EORGIA INSTITUTE OF TECHNOLOGY

OFFICE OF CONTRACT ADMINISTRATION

misc.

[X] ORIGINAL

[ ] REVISION NO. ____

PROJECT ADMINISTRATION DATA SHEET

roject No. G 33-601 DATE: 8-7-81

roject Director: H. H. Herman School/~~Lab~~ Chemistry

ponsor: American Heart Association, Georgia Affiliate, Inc, 2581 iedmont Road NE, P.O. Box 13589, Atlanta, Ga 30324

ype Agreement: Grant-In-Aid Agreement (No Number)

ward Period: From 7-1-81 To 6-30-82 (Performance) 6-30-82 (Reports)

ponsor Amount: $ 21,934 Contracted through:

ost Sharing: N/A GTRI/~~GIT~~

itle: Bioassay of Novel Antihypertensive Agents

DMINISTRATIVE DATA

OCA CONTACT Don Hatley

) Sponsor Technical Contact: Mr James E. Allen Executive Director

) Sponsor Admin./Contractual Contact: above address phone - (404) 261-2260

eports: See Deliverable Schedule Security Classification: N/A

efense Priority Rating: N/A

STRICTIONS

e Attached N/A Supplemental Information Sheet for Additional Requirements.

avel: 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. N/A - None Proposed.

uipment: Title vests with Sponsor, since Agreement is silent regarding Title.

MMENTS: Sponsor's Research Policies re expenditures of Grant funds referred to in par. 4 of agreement are not available at time of initiation. Copies will be distributed as soon as received by OCA.

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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 6/7/83

Project Title: Bioassay of Novel Antihypertensive Agents

Project No: G-33-601

Project Director: Dr. Heath H. Herman

Sponsor: American Heart Association, Georgia Affiliate

Effective Termination Date: 6/30/82

Clearance of Accounting Charges: 6/30/82

Grant/Contract Closeout Actions Remaining:

- [ ] Final Invoice and Closing Documents
- [X] Final Fiscal Report
- [X] Final Report of Inventions
- [X] ~~Govt.~~ Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other _____

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

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FORM OCA 10:781

TERMINAL REPORT

I. Project Investigator: Heath H. Herman, Ph. D.

Project Title: Bioassay of Novel Antihypertensive Agents

Period of Support: July 1, 1981 to June 30, 1982

II. Project Report

The research work supported by the American Heart Association Georgia .liate in the above-mentioned project has been quite productive. This project as its purpose the initial enzymatic investigation and the initial bioassay of enrgic activity of a number of novel adrenergic neurotransmitter analogs which e considered to have potential as new antinypertensive proto-drugs. In the work formed under the auspices of this grant we have used a continuous O_2 monitor ay developed in this laboratory (Phillips, et al.(1980) Fed. Proc. 39, 2936) to form the enzymatic assays for dopamine-beta-hydroxylase (DBH) activity and for mo pamine oxidase (MAO). We have used three different types of bioassay methods in testing of our prototype compounds. These were: (1) rabbit ileal segment con-ction assays of adrenergic receptor agonism and antagonism; (2) anesthetized dog od pressure and heart rate monitoring with intravenous infusion of prototype pounds; and (3) spontaneously hypertensive rat (SHR) tail-cuff systolic blood ssure monitoring with subcutaneous application of prototype compounds.

By way of background, we have designed and synthesized prototypic compounds of ee distinct classes which were the subjects of investigation in this project. se classes of compounds were: (1) Ketone-generating analogs; (2) Olefinic analogs (3) Sulfur-containing analogs. Our results with each of these classes will be cussed separately (see original proposal for more detail).

one-Generating Analogs

In the original proposal, we mentioned our discovery of the oxygenation of S-zylic alcohols by DBH. This work was published (JBC 256, 2258, 1981) and a

int is attached to this report. Two extensions of this enzymatic ketonization were also published during the grant period and are also attached (JBC 256,8470, and BBRC 104, 38, 1981). We have recently found that S-octopamine is a poor trate for MAO, with a K_m at least one power of ten greater than comparable subtes, althougn this work is still preliminary. In in vitro and in vivo experiation using the methods outlined earlier, we have found that: (1) S-octopamine esses very little alpha- or beta-adrenergic agonistic activity; (2) this compossesses very little adrenergic antagonistic activity in vitro (at least 10^2 than propranolol) and similarly, in vivo, appears to exert very little adrenerantagonistic activity; and (3) at large doses (ca. 1 mg/kg) S-octopamine causes ndirect sympathomimetic effect in anesthetized dogs when given intravenously.

