hypertension in the SHR. These observations support the hypothesis that a CCK8S-mediated attenuation in dopaminergic neurotransmission in the NA plays a role in the pathogenesis of hypertension in the SHR. The striatum, which includes the NA, olfactory tubercle, and caudate-putamen, is a large group of neural structure traditionally associated with motor function. This function has been ascribed to the striatum because of the psychomotor effects associated with DA depletion in the dorsal striatum (i.e. caudate nucleus and putamen) of experimental animals or humans with parkinson's disease (Albin et al., 1989; Wichmann & DeLong, 1993). However, recent studies have focused on the robust interconnections between the NA located in the ventral striatum and the rest of the limbic system which is known to play a major role in cardiovascular regulation (Zahm & Brog, 1992). For example, the NA is
densely innervated by the BLA while the NA projects strongly to the LHA (Heimer et al., 1991; Zahm & Brog, 1992; Zahm & Heimer, 1993). Both the BLA and the LHA are involved in the integration and production of complex behaviors and the autonomic responses that accompany these behaviors (for review see Loewy, 1991). For example, chemical and electrical stimulation of the BLA and the LHA in the rat produces attack behavior with large increases in blood pressure and heart rate (Yardley & Hilton, 1986; Maskati & Zbrozyna, 1989). Support for the importance of a relay from the amygdala to the NA comes from the observation that electrical stimulation of the NA inhibits the cardiovascular effects elicited from stimulation of the BLA (Haifa et al., 1989).

Consequently, the hypothesis that neurons in BLA project to the same location as neurons in the NA that project to the LHA was tested by a combined retrograde and anterograde tract-tracing study.

6.4 Organization in the Nucleus Accumbens of Afferents from the Amygdala and Efferents to the Hypothalamus

These experiments tested the hypothesis that the terminal field of the afferent projections from the BLA to the NA overlap with the origin of the efferent projections from the NA to the LHA. Microinjections of the anterograde tracer FR in the BLA labeled fibers and terminals in the NA. The highest density of FR labeling was found in the posteromedial region of the NA in an area corresponding to the accumbal shell. Less intense FR labeling was found in the rostral portion of the caudate-putamen and very sparse labeling was found in the olfactory tubercle. A previous report using Phaseolus
*vulgaris-leucoagglutinin* (PHA-L) as an anterograde tracer found similar projection patterns from the BLA to the striatum (Kita & Kitai, 1990). The experiments also showed that microinjections of the retrograde tracer FG in the LHA labeled densely the somas and dendrites of neurons in the shell region of the NA. This pattern is consistent with published reports that found that the accumbal shell sends some efferent projections to the rostrocaudal extent of the LHA (Groenewegan et al., 1984; Heimer et al., 1991; Zahm & Heimer, 1993). However, the density of this striatal-hypothalamic pathway had not been adequately described. This investigation found that a large number of neurons were labeled following relatively large injections of FG in LHA providing support for the importance of a striatal-hypothalamic pathway. More importantly, the terminal field of the BLA-NA pathway was found to overlap with the origin of accumbal neurons that project to the LHA. Under high magnification, FR-labeled fiber terminals appeared to make contact with numerous FG-labeled cell bodies and dendrites. These observations provide anatomical evidence for a relay in the NA from the amygdala to the hypothalamus.

Double-labeling with retrograde and anterograde fluorescent tract-tracers is a useful approach to demonstrate synaptic inputs to neurons with known projection patterns. However, it is appropriate to consider some of the methodological problems of the tract-tracing experiments. As with other tracer substances, the possibility that FR and FG are picked-up by fibers of passage cannot be excluded with absolute certainty. Nance and Burns (1990) showed that central injections of cell body specific neurotoxins along with FR greatly reduced the amount of anterograde labeled fibers observed suggesting that most of the FR uptake occurred in the soma and not fibers passing through the injection.
area. In addition, injections of FR into a fiber tract such as the fimbria resulted in minimal anterograde labeling (Schmued et al., 1990) or injections of FG in intact fibers also showed minimal transport in either retrograde or anterograde directions (Schmued & Fallon, 1986). Therefore, injections of these fluorescent tracers as well as other tract-tracing substances would be expected to cause some uptake and transport by neuronal fibers passing through the damaged area. However, the use of fine micropipettes and the slow injection of small amounts of fluid would cause a minimal amount of tissue damage at the injection site. Both FR and FG appear to be sensitive tract-tracing substances that do not require histochemical or immunohistochemical processing, and for these reasons, are practical for double-labeling studies (Nance & Burns, 1990). Another limitation of this investigation is the conclusion that can be inferred from the apparent contacts between FR-labeled fiber terminals and FG-labeled cell bodies observed using fluorescent microscopy. While these apparent contacts are suggestive of synapses, ultrastructural confirmation using electron microscopy is necessary to show direct inputs between fiber terminals and other cellular elements.

