RESEARCH AGREEMENT

THIS AGREEMENT, dated as of 1st July 1992, by and between GLAXO GROUP RESEARCH LTD whose address is Glaxo House, Berkeley Avenue, Greenford, Middlesex, UB6 0NN, United Kingdom (hereinafter called "GLAXO") of the first part and VANDERBILT UNIVERSITY SCHOOL OF MEDICINE, whose address is Nashville, Tennessee 37232, USA (hereinafter called the "University") of the second part.

and Jackie D. Corbin, Ph.D.

WHEREAS:

A GLAXO wishes to sponsor a programme of research as described in Schedule 1 hereto (hereinafter called "the Programme of Research") and to obtain an exclusive license to inventions arising from such research; and

B The University is willing to arrange for Prof J Corbin (hereinafter called "the Principal Investigator") to conduct the Programme of Research at the University and with the objective of furthering the University's goals of research, teaching, education and public service;

NOW, THEREFORE, in consideration of the mutual benefits to be derived therefrom, the parties hereto agree as follows:

I. Statement of Work

(a) The Programme of Research shall be conducted at the University under the direction of the Principal Investigator, and shall be as described in Schedule I attached hereto and incorporated into this Agreement by reference. The content of Schedule I may be modified from time to time by the Principal Investigator upon prior agreement between the Principal Investigator and GLAXO.

(b) The University, under the direction of the Principal Investigator, shall exercise reasonable efforts to carry out the Programme of Research in accord with accepted standards for the conduct of scientific research.

II. Period of Performance

The Programme of Research shall be funded for a three year period and shall commence on or about July 1, 1992 and shall terminate on June 30, 1995. This Agreement may be renewable for additional periods upon the mutual consent of the parties.

III. Payment

As consideration for the University undertaking the Programme of Research GLAXO will make payments for the Programme of Research to the University in accordance with Schedule 2 attached hereto and incorporated into this Agreement by reference. Title to any and all supplies and equipment purchased by the University for use in the

Programme of Research shall be in the University and shall be free of all claims, liens or encumbrances of GLAXO or anyone claiming under or through GLAXO. A final financial accounting of all costs incurred and all funds received by the University under this Agreement, together with a check for the unexpended balance, if any, will be submitted to GLAXO within ninety (90) days following the termination of the Programme of Research.

IV. Rights in Inventions and Know-How

(a) Ownership of Inventions: Any invention in the Field, below defined, made by the Principal Investigator or any other person who participates in the Programme of Research at the University (other than GLAXO employees) and conceived or reduced to practice during the course of and under the Programme of Research shall be the property of the University subject to the rights agreed herein to be granted to GLAXO under a License Agreement to be entered into between the parties.

The University shall promptly and confidentially disclose any Invention in the Field to GLAXO in writing after disclosure has been made to the University's Office of General Counsel by the investigators working on the Programme of Research.

(b) Exclusive License: GLAXO shall have an option to execute a License agreement with respectto any Inventions. The option shall expire six months from the date when a patent application is first filed covering any such Inventions unless exercised in writing by GLAXO.

The License Agreement shall provide for GLAXO to be granted an exclusive worldwide licence, with right to sublicense, to make, use and sell the Inventions, subject to agreement by the parties, following good faith negotiations of specific terms, including royalty rates, and subject to the rights of the Federal Government, as required by applicable law and regulation.

GLAXO's license shall encompass a non-exclusive right to use and disclose all Know-how arising from the Programme of Research, and such shall be royalty free. Additionally, GLAXO shall have the unlimited right, without payment, to use non-patentable Know-how in the furtherance of its own research.

To the extent the University may legally do so, it shall grant to GLAXO a non-exclusive license under any or all Underlying Patents to the extent necessary to practise the Inventions covered by the Patents to be licensed pursuant to this Agreement and subject to any then-outstanding licenses or options previously granted by the University to Third Parties (as hereafter defined). The parties agree to negotiate in good faith the terms of any such non-exclusive license including any royalty to be paid.

