

PROJECT ADMINISTRATION DATA SHEET

ORIGINAL  REVISION NO. \_\_\_\_\_

Project No. G-33-S02<sup>W02</sup>\* ~~GTR/GIT~~ DATE 1/31/83

Project Director: Sheldon W. May School/~~Lab~~ Chemistry

Sponsor: NIH, National Heart, Lung & Blood Institute

Type Agreement: Grant No. 5 R01 HL28167-02 BNP

Award Period: From 1/1/83 To 12/31/83 (Performance) \_\_\_\_\_ (Reports) \_\_\_\_\_

Sponsor Amount: Total Estimated: \$128,552 Funded: \$ 128,552

Cost Sharing Amount: \$ 6,766 Cost Sharing No: G-33-312

Title: Novel Antihypertensives: Rational Design & Evaluation

ADMINISTRATIVE DATA

OCA Contact Frank Huff

1) Sponsor Technical Contact:  
Mr. Armando Sandoval  
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2) Sponsor Admin/Contractual Matters:  
Mrs. Margaret Heydick  
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Defense Priority Rating: none

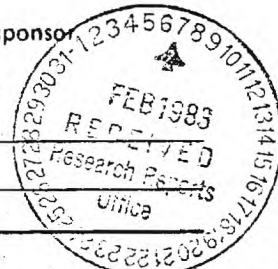
Military Security Classification: none  
(or) Company/Industrial Proprietary: \_\_\_\_\_

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval - Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of \$500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT



COMMENTS:

Notice of Award dated 1/17/83 provides funding of \$87,947 for direct costs in year two (calendar year 1983). The sponsor anticipates the program will continue through calendar year 1984.

This project is continuation of G-33-S01 \*

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- Other

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 1/30/84

Project No. G-33-W02

School/Dept Chemistry

Includes Subproject No.(s) \_\_\_\_\_

Project Director(s) S. W. May ~~OSP~~ / GIT

Sponsor NIH, National Heart, Lung, & Blood Institute

Title Novel Antihypertensives: Rational Design & Evaluation

Effective Completion Date: 12/31/83 (Performance) \_\_\_\_\_ (Reports)

Grant/Contract Closeout Actions Remaining:

- None
- Final Invoice or Final Fiscal Report
- Closing Documents
- Final Report of Inventions
- Govt. Property Inventory & Related Certificate
- Classified Material Certificate
- Other Annual Report of Expenditures

Continues Project No. G-33-W01

Continued by Project No. G-33-W03

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<b>SECTION IV PROGRESS REPORT SUMMARY</b>		GRANT NUMBER HL28167	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR <b>MAY, SHELDON W.</b>		PERIOD COVERED BY THIS REPORT	
NAME OF ORGANIZATION <b>GEORGIA INSTITUTE OF TECHNOLOGY</b>		FROM 01/01/83	THROUGH 12/31/83
TITLE <i>(Repeat title shown in item 1 on first page)</i> <b>NOVEL ANTIHYPERTENSIVES: RATIONAL DESIGN AND EVALUATION</b>			

(SEE INSTRUCTIONS)

PUBLICATIONS

H.H. Herman, S.H. Pollock, S.R. Padgette, J.R. Lange and S.W. May, "The Effects of Phenyl-2-Aminoethylsulfide, A Novel Dopamine- $\beta$ -Hydroxylase Substrate, On the Cardiovascular System of the Anesthetized Dog," Journal of Cardiovascular Pharmacology, **5**, 725-730 (1983).

S.W. May, P.W. Mueller, S.R. Padgette, R.S. Phillips, and H.H. Herman, "Dopamine- $\beta$ -Hydroxylase: Suicide Inhibition by the First Olefinic Substrate, 2-Phenyl-2-Aminoethylethene," Biochem. Biophys. Res. Commun., **110**, 161-168 (1983).

S.H. Pollock, S.R. Padgette, H.H. Herman, J.H. Han and S.W. May, "Novel Antihypertensive Agents: Dopamine- $\beta$ -Hydroxylase Substrate Analogs," Fed. Proceed. **42**, 2260 (1983).

Phenyl Aminoethyl Sulfides: Enzymatic and Medicinal Properties of a Novel Class of Dopamine-B-Hydroxylase Substrates. under submission to J. Medicinal Chemistry.

PROGRESS REPORT

The goal of our research program is to evaluate, at both the cellular and enzymatic levels, novel compounds of our design which represent analogs of catecholamines and related compounds. Our compounds are designed as either pro-drugs, novel enzyme substrates, or suicide inactivators, and they fall into three general classes--ketone-generating, heteroatom-containing, and olefin-containing neurotransmitter analogs.

The following paragraphs summarize our progress during the second year of this project.

Heteroatom-Containing Analogs: Phenyl-2-aminoethyl sulfide (PAES) was designed, synthesized and characterized in our laboratories as a novel synthetic substrate for dopamine-beta-hydroxylase (DBH). We discovered the conversion of PAES by DBH to the corresponding sulfoxide, phenyl-2-aminoethyl sulfoxide (PAESO) and have investigated the kinetics and mechanism of this oxygenation reaction. These findings represent the first demonstration of oxygenation by DBH of a heteroatom in a synthetic substrate analog. One of the most striking aspects of these findings is the fact that DBH oxygenates PAES more rapidly than the corresponding aliphatic carbon analog.