6.5 Interpretation of Results

In both rats and humans with essential hypertension, the hemodynamic pattern during the development of high blood pressure is characterized by an enhanced cardiac output (Lundin & Hallback-Nordlander, 1980). The enhanced cardiac output has been hypothesized to cause structural changes in the heart and blood vessels and functional changes in the control mechanisms that regulate blood pressure (Folkow, 1982).
Eventually, these structural and functional changes are believed to maintain blood pressure at ever increasing levels. As Folkow (1982) clearly presented in his detailed review of the literature, the enhanced cardiac output in individuals prone to hypertension may be due to individuals' behavioral and cardiovascular hyperreactivity to stimuli in their environment. In fact, this hyperreactivity may represent one of the inherited predisposing factors in both rat and human genetic hypertension. Therefore, it is necessary that we understand the brain mechanisms involved in an organism's reactivity to its environment.

The mesostriatal dopaminergic system provides a striking example of how a small number neurochemically distinct neurons can exert considerable control over brain function and behavior. For example, bilateral lesions of this system in the rat produces a severe and generalized state of behavioral unresponsiveness (Mogenson, 1987). To explain this behavioral unresponsiveness, Mogenson (1987) has provided strong evidence that activation of the mesostriatal dopaminergic neurons has a modulating influence on the relay of information in the NA from limbic structures and neocortex to extrapyramidal motor areas. Evidence for this comes from studies in which activation of NA neurons by stimulation of the amygdala or hippocampus is reduced by the stimulation of dopaminergic cells in the ventral tegmental area (Yim & Mogenson, 1982; Yang & Mogenson, 1984). A similar attenuating effect of excitatory inputs to the NA is produced by the exogenous application of DA to NA neurons (Yim & Mogenson, 1982; Yang & Mogenson, 1984). This may reflect important limbic-motor integrative activity that contribute to behavioral response initiation. Accordingly, the mesostriatal system can be viewed as a level-setting system which determines the threshold for an organism's behavioral responding.
Therefore, a reduction in dopaminergic activity could result in a disinhibition of forebrain activity and cause a reduction in the threshold for behavioral responding. This could make an organism hyperreactive to the environment.

In addition to its well known role as a modulator of behavioral responsiveness, DA is involved in cardiovascular regulation and possibly the pathophysiology of hypertension. For example, a decrease in DA content and release has been documented in the dorsal and ventral striatum of the adult hypertensive SHR (Linthorst et al., 1991; Sutoo et al., 1992). In addition, systemic administration of the centrally acting DA agonist bromocriptine and i.c.v. injections of DA have antihypertensive effects in the SHR (Van den Buuse & De Jong, 1991). Although this suggests that the high blood pressure of adult SHRs is due to a DA insufficiency that can be corrected by exogenous administrations of DA, the neural mechanisms that lead to this insufficiency remain unknown.

Cholecystokinin is co-localized with DA in neurons of the ventral tegmental area that project to the NA (Hökfelt et al., 1980; Seroogy et al., 1987, 1988a, 1988b, 1989a, 1989b). In addition, CCK8S is found in numerous cortical and amygdaloid neurons which project to the NA (Meyer & Protopapas, 1985; Zabroszky et al., 1985). Functionally, CCK8S has been shown to affect the release of DA in the NA and to interact with DA to modulate behavioral responding (Crawley & Corwin, 1994). The major hypothesis of the present thesis was that the DA insufficiency in the ventral striatum of the SHR is due to changes in the modulation of DA release by CCK8S. The receptor autoradiography experiments demonstrated an upregulation of CCK8S receptors in the NA of young prehypertensive and adult hypertensive SHRs. These results are supported by the
microdialysis experiments showing that the CCK8S-induced release of DA in the NA of SHRs is enhanced compared to normotensive rats. Furthermore, daily subcutaneous injections of the CCK8S antagonist proglumide, which would be expected to further attenuate the release of DA in the NA, aggravated the hypertension in the SHR. These observations support a role for CCK8S in the pathophysiology of hypertension. As the results of the experiments suggest, changes in CCK8S neurotransmission in the SHR could disrupt the normal modulation of dopaminergic activity in the ventral striatum.