Within 6 (six) months after GLAXO exercises the option to a license GLAXO and the University shall negotiate in good faith the terms of the License Agreement. In such terms, a reasonable royalty shall not exceed seven percent (7%) for Patents covering such Invention, based upon Net Sales of any product embodying the Invention, provided that royalties otherwise payable may be offset by GLAXO's worldwide reasonable costs of patenting the Invention (pursuant to subparagraph IV(c) hereof) and maintaining and enforcing the patent; and provided, however, that the amount of such offset shall be mutually agreed upon but in no event shall exceed one half of the royalty otherwise payable. The royalty terms to be negotiated in good faith may include an initial license fee not to exceed One Hundred Thousand Dollars ($100,000), payable to the University upon execution of the License Agreement. Annual minimum royalties may also be included in the royalty terms to be defined in the License Agreement.

Criteria of due diligence to be met by GLAXO in commercializing a licensed Invention shall also be negotiated in good faith and set forth in the License Agreement.

If the parties fail, after good faith negotiation, to conclude the License Agreement within 6 (six) months of beginning negotiations, the University shall be free to undertake and conclude licensing negotiations with a Third Party, with no further obligation to GLAXO except as provided in IV(c) (i).

For the avoidance of doubt the parties agree that all rights to licences under any patents or know-how arising under the earlier agreement of 1st July 1989 between the parties hereto shall remain accrued.

(c) Patenting

(i) The University shall, at its discretion, have the right to seek patent protection for Inventions at GLAXO's expense and in the name of the University, using independent patent counsel satisfactory to GLAXO, but chosen by the University to represent it. The University shall provide regular reports on the progress of patent applications. GLAXO shall inform the University in which countries it desires the University to seek patent protection for such Inventions, and the University shall make such filings at GLAXO's expense. The University shall provide GLAXO sufficient opportunity to review and approve any patent applications or other filings and correspondence prior to their submission by the

University to the appropriate Patent Office, and shall work in good faith with GLAXO's patent counsel to resolve any disputed provisions before submission to the Patent Offices. The University shall also promptly provide GLAXO with copies of all documents and correspondence received by the University from the Patent Offices. The University and the Principal Investigator and any other inventors will execute all documents and perform all acts, at GLAXO's expense, reasonably necessary to prosecute, maintain and enforce the Patents. GLAXO shall be reimbursed for expenses incurred by it in the filing of a patent application covering an Invention, on which it determined not to exercise its option to License, or for which no License Agreement was entered into, but such reimbursement shall only be made by the University to GLAXO from monies received by the University either as a license fee or royalty payments from licensing of the Invention to a Third Party.

(ii) GLAXO shall promptly advise the University of any decision not to finance the filing, prosecution, or maintenance of a Patent in adequate time to allow the University, at its own cost, to effectuate such filing, prosecution, or maintenance if it so desires; and shall, at the request of the University, take whatever steps may be necessary to return to the University all rights which GLAXO may have thereto.

(iii) Although the University shall use reasonable efforts to obtain Patent protection, it does not warrant that it will be able to do so and it shall not be a breach of this Agreement and the University shall not have liability if the United States Patent Office or any foreign Patent Office refuses to grant a Patent.

(d) Affiliates: Any right of GLAXO pursuant to this Article IV shall also be vested in any Affiliates of GLAXO.

V. Warranty

The University warrants that it has not previously and is not currently receiving grant support and is not consulting with any Third Party which support or consultancy would or could entitle such Third Party to rights to any Invention, subject to any rights acquired by the Federal Government from previously funded research.

GLAXO agrees that the University may supplement the Programme of Research funds by seeking and securing monies by means of grants from agencies of the United States Government. The University will grant GLAXO an exclusive license to any Invention in the Field

developed from the joint support of GLAXO under this Agreement and any Federal funds to be applied in the future to the Programme of Research, subject to the reservation to the Government of a non-exclusive, fully paid-up license to practice the Invention. The University shall receive GLAXO's written permission before performing services for non-governmental entities which may directly or indirectly conflict with GLAXO's interest.

By entering into this Agreement, it is understood that the University presently has no such conflicting interests, agreements or obligations with any Third Party, other than to the Federal Government as aforesaid.