inic Analogs

In our original proposal we suggested working with two olefinic prototypic ounds. One of these, 1-phenyl-(1-aminomethyl)ethene (PAME), has proved to be a exciting find. To recapitulate, we theorized that an unsaturated functionality ted at the benzylic position in a molecule designed to resemble a DBH substrate d "fool" the enzyme into attempting to oxygenate this double bond with the reing formation of a highly reactive epoxide (see original proposal for structure details). This prediction has now been almost completely realized. PAME is an llent substrate for oxygenation by DBH and the 1:2 oxygen to electron stoichioy we have determined is diagnostic of oxygenation by the normal monooxygenase way. We have determined a K_m of 8.3 mM and a k_{cat} of 10/sec for PAME using the en monitor kinectics assay method. Most importantly, we have conclusively nstrated time-dependant inactivation of DBH by PAME and have shown that this invation is irreversible to dialysis and is ascorbate dependent. Additionally, we now identified the ultimate product of DBH oxygenation of PAME as the 1-phenylinomethyl-glycol which we contend is derived from the initial epoxide by hydrol-. In similar oxygen-uptake experiments, we have demonstrated that PAME is an exent MAO substrate, with a K_m of 1.2 mM and a k_{cat} of 0.5/sec. In in vitro and

in vivo experiments, we have found that PAME is not a direct adrenergic agonist ither alpha or beta receptors and is a very weak adrenergic antagonist. It is ver, a potent indirect sympathomimetic with characteristics similar to those of topamine discussed previously. The work with PAME discussed above is currently g organized into a large paper which will be submitted this fall to the Journal iological Chemistry. Because PAME will be the first demonstration of the oxi- on of an olefin by DBH, and because of its potential significance for medical arch (discussed here in the conclusions), we believe that this paper will be e significant.

ur-Containing Analogs

Our final class of novel neurotransmitter analogs has proved to be the most int- ting of all, and the work reported below holds the most promise for an eventual lopement into an actual clinical tool. To recapitulate, we had earlier found DBH catalyzed the oxygenation of benzylic sulfides when the sulfur atom was in- orated into a phenylethylamine-like molecule of our own design (JACS 102, 5981,). Subsequent work led to the developement of a series of para-substituted sul- analogs whose kinetic characteristics in DBH-catalyzed oxygenations led to new ence for the enzymatic mechanism (see attached reprint, JBC 256, 8470, 1981). ork carried out under the auspices of this grant, we have found that our proto- sulfur compound, phenyl-2-aminoethylsulfide (PAES), is quite an excellent MAO trate, with a K_m of 0.08 mM and a k_{cat} of 0.63/sec. Thus, PAES is the best sub- te for MAO of all of our prototypic compounds, a fact of some significance, as be discussed below. In in vitro and in vivo adrenergic receptor assays, PAES our other analogs, proved to have very little alpha- or beta-adrenergic agonist vity. Similarly, PAES exerts a very small adrenergic antagonism at the receptor l. Again, as was the case for our other two classes of compounds, PAES proved to rt significant indirect sympathomimetic activity in intravenous infusions into sthetized dogs at large doses (ca. 1 mg/kg). In a detailed series of dose res- ce experiments with PAES infusion into anesthetized dogs however, a number of

king findings were observed. First, the supposition that PAES was exerting an rect sympathomimetic effect was confirmed in a number of experiments in which its cts were completely blocked by cocaine pretreatment. Secondly, we have demonted that reserpine pretreatment (3 mg/kg, 24 hours) also completely blocks the rect sympathomimetic effects of PAES. Thus, PAES is somehow interfering with a anism involving the catecholamine reuptake system and vesicular storage of neurosmitter in adrenergic neurons. These findings led us to a series of in vivo riments using anesthetized dogs in which the ability of the sympathetic reflex rol of blood pressure was examined after pretreatment with a fixed dose of PAES. results of these experiments were quite remarkable. In one series of experiments ised hydralazine, a known vasodilator, to elicit a sympathetic reflexive increase eart rate. After pretreatment with 4 mg/kg PAES, this reflexive tachycardia to hydralazine was abolished, suggesting that sympathetic nerve pathways were ole to respond to the hypotension caused by hydralazine. Similarly, the $beta_2$ ponse in arterial smooth muscle to norepinephrine (a relaxation leading to deases in blood pressure) were much prolonged after pretreatment with the same dose PAES, again suggesting a diminuition of sympathetic reflex activity. These rets have been incorporated into a manuscript presently under review for publication the Journal of Cardiovascular Pharmacology and a copy of this manuscript is ended herein.