The mechanisms by which an interaction between CCK8S and DA could play in the pathogenesis of hypertension remains theoretical at the present. Tissue levels of CCK8S have been reported to be lower in the cortex, hippocampus, and brainstem of SHRs (Jarrot et al., 1987; Shulkes et al., 1989). Consequently, depletion of CCK8S in neurons that project to the NA may lead to the upregulation of CCK8S receptors that are involved in the regulation of DA release in the NA. Indeed, stimulation of CCK8S receptors in the NA of SHRs produced an enhanced release of DA in this area of the striatum when compared to WKYs. Therefore, a decrease in CCK8S neurotransmission in the NA of SHRs could be responsible for an attenuation in dopaminergic activity in the NA. However, the neural mechanism by which changes in DA neurotransmission in the ventral striatum could lead to hypertension are not yet understood.

A dense projection from the NA to the hypothalamus distinguishes this part of the striatum from that of the caudate-putamen (for discussion see Zahm & Heimer, 1993). The functional significance of a striatal-hypothalamic pathway has not been investigated. However, the shell of the NA receive numerous projections from the prefrontal cortex,
hippocampus, insular cortex, and the BLA (Groenewegen et al., 1982, 1987; Sesack et al., 1989; Hurley et al., 1991; McDonald, 1991; Yasui et al., 1991; Berendse et al., 1992). These association areas of the brain have been implicated in higher level information processing such as memory, learning, emotions, and motivation as well as the control of the autonomic nervous system (Neafsey, 1990). The shell of the NA with its input from association areas in the cortex and its projections to autonomic areas in the hypothalamus provides a powerful system to integrate higher level of information processing with the autonomic nervous system. Consequently, an amygdala-accumbus-hypothalamus pathway may be part of a neural system involved in the integration of complex behavior such as emotions with the autonomic response that accompany these behaviors. For example, electrical stimulation of the BLA and the LHA produce a defense reaction with a similar cardiovascular response as observed in SHRs exposed to alerting and noxious stimuli (Iwata et al., 1987; Haifa et al., 1989). Furthermore, the defense reaction elicited from the amygdala is believed to be mediated by unknown relays to the hypothalamus or other regions of the brain (Hopkins & Holstege, 1978; Schwaber et al., 1980; Price & Amaral, 1981). The NA may serve as this critical relay because of the overlap of BLA inputs to the NA and the source of projection neurons form the NA to the LHA. Consequently, DA by altering the relay of information in the NA from the amygdala to the hypothalamus may modulate the defense reaction and the cardiovascular hyperreactivity seen in the SHR (see Figure 33). Indeed, electrical stimulation of the NA inhibits the defense reaction elicited by stimulation of the amygdala (Haifa et al., 1989).
The modulation of inputs from the amygdala to the NA by DA is supported by electrophysiological evidence (see review by Mogenson, 1987). For example, the excitability of axonal terminals in the NA was enhanced by stimulation of the ventral tegmental area (Yang & Mogenson, 1986). Since neurotransmitter release is dependent on the amplitude of the action potential arriving at a fiber terminal, then moderate depolarization of axon terminals would increase the resting membrane potential and reduce the release of transmitter substances (Takeuchi & Takeushi, 1962; Kusano et al., 1967). From Mogenson's experiments, one can conclude that DA release in the NA acts on presynaptic terminals from the amygdala to modulate the release of their neurotransmitters. Indeed, DA has been shown to reduce the release of the excitatory amino acid glutamate, the neurotransmitter probably used by excitatory afferent inputs to the NA (Mitchell & Doggett, 1980; Rowland & Roberts, 1980; Godukhin et al., 1984). Therefore, DA appears to inhibit the activity of presynaptic excitatory afferent inputs from the amygdala to the NA causing a decrease in the excitability of postsynaptic neurons in the NA. It is hypothesized that a decrease in DA activity in the NA of the SHR causes an attenuation in the inhibitory effects of DA on excitatory inputs from the amygdala. Consequently, an increase in the activity of the amygdala-accumbus-hypothalamus circuitry could be responsible for the cardiovascular hyperreactivity believed to be an important factor in the development of hypertension. As outlined before, changes in CCK8S modulation of DA release in the NA may be responsible for the attenuation of DA activity in the ventral striatum of the SHR and may lead to an over-activity in the pathways that control behavioral and cardiovascular reactivity (summarized in Figure 34).
Figure 33. Diagram showing the anatomical connections in the posterior portion of the NA that could be involved in a DA-mediated modulation of information from the amygdala to the hypothalamus. The BLA sends excitatory impulses to the NA where projection neurons innervate the LHA. In turn, the LHA influences cardiovascular centers in the medulla and spinal cord that innervate the heart and the vasculature. As described in the text, stimulation of the BLA and the LHA produce strong cardiovascular responses in addition to the behaviors that comprise the defense reaction. Dopaminergic neurons in the ventral tegmental area (VTA) project to NA and influence the relay of information from the amygdala to the NA (inset). As described earlier, DA in the NA has an inhibitory effect on excitatory impulses from the amygdala. In addition, CCK8S stimulates the release of DA in the posterior region of the NA thereby accentuating DA inhibitory influence on the relay of information from the amygdala.
Medulla
Spinal Cord
Figure 34. Flowchart showing the role that CCK8S and DA could play in the development of hypertension. A. Normal CCK8S and DA activity in the NA attenuate the relay of information from the BLA to the LHA. This would lead to a normal reactivity to stimuli in the environment as is the case in the WKY. B. Low levels of CCK8S and the subsequent reduction in CCK8S-induced release of DA could lead to an over-activity of the amygdala-accumbens-hypothalamus circuitry responsible for the cardiovascular responses that accompany emotional arousal. This may result in the cardiovascular hyperreactivity that has been shown to trigger the development of hypertension in the SHR.
Environmental Stimuli