VI. Confidentiality/Publication

(a) For purposes of expediting the progress of the Programme of Research, it may be desirable or necessary for each party, during the life of this Agreement, to disclose to the other party certain confidential or proprietary information so identified and in its possession relative to the Programme of Research. In such event, the receiving party agrees to preserve such information as confidential. The obligation of confidentiality shall not apply to any information which:

(i) is now in the public domain or which becomes generally available to the public through no fault of the receiving party; or

(ii) is already known to, or in the possession of, the receiving party as can be demonstrated by documentary evidence; or

(iii) is disclosed on a non-confidential basis from a Third Party having the right to make such disclosure; or

(iv) is independently developed by the receiving party as can be demonstrated by documentary evidence.

GLAXO shall direct all such confidential information to the Principal Investigator, unless otherwise requested by the University, and the Principal Investigator and the University shall direct all such confidential information to Dr B Ross or another GLAXO designee.

(b) The University and its employees shall have the right, consistent with academic standards, to publish or present the results of research performed under this Agreement, provided such publication or presentation does not disclose proprietary trade secrets or confidential information of GLAXO. The University agrees to submit all publications and presentations describing the methods or results of the Programme of Research to GLAXO

thirty (30) days prior to submission of such proposed publication or presentation to a journal editor, or other Third Party. GLAXO shall have thirty (30) days after the receipt of the publication or presentation to review it. Upon notice by GLAXO that GLAXO reasonably believes a patent application relating to an Invention should be filed prior to the publication or presentation and if GLAXO and the University cannot agree on revising the publication or presentation such that in GLAXO's opinion it can be published or presented prior to the filing of a patent application, submission of the publication or presentation shall be delayed for up to ninety (90) days from the date GLAXO so notifies the University or until a patent application or applications are filed, whichever comes first.

VII. Reports

The University will provide GLAXO with progress reports on the Programme of Research at such times and in such form as GLAXO may reasonably request, with a final report on the Programme of Research due sixty (60) days after termination or expiration of this Agreement. GLAXO shall have the right at reasonable times to discuss the reports and the progress of the Programme of Research with and suggest avenues of research to the Principal Investigator.

VIII. Termination

(a) If either party breaches any of the warranties, terms or conditions of this Agreement and fails to remedy that breach within thirty (30) days after receipt of notice of such breach from the other party, the party giving notice may, at its option and in addition to any other remedies it may have in law or in equity, terminate this Agreement by sending written notice of termination to the other party.

(b) If the Principal Investigator is unable or unwilling to continue to serve as the Principal Investigator, or if in GLAXO's judgement the progress of the Programme of Research significantly fails to meet the specific aims indicated in Schedule 1, GLAXO may terminate this Agreement and its funding thereunder on thirty (30) days prior written notice.

c) If the Principal Investigator ceases employment at the University and becomes employed at a different institution in which he is able to continue the Programme of Research undertaken pursuant to this Agreement, GLAXO will continue to support the Programme of Research at the other institution, subject to an agreement between the University and the other institution as to ownership of any inventions and patents developed from the research begun at the University and an agreement between the University and the other institution concerning negotiation of a

license agreement with GLAXO, including an equitable sharing of royalty payments.

(d) Upon notice of termination pursuant to this Paragraph VIII, the University will proceed in an orderly fashion to terminate any outstanding commitments and to phase-down the Programme of Research, unless it desires to proceed further at its own expense or with the support of a Third Party. GLAXO shall reimburse the University for its reasonable costs to the date of termination, including irrevocable commitments existing at the time the notice of termination is received. In no case will reimbursement under this provision exceed the total Programme of Research cost set forth in Schedule 2

(e) The provisions of Paragraphs IV, V, VI, VII, XII, XVI, XVII and XVIII will survive the termination or expiration of this Agreement.

(f) Upon termination of this Agreement, the University shall, if requested to do so by GLAXO,

(i) provide a copy of all Know-how, as defined in Article XIX(D), arising from the Programme of Research to GLAXO, and/or

(ii) return all confidential information disclosed to the University by GLAXO, except one copy which may be retained by the Counsel's office or similar equivalent for archival purposes.

IX. Use of Name

Neither party shall utilize the other's name in any promotional or advertising context or press release without the prior written consent of the other party. GLAXO may request that the University include an appropriate acknowledgement of GLAXO's sponsorship of the Programme of Research in any University publication discussing such research.

X. Notice

Any notice or other communication required or permitted under this Agreement shall be in writing and will be deemed given as of the date it is

(a) mailed, postage prepaid, first-class, certified mail, return receipt requested; or

(b) sent, shipping prepaid, receipt requested, by national courier service,

to the party at the address listed above or subsequently specified in writing.