Finally, we have obtained evidence for a direct antihypertensive effect for PAES examining its effects on SHR. Using age-matched SHR males we have examined the ects of chronic administration of 5, 10, 25, and 50 mg/kg subcutaneous injections PAES, measuring the systolic blood pressure of the conscious animal using an inect tail-cuff plethysmographic method. The animals remained conscious at all es during the experimentation, betrayed no behavioral changes as a result of the g treatment, and sustained no weight losses as a result of the treatment. We have nd that the drug is non-toxic in this model up to the highest levels tested so far

that its blood pressure-lowering effects are reversible after withdrawal of the g. In our most striking experiment to date, groups of SHR and saline-treated SHR trols received either 50 mg/kg PAES or saline (1 mg/kg) s.q. at 24 hour intervals seven consecutive days. Blood pressure monitoring revealed a 15-20 % reduction the PAES-treated group, apparent at 24 hours, and maintained for seven days. se results suggest that the antihypertensive potential of these compounds is well th continued investigation.

iclusions-- Lay Summary

Our success in the synthesis, enzymatic testing, and biassay of these three new asses of adrenergic analogs is a result of an exciting and highly productive collaration between biochemists at Ga. Tech and pharmacologists at Mercer University uthern School of Pharmacy. We cannot emphasize too strongly the the importance of r findings with respect to the biological activity of our sulfur-containing analog, ES. Its direct antihypertensive activity and its ability to act synergistically th a currently-used vasodilatory antihypertensive, hydralazine, to remove the flexive cardioacceleratory side-effects suggest that PAES has a high potential for e eventual developement into an important clinical tool. The parallel indications indirect sympathomimesis with test compounds from the other two classes suggest at they too might exhibit similar effects upon closer examination. Additionally, r findings with respect to our olefinic analogs suggest that they could be used to lectively down-regulate sympathetic activity by inactivating dopamine beta-hydroxyse molecules and thus lowering norepinephrine synthesis.

III. Collaborators

Dr. Stanley H. Pollock, Assoc. Proffessor of Biomedical Sciences at Mercer iversity Southern School of Pharmacy was the Collaborating Investigator on this ant. His help has proved to be invaluable in the critical pharmacological evaluaon of our test compounds. In the design of bioassay experiments, the interpretaon of biological results and the training of technicians and students in the work

rformed under this grant, he has proved to be a great asset to this effort. Also, . Sheldon W. May, Professor of Biochemistry at Ga. Tech, who was a consultant on is project has contributed quite significantly to the planning and execution of t ese experiments. He is an expert in the oxygenation of olefinic substrates by a mber of enzymes, and it was in his laboratories that PAME was conceived and tested veral of his graduate students have worked on this project and have both learned d contributed to the work of this project. Our technician, Julie Lange, was first undergraduate researcher in Dr. May's group who, after graduation, worked full me on this project over the last year. Stephen Padgette and Jin Hee Han, both aduate students at Ga. Tech, have worked on this project and have had the opportunity to expand their biochemistry training to include pharmacology and bioassay xperimentation.

IV. Publications Resulting

A. Abstracts:

Herman, H.H., Mueller, P.W., Padgette, S.R., Pollock,S.H., Phillips,R.S. AND May, S.W. (1982) Fed. Proc. Amer. Soc. Exptl. Biol. 41, 1588.

Herman, H.H., Pollock, S.R., Han, J.H., Lange, J.R., and May, S.W. (1983) Fed. Proc. (in preparation).

B. Presentations

abstract above was presented at the national meeting of FASEB in New Orleans, La. in April, 1982 by Heath H. Herman.

C. Manuscripts

Herman, H.H., Pollock, S.R., Padgette, S.R., and May, S.W. (1982) Phenyl 2-Aminoethyl Sulfide: Effects on the Cardiovascular responses of the Anesthetized Dog. J. Cardiovas. Pharm. (submitted and provisionally accepted - MS #8293).

Herman, H.H., Padgette, S.R., Mueller,P.W., Phillips, R.S., and May, S.W. (1982) Phenyl 1-Aminomethyl Ethene: A Novel Olefinic DBH Substrate and Neurotransmitter Analog. J. Biol. Chem. (in preparation).

V. Research Continuation

Unfortunately, the work of this project cannot continue at the pace of

e past year. Our inability to secure a Continuation from the Ga. Affiliate of e AHA at its May, 1982 meeting (when none of these results were apparent) has ant that we have had to let go our technician, Julie Lange. Also, because of the gh cost of the animals necessary for the indicated experimentation, we will have greatly reduce this aspect of our work.

We will apply for another grant from the Ga. Affiliate of the American Heart sociation in the coming weeks, however, although at best this will mean that ncentrated work will begin again in the summer of 1983. A limited amount of rk will continue on this project using the laboratories of Dr. S. W. May at . Tech, under whom Dr. Herman will work as a researcher on an unrelated grant.