

PROJECT ADMINISTRATION DATA SHEET

ORIGINAL REVISION NO. _____

Project No. G-33-S01 (G-33-377 cost sharing) DATE 2/22/82

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

Sponsor: NIH, National Heart, Lung, and Blood Institute

Type Agreement: Grant No. 1 R01 HL28167-01 BNP

Award Period: From 1/1/82 To 12/31/82 (Performance) _____ (Reports) _____

Sponsor Amount: \$131,670 Contracted through: _____

Cost Sharing: \$ 6,930 GTRI/~~GRF~~ _____

Title: Novel Antihypertensives: Rational Design and Evaluation

ADMINISTRATIVE DATA

OCA Contact Don Hasty x4820

1) Sponsor Technical Contact:

Mr. Armando Sandoval

National Heart, Lung & Blood Inst.

NIH

Bethesda, MD 20205

(301) 496-7255

Defense Priority Rating: none

2) Sponsor Admin/Contractual Matters:

Ms. Margaret E. Heydrick

Nat'l Heart, Lung & Blood Inst.

NIH

Bethesda, MD 20205

(301) 496-7255

Security Classification: none

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 1/5/82 provides funding of \$86,368 for direct costs in the first year. The sponsor anticipates the program to go through 12/31/84.



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SPONSORED PROJECT TERMINATION SHEET

Date 6/30/83

Project Title: Novel Antihypertensives: Rational Design and Evaluation

Project No: G-33-S01

Project Director: Dr. Sheldon W. May

Sponsor: DHHS; PHS; NIH - National Heart, Lung and Blood Institute

Effective Termination Date: 12/31/82

Clearance of Accounting Charges: 12/31/82

Grant/Contract Closeout Actions Remaining:
None

- Final Invoice and Closing Documents
- Final Fiscal Report
- Final Report of Inventions
- Govt. Property Inventory & Related Certificate
- Classified Material Certificate
- Other Annual Report of Expenditures (01 year)

NOTE: Continued by G-33-S02

Assigned to: Chemistry (School/~~Laboratory~~)

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APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1	GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT	HL28167	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial) MAY, SHELDON W.	PERIOD COVERED BY THIS REPORT	
NAME OF ORGANIZATION GEORGIA INSTITUTE OF TECHNOLOGY	FROM 01/01/82	THROUGH 12/31/82

TITLE (Repeat title shown in Item 1 on first page)

NOVEL ANTIHYPERTENSIVES: RATIONAL DESIGN AND EVALUATION

- List all publications, not previously reported, resulting from work supported by this grant (author(s), title, page numbers, year, journal or book). List manuscripts separately as submitted for publication or accepted for publication.
- Provide two reprints of publications not previously submitted to the awarding unit.
- Progress Report. (See instructions)

- S.W. May, R.S. Phillips, H.H. Herman and P.W. Mueller, "Bioactivation of Catha-Edulis Alkaloids: Enzymatic Ketonization of Norpseudoephedrine", Bioch. Biopy. Res. Commun. 104, 38-44, (1982).

H.H. Herman, P.W. Mueller, S.R. Padgett, S.H. Pollock, R.S. Phillips and S.W. May, "The Effects of Novel Neurotransmitter Analogs Upon Adrenergic Structures and Cardiovascular Responses", Fed. Proceed. 41, 1588 (1982).

H.H. Herman, S.H. Pollock, S.R. Padgett, J.R. Lange and S.W. May, "The Effects of Phenyl-2-Aminoethylsulfide, A Novel Dopamine- β -Hydroxylase Substrate, On the Cardiovascular System of the Anesthetized dog", submitted to Journal of Cardiovascular Pharmacology.

S.W. May, P.W. Mueller, S.R. Padgett, R.S. Phillips, and H.H. Herman, "Dopamine- β -Hydroxylase: Suicide Inhibition by the first Olefinic Substrate, 2-Phenyl-2-Aminomethylethene", submitted to Biochem. Biophys. Res. Commun.

3. Progress Report

The goal of our research program is to evaluate--at both the cellular and enzymatic levels--novel compounds of our own design which represent analogs of catecholamines and related compounds. Our compound 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 first year of this project.

Heteroatom-containing analogs; In previous work, Phenyl-2-aminoethylsulfide (PAES) has been designed, synthesized and characterized in our laboratories as a novel synthetic substrate for dopamine- β -hydroxylase (DBH). We have discovered the conversion of PAES by DBH to the corresponding sulfoxide, phenyl-2-aminoethylsulfoxide (PAESO) and have investigated the kinetics and mechanism of this oxygenation reaction. These findings represented the first demonstration of oxygenation of a hetroatom by a specific hydroxylase such as DBH. One of the

more striking aspects of these findings is the fact that DBH oxygenates PAES more rapidly than the corresponding aliphatic carbon analog. In addition, we have established that the stereochemistry of the sulfoxidation reaction is fully consistent of that of other DBH reactions.

This work has now been extended in two directions. In the first place, we have just recently discovered that selenium analogs are also excellent substrates for DBH, and it is therefore likely that other heteroatom will also be oxygenated by this enzyme. Thus, these findings open up many new approaches for the design of heteroatom-containing DBH substrates, and for the evaluation of these compounds at both the enzymatic and cellular levels. We are now completing characterizations of the selenium-oxidizing activity of DBH, and a manuscript describing these results will be submitted for publication shortly.

The second direction in which we have extended our studies with PAES is the in vivo characterization of the effects of this compound in anesthetized dog. We have 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. Further experiments are in progress in order to evaluate the clinical implications of these findings, but we are most encouraged and excited by the results in hand to date.

Olefinic Substrate Analogs: The results which we have obtained with this class of compounds during this past year have been very exciting to us. In the first place, we have now definitely established that our prototypic olefinic substrate, PAME, is both an excellent substrate for DBH and is, indeed, a suicide inactivator. A body of experimental evidence is now in hand to clearly support this conclusion. In the first place, after an extensive and difficult series of experiments, we have 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. We are now just completing experiments with tritiated PAME, which has been prepared by the low pressure tritiation procedure described in our original proposal.

With this extensive body of experimental evidence now in hand, we will now proceed to the evaluation of the in-vivo effects of PAME and to the design of olefinic substrates of refined structure. Because of its simple chemical structure, there is no doubt in our minds that PAME will be readily taken up presynaptically, and thus we are especially interested in observing the effects of this compound in our bioassays. These experiments will be carried out during the second year of this project.

Studies With Other Substrates Analogs: As described in our recent BBRC paper (ref. 1 above), it is our contention that the ketonization activity which we have demonstrated for DBH is relevant to the bioactivation of norpseudoephedrine and related compounds which contain the S-alcohol functionality at the benzylic position. Based on a large body of experimental evidence in the literature, we have no doubt that such compounds should be taken up and have access to DBH within synaptic vesicles. We are currently exploring the approach of producing pro-pro-drugs of this class through the resolution of dihydroxyphenylserines to obtain the diastereomer which will generate the S-ol after enzymatic decarboxylation, and thus will eventually generate the corresponding ketone after DBH ketonization. Experiments along these lines will be carried out during the coming year of this project.

Finally, we have initiated the syntheses of second-generation substrate analogs from our three basic classes. For example, the PAES analog containing an α -methyl substituent is being prepared, since this analog will be much less susceptible to metabolism by MAO. Similarly, ring-oxygenated analogs which will have altered susceptibility to COMT are being prepared. It is our intention to examine the effects of such structural modifications on the activities of our compounds, and on the levels of naturally occurring catecholamines after administration of these compounds.