XI. Independent Contractor

The University's relationship to GLAXO under this Agreement shall be that of independent contractor and not an agent, joint venturer or partner of GLAXO.

XII. Liability/Insurance

(a) The University agrees that GLAXO shall not be liable for, or need to indemnify the University for, damage to or loss of property or injury to or death of any persons to the extent arising out of or in connection with the University's performance of this Agreement to the extent such damages, losses or injuries occur on the University's premises and result from the negligence or intentional wrongful acts of the University faculty, staff or students.

(b) GLAXO agrees to indemnify and hold harmless the University, the Principal Investigator, members of his research team and other of the University's employees, trustees, officers, affiliates and their agents (hereinafter referred to collectively as the "Indemnified Persons") from and against any and all liability, claims, lawsuits, losses, damages, costs or expenses (including attorney's fees), which the Indemnified Persons may hereafter incur, suffer or be required to pay by reason of the manufacture, use, sale, performance, lease or other commercialization or distribution by GLAXO, its Affiliates, licensees or agents of products, devices, processes or procedures first conceived or reduced to practice at the University, by the Principal Investigator and the members of his Research Team under the Programme of Research, except to the extent that such liability, claims lawsuits, losses, damages, costs or expenses (including attorneys' fees) result from the negligence or intentional wrongful acts of the University, the Principal Investigator or said Research Team. The University shall notify GLAXO upon learning of the institution or threatened institution of any such liability, claims, lawsuits, losses, damages, costs and expenses, and the University shall cooperate with GLAXO in every proper way in the defence or settlement thereof at GLAXO's request and expense.

(c) GLAXO shall, upon the entry of any product, device, process or procedure resulting from the Programme of Research into FDA Phase III clinical trials and for so long as GLAXO or its Affiliates or any of their sublicensees or agents shall sell any products, devices or procedures which were invented or developed during the Programme of Research or licensed under a License Agreement to be entered into between the parties, or shall practice any process or method which incorporates the Programme of

Research, Know-how or an Invention, secure and maintain in full force and effect a policy or policies of general liability insurance (with product liability endorsements) with limits of not less than One Million Dollars ($1,000,000) per person and Three Million Dollars ($3,000,000) per occurrence with no aggregate annual limit, or adequately self-insure. Such coverage(s) shall be purchased from a carrier or carriers reasonably acceptable to the University, which carrier(s) shall provide certificates evidencing the coverage(s) maintained and specifying that the University will be given no less than thirty (30) days prior written notice of any change in or cancellation of such coverage(s).

XIII. University's Disclaimers

(a) Disclaimers: Neither the University nor any of its faculty members, researchers, trustees, officers, employees, or agents assume any responsibility for the manufacture, product specifications, or the use of administration of drugs, compounds or other substances which are manufactured by or for or which are sold by or for GLAXO or by any of its Affiliates or sublicensees and which are the result of research undertaken pursuant to this Agreement. All warranties in connection therewith shall be made by GLAXO (or the particular GLAXO Affiliate or sublicensee that is involved) as the manufacturer, seller, or administrator thereof and none of such warranties shall directly or by implication in any way obligate the University or any of its faculty members, researchers, trustees, officers, employees, or agents.

(b) Disclaimer of Warranty: THE UNIVERSITY MAKES NO WARRANTIES OR REPRESENTATIONS INCLUDING, BUT NOT LIMITED TO, WARRANTIES OF FITNESS OR MERCHANTABILITY, REGARDING ANY DRUG, COMPOUND OR OTHER SUBSTANCE PRODUCED BY OR RESULTING FROM THE PROGRAMME OF RESEARCH.

XIV. Assignment

Neither party may assign, delegate or otherwise transfer any of its rights or obligations under this Agreement to any Third Party without the prior written consent of the other party.

XV. Entire Agreement

This document contains the entire agreement and understanding between the parties as to its subject matter. This Agreement can only be modified by written agreement duly signed by persons authorized to sign agreements on behalf of GLAXO and the University.

XVI. Severance

Each clause of this Agreement is distinct and severable. If any clause is deemed illegal, void or unenforceable, the validity, legality or enforceability of any other clause or portion of this Agreement will not be affected thereby.