This early work in vitro suggested the possibility that PAES might be active in vivo, and affect adrenergic neuronal function by virtue of its ability to compete with dopamine, the normal substrate for DBH oxygenation. Such a competition could lead to a reduction of the catecholamine stores in adrenergic tissues and thus act to lower the levels of sympathetic tone in a manner which could have important implications for the lowering of systemic blood pressure.

In our initial experiments, we found that PAES possesses very little, if any, direct adrenergic agonist activity, but exhibits indirect sympathomimetic activity at relatively high doses (4 mg/kg). This assertion that PAES is a new indirect sympathomimetic is supported by our findings that pre-treatment with cocaine completely abolishes its sympathomimetic activity, and that its effects are diminished in consecutive stimuli. Additionally, we have found that PAES infusion almost completely blocks the reflex tachycardia elicited by hydralazine, a direct vasodilator. In contrast, the product of DBH oxygenation of PAES, PAESO, has been found to possess neither direct or indirect sympathomimetic activity. If PAES is being converted to PAESO by DBH in the adrenergic nerve terminal, thus competing with DBH oxygenation of dopamine to norepinephrine, then the inactivity of PAESO could have important clinical implications, since it would result in PAES being converted into a "false transmitter." Thus, the exciting possibility arises that through the use of PAES, norepinephrine stores in peripheral adrenergic structures could be lowered in a controlled manner.

Within the past nine months, we extended our work to three "second generation" derivatives of PAES; namely, the  $\alpha$ -methyl, the  $\alpha$ -methyl-p-hydroxy, and the p-hydroxy derivatives. These derivatives were designed to exhibit an altered profile of central vs. peripheral action, and to exhibit enhanced resistance toward MAO catabolism. Kinetic characterization of these compounds was carried out in vitro with both DBH and MAO. As expected, all were excellent DBH substrates and the  $\alpha$ -methyl derivatives were MAO resistant. Direct bioassays of antihypertensive activity were then carried out in spontaneously hypertensive rats, a common animal model for hypertension.

In the group of animals which received HOPAES injections, the blood-pressure reduction was quite obvious, reaching a maximum reduction of 25% at one hour post-injection, with a gradual return of the blood pressure to hypertensive levels after three hours. In contrast, the time course of the blood pressure reduction observed with an identical dose of HOME-PAES is considerably broader, extending for four to five hours. Since these two compounds differ structurally only in that HOME-PAES possesses an alpha-methyl group which makes it MAO-inactive, the prolonged duration of the hypotensive activity of HOME-PAES might suggest that MAO is playing a significant role in the termination of the activity of these compounds. In this work we have thus demonstrated that DBH-active substrate analogs containing a benzylic sulfur atom are capable of eliciting a hypotensive effect in a commonly-used animal model for hypertension.

During the coming year, we will extend these studies to direct measurement of blood pressure effects in SHR, to assessing effects on atrial muscle preparation, and to obtaining direct chemical evidence for the buildup of PAESO and corresponding sulfoxidation products in biological fluids.

An exciting new direction is our conclusive demonstration during the past summer that selenium analogs of PAES are DBH substrates and exhibit striking antihypertensive activity in SHR. Manuscripts detailing these findings, and possibly a patent, will be written during the next several months. We will be working during the coming year on the potency and mechanism of action of these exciting new compounds.

Olefinic Substrate Analogs: The results which we have obtained with this class of compounds during this past year have been very exciting to us. We published evidence that our prototypic olefinic substrate, PAME, is both an excellent substrate for DBH and is, indeed, a suicide inactivator. After an extensive and difficult series of experiments, we developed a protocol to identify the product of the oxygenation of PAME, and to compare its structure with the corresponding synthetic diol which we have succeeded in preparing and characterizing. These experiments were made especially difficult by the fact that the epoxide product of PAME is expected to be highly reactive and unstable, due to its benzylic structure. We have thus unequivocally established that DBH is indeed capable of carrying the oxygenation of olefins, this once again representing a totally new activity for this enzyme. Inactivation studies with PAME have now established that it exhibits all of the characteristics expected for a suicide inactivator. Inactivation is irreversible, exhibits time and concentration dependencies, and is protected against by substrates. The inactivation reaction exhibits first-order kinetics and the data give a linear double reciprocal plot. DBH inactivated by PAME is not reactivated upon extensive dialysis nor upon extended incubation in the presence of nucleophiles. Furthermore, the stoichiometry for PAME oxygenation is precisely that expected for an oxygenase reaction.

Work in progress has now completed the characterization of the pH dependence of PAME reaction and inactivation, and during the coming year we will be characterizing partition ratios and inactivation stoichiometry using tritiated PAME. Bioassays of our olefins using dogs, SHR and isolated heart atrial tissue have begun and will represent a major area of concentration during the coming year.