Amygdala

DA CCK8S

Nucleus Accumbens

Lateral Hypothalamus

Normal Cardiovascular Reactivity

Normotension

A

Environmental Stimuli

Amygdala

DA CCK8S

Nucleus Accumbens

Lateral Hypothalamus

Cardiovascular Hyperreactivity

Hypertension

B
7. SUMMARY

The autoradiography experiments demonstrated an increased density of CCK8S receptors in the caudal portion of the NA of young prehypertensive and adult hypertensive SHRs (Kirouac & Ganguly, 1993, 1995). An upregulation of CCK8S receptors is not secondary to a rise in blood pressure because these changes occurred prior to the onset of hypertension. The microdialysis experiments indicated that CCK8S-induced DA release in the posterior portion of the NA is greater in the SHR than in the WKY (Kirouac & Ganguly, in press). These observations support the hypothesis that alterations in CCK8S mediated release of DA may be responsible for the attenuated release of DA in the ventral striatum of SHRs and may have a causal role in the development of hypertension. In addition, the observation that daily treatment with the CCK8S antagonist proglumide enhanced hypertension in SHRs further supports this hypothesis. The tract-tracing experiments were conducted to provide preliminary evidence for the relay of information in the NA from the amygdala to the hypothalamus (Kirouac & Ganguly, in press). These anatomical experiments showed that terminal fields from neurons originating in the BLA overlap with accumbal neurons which project to the lateral hypothalamus. An amygdala-accumbus-hypothalamus pathway may be involved in the integration of neural activity that controls responses in the autonomic nervous system during emotional arousal. Consequently, an attenuation in dopaminergic neurotransmission in the NA mediated by CCK8S may result in changes in the activity of brain circuitry involved in emotional arousal. This may lead to the behavioral hyperreactivity that has been shown to be an important factor in the development of hypertension.
8. REFERENCES