XVII. Waiver

The failure of a party in an instance to insist upon the strict performance of the terms of this Agreement will not be construed to be a waiver of any of the terms of the Agreement, either at the time of the party's failure to insist upon strict performance or at any time in the future, and such terms will continue in full force and effect.

XVIII. Governing Law

The construction and performance of this Agreement will be governed by the laws of England

XIX. Definitions

a) "Affiliate" shall mean any company controlling, controlled by, or under common control with GLAXO.

b) "Field" shall be the subject matter of the research described in the Programme of Research and amendments thereto.

c) "Inventions" shall mean all inventions in the Field conceived or reduced to practice during the course of and under the Programme of Research which are made, in whole or in part, by any non-GLAXO employee participating in the Programme of Research.

d) "Know-how" shall include, without limitation, all technical data, information, materials and non-patentable or non-patented discoveries arising out of the Programme of Research.

e) "Net Sales" shall mean the gross invoice price of the sold product less (i) charges for transportation, insurance, taxes and duties or governmental charges shown on the invoice, or otherwise capable of documentation, (ii) credits for trade, cash or other discounts actually granted, and (iii) amounts repaid or credited by reason of rejections, recalls or return of goods or because of retroactive price reductions.

(e) "Patents" shall include all patent applications covering Inventions, together with any patent resulting therefrom, including divisionals, continuations, continuations-in-part, reissues, extensions of term, registrations and confirmations thereof. So defined, the term "Patents" does not include patents on inventions reduced to practice prior to the commencement date of the agreement between the parties hereto and dated 1st July

1989 or not reduced to practice or conceived during the course of and under that agreement or during the course of and under the Programme of Research, which patents (if any) shall be referred to herein as the "Underlying Patents".

g(f) "Third Party" shall mean any person other that the University and GLAXO Group Research Limited and the Affiliates of GLAXO Group Research Limited as hereinunder defined

Schedule 1

[PROGRAMME OF RESEARCH]

Synthesizing cGMP Analogs and Phosphodiesterase Inhibitors to Probe the Mechanisms and Roles of Smooth Muscle Protein Kinases and Phosphodiesterases

PRINCIPAL INVESTIGATOR: Jackie D. Corbin

INTRODUCTION

During the tenure of our current Glaxo Cardiovascular Discovery project, we have developed a diverse catalog of highly potent cGMP analogs for relaxing vascular smooth muscle, which surpass the potencies of those previously available (see enclosed manuscript by Sekhar, et al.). These new compounds have provided for important new insights into the basic mechanisms of regulation of the protein kinases as well as into their involvement in relaxation. It is anticipated that some of the compounds generated by this project, and by future projects, will be used by Glaxo scientists at various international sites for intact tissue and animal testing as well as for other experiments. It is well-known from the work of many groups, including our own, that the cGMP-dependent protein kinase (cG kinase) has important disease-related functions other than the induction of vascular smooth muscle relaxation. These include airway smooth muscle relaxation (asthma), corpus cavernosum relaxation (male impotence), and platelet aggregation inhibition (cardiovascular malfunction). Additionally, four groups have reported within the last year that nerve cell long-term potentiation (memory) is mediated by cGMP. Our cGMP analogs and phosphodiesterase (PDE) inhibitors are likely to be effective in many of these systems. The following proposal extends the Corbin/Glaxo collaboration. The emphasis is shifted to include consideration of both protein kinase and PDE since, as will be discussed below, it has become clear that these two are very related entities when considering regulation of smooth muscle relaxation. We will focus on the mechanisms of cyclic nucleotide action, which has been our long-term research interest.

DESCRIPTION

1. cGMP analogs. A new group of analogs will be designed and synthesized in order to explore fundamental mechanisms of cG kinase activation and cGMP-binding PDE (cG-binding PDE) action as well as to establish the pathway(s) of cGMP and cAMP relaxation of smooth muscle. The structures of the new analogs will be based on the advances achieved in analog design during the last two years of this project. We believe that the efficacy of analogs for either cG kinase activation or for smooth muscle relaxation can be significantly improved. Furthermore, the resistance of cGMP to hydrolysis by PDEs or its use as an antagonist of PDE catalytic activity has not been a major consideration in previous studies.

(A) We will attempt to answer the basic question: Why do the type Iα and type Iβ isozymes of cG kinase have profoundly different cGMP analog specificities (see enclosed manuscript by Sekhar, et al.)? These isozymes have identical amino acid sequences within their cGMP-binding sites. Type Iα and Iβ apparently are generated by an mRNA alternative splicing mechanism, and differ only in the first ~100 amino-terminal residues out of a total of ~650 residues. This portion of the protein includes the "dimerization" and "inhibitory" domains of these enzymes. The inhibitory domain is the region that maintains the kinase in an inactive form in the absence of cGMP. We theorize that a segment within

this domain directly or indirectly influences one or both of the two cGMP-binding sites of the protein kinase to modify cGMP analog specificity. Such an influence could pertain to either or both of the two isozymes. Proteolytic enzymes will be used to truncate both isozymes at discrete sites that we have already established (between residues 40-70) in the inhibitory domains of types Iα and Iβ. After purification of the truncated forms, studies of cGMP analog specificities for kinase activation and binding will be performed. Certain truncations should produce identical cGMP analog specificities of the two isozymes. In fact, a truncated form of one isozyme could exhibit the identical analog specificity of the intact form of the other isozyme. When we are successful in expressing the cDNA of cG kinase, mutagenesis within the amino-terminal 100 residues will allow much more precise truncations than can be obtained by proteolysis. It is expected that the results of these experiments may define the contact point(s) between the inhibitory domain and the cGMP-binding domains of the kinase, thus revealing an important aspect of the mechanism of cGMP activation of the enzyme, which is one of the ultimate goals of this laboratory.

(B) A concerted effort will be made to synthesize analogs that are specific for the type Iβ isozyme of cG kinase. Although analogs that are highly specific for type Iα have been successfully developed, analogs with a similar specificity for the Iβ form have been elusive. This problem is quite germane to the overall objectives since we have determined in the current project that human aorta, like the bovine tissue, contains nearly equal concentrations of the Iβ and Iα isozymes. If this particular aim is successful, then we should be able to establish whether or not the type Iβ form, in addition to type Iα, mediates the cGMP stimulation of muscle relaxation. Future investigations of the kinases, such as analyses of mixtures of the two isozymes and studies of the roles of each of the two cGMP-binding sites of type Iβ (see section A), will also be facilitated.

(C) We will expand on our most significant finding that analogs modified at the 1,2- and 8-positions of the guanine moiety of cGMP are the most potent cG kinase activators and muscle relaxants. We originally proposed to synthesize such analogs because 1,2-modified analogs bind to one of the two cGMP-binding sites of the cG kinase and 8-modified analogs bind to the other site. As predicted, when these two structural elements were combined, the resulting compounds were even more efficacious because they bound well to both binding sites. For reasons that are difficult to explain, these analogs were also quite potent activators of both cG kinase type Iα and Iβ. We now believe that the high potencies of these analogs in intact tissues are due not only to the high kinase affinity of 1,2- and 8-modifications, but also to a strong PDE resistance and antagonism due mainly to the 8-modifications. The latter properties also contribute to the long-lasting analog effect observed in the intact tissue (and presumably in the intact organism). We will emphasize PDE resistance in planning new analogs with improved potencies, and we will use the purified PDE (see below) as well as the kinase for initial screening. Thus, when considering double modifications (single analogs modified at both the 1,2- and 8-positions), the PDE resistance of the parent compound will be more carefully scrutinized. In our preliminary work, we have found that some cGMP analogs are not only PDE-resistant, but they also act like methylxanthine inhibitors of the enzyme by binding tightly to its catalytic site. This would result in elevation of tissue levels of cyclic nucleotides. An ideal analog for the intact tissue could be one that penetrates the cell membrane well and combines a high kinase affinity with a high PDE affinity, provided that the analog is not degraded when bound to the PDE. We will continue to use the pig coronary arteries as a final test system.

(D) Using the pig coronary arteries, we will test combinations of compounds that are potentially synergistic for relaxation. Combinations would include: (1) two cGMP analogs, each selective for one or the other of the two cGMP-binding sites of the kinase; since we know from our previous work that the two cGMP-binding sites of the kinase are positively cooperative, the combination of two such analogs should theoretically be synergistic in activating cG kinase and in relaxing the muscle; (2) two cGMP analogs, one selective for type Iα and the other for type Iβ cG kinase; (3) a cGMP analog and a cAMP analog (both cG kinase and cA kinase could be involved in inducing relaxation); and (4) a cGMP analog and a PDE inhibitor (see below).

2. Inhibitors of a cGMP-Binding PDE. We have a long-standing interest in this enzyme, and recently, in collaboration with Joe Beavo's group, we have cloned the cDNA for the bovine enzyme. Along with the cG kinase, the cG-binding PDE is present in high concentrations in vascular smooth muscle. This is probably not a coincidence, and we have recently found that the cG kinase phosphorylates the cG-binding PDE. As noted above, PDE resistance of cGMP analogs, in addition to potency in activating cG kinase, probably plays a key role in determining their efficacies for relaxing smooth muscle. Furthermore, since the cG-binding PDE presumably regulates cGMP levels in vascular smooth muscle, inhibitors of this enzyme should also be effective relaxants; thus, combinations of cGMP analogs and cG-binding PDE inhibitors should be synergistic for relaxation. These ideas will be tested. It will be necessary to measure the tissue levels of cGMP and cAMP in these experiments. Elevations in the levels of these cyclic nucleotides would be consistent with PDE inhibition. We will design PDE inhibitors based on the theory that the potencies of existing inhibitors, such as 3-isobutyl-1-methylxanthine (IBMX) and zaprinast, could be enhanced by appending groups that would allow the inhibitors to more closely resemble the entire cGMP molecule. The existing inhibitors resemble mainly the guanine component. Our preliminary results indicate that this strategy works. We have synthesized one compound, 8-(4-OH-phenylthio)-IBMX (Table 1) that is about 160-fold more potent than the parent IBMX, and 6-fold more potent than the best existing inhibitor, zaprinast. We also have access to PDEs other than cG-binding PDE to use as controls in these studies, and we have developed assays for measuring PDE activities in crude mixtures. It should be pointed out that results from our laboratory have indicated a communication between the cGMP-binding site(s) and catalytic site of the PDE. PDE inhibitors or cGMP analogs found to be specific for either of these sites will be used for studies of effects of this communication.

Table 1. Proposed new compounds. The compounds have been selected based on our previous experience, and their use should address each of the questions mentioned above. Some cGMP analogs (such as modifications at both the 8- and 1,2-positions) should be quite potent for cG kinase (both isozymes) activation and smooth muscle relaxation; some (modifications at the 1,2-position) should be type Iβ-specific; some (modifications at the 8-position or at both the 8- and 1,2-positions) should be PDE-resistant or PDE-antagonistic; and some (such as modifications of IBMX and zaprinast), including certain cGMP analogs (such as modifications at the 1,2-position or at both the 1,2- and 8-positions) should be PDE inhibitors. From our previous experience, early results will suggest second and third generation analogs to synthesize. Methods for synthesizing analogs have already been published by ourselves or others, or have been developed recently in our laboratory.

A. **cGMP Analogs** (PET=phenyletheno, PT=phenylthio)

1,N^2-PET-cGMP derivatives	8-PT-cGMP derivatives
8-Br-(2,4-diOH-PET)-	8-(2,4-diOH-PT)-
8-Br-(2-NH_2-PET)-	8-(2-NH_2-PT)-
8-Br-(4-OH-PET)-	8-(4-OH-PT)-
8-Br-(4-NH_2-PET)-	8-(4-NH_2-PT)-
Repeat above with 8-I- instead of 8-Br-, if appropriate	Combine PET changes with 8-halo- or 8-PT- changes, where appropriate

B. **PDE inhibitors** (PT=phenylthio, PA=phenylamino)

IBMX derivatives

8-SH-	8-PA-	8-(4-OH-PA)-
8-NH_2-	8-(4-F-PA)-	8-(4-OH-PT)-
8-CF_3-	8-(4-Cl-PT)-	8-(2,4-diOH-PT)-
8-Br-	8-(4-NO_2-PT)	8-(4-OH-phenyl)-
		8-(4-halo-phenyl)-

4-aminodiphenyldisulfide

Zaprinast derivatives (with carbon instead of nitrogen at 8-position) - 8-Br-, 8-PT-, 8-(2,4-diOH-PT)-, Modifications of 2-phenyl ring

Controls

Zaprinast Dipyridamole Papaverine 8-methoxymethyl-IBMX

Preliminary results indicate a poor correlation between potency of compounds for inhibiting the cGMP-binding PDE and for relaxing smooth muscle. Furthermore, the most potent PDE inhibitor is <one-tenth as potent as the best cGMP analog for relaxing the muscle.

TIMETABLE AND EXPECTED ACHIEVEMENTS

It is anticipated that all of the proposed compounds can be synthesized and tested within two years of the grant period. Based on results of the first generation of compounds, a second generation can be analyzed during the third year. We hope to have achieved the following by the end of the third year:

(1) Improvement(s) in cGMP analog affinity for cG kinase of >10-fold and in efficacy for smooth muscle relaxation of >10-fold. Theoretically, even greater

improvements are probable, even if none of the potentially synergistic combinations are successful.

(2) Better cGMP-binding site and isozyme-specific cGMP analogs. We are especially in need of a type Iβ-specific analog that would allow studies of the roles of the binding sites of this enzyme and investigations of the roles of the enzyme in intact cells.

(3) Identify the points of contact between the inhibitory domain and the cGMP-binding sites of the protein kinase. This will be done through the use of cGMP analogs and truncated isozymes. The results should allow a better insight into the mechanism of cGMP activation of the kinase, a long-range goal of this laboratory.

(4) Determine if the cG-binding PDE plays a significant role in cGMP analog efficacy for smooth muscle relaxation. This will be addressed by synthesizing cGMP analogs that are PDE-resistant and that block PDE hydrolysis of endogenously generated cyclic nucleotides. Theoretically, these compounds should be more potent than classical PDE inhibitors.

(5) An improvement in cG-binding PDE inhibitor potency of >100-fold. These compounds, which would be based on structures of classical inhibitors, should be more potent relaxants as well. If not, then perhaps we will gain new knowledge of the target(s) of these compounds in intact tissues.

(6) Construct more precise maps of the cGMP-binding sites of the protein kinase and of the cGMP-binding and catalytic sites of the PDE. This would permit many mechanistic studies of the enzymes, as well as the design of more efficacious compounds in the future. This aim should be facilitated by other projects underway in this laboratory that utilize site-directed mutagenesis to study the cyclic nucleotide sites of both enzymes.

IN WITNESS WHEREOF, the parties hereto have caused this Agreement to be duly executed as of the day and year first above written.

"GLAXO"	"UNIVERSITY"
By: B.J. Price.	By: See attached
Name: DR. BJ. PRICE	Name:
Title: RESEARCH DIRECTOR	Title:
Date: 29/10/92	Date:

"PRINCIPAL INVESTIGATOR"	APPROVED BY:
_____	_____
Date: _____	Date: _____
_____	

VANDERBILT UNIVERSITY MEDICAL CENTER

Recommended By:

Jackie D. Corbin

Jackie D. Corbin, Ph.D.
Principal Investigator

9/1/92
Date

Daryl K. Granner

Daryl K. Granner, M.D.
Chair, Department of Molecular Physiology
and Biophysics

8-24-92
Date

John E. Chapman

John E. Chapman, M.D.
Dean, School of Medicine

9.14.92
Date

Approved By:

Joel G. Hardman

Joel G. Hardman, Ph.D.
Associate Vice Chancellor
for Health Affairs

9-14-92
Date

Schedule 2

[SCHEDULE OF PAYMENTS]

		1992-93	1993-94	1994-95
Salary and Fringe Benefits				
Jackie Corbin, Professor	25%	$ 30,950	32,800	$ 34,780
Jack Wells, Professor	10%	9,590	10,170	10,780
Sharron Francis, Assoc. Prof.	25%	15,825	16,780	17,790
Raja Sekhar, Research Associate	100%	28,800	30,530	32,360
Ken Grimes, Research Assistant	100%	33,800	35,830	37,980
Jeff Smith, Graduate Student	100%	14,200	15,060	15,960
	Total	$133,165	$141,170	$149,650
*Equipment (Iris Graphics Terminal)		28,000	0	0
software and tech assist		2,000	2,000	2,000
Supplies		35,000	37,100	39,330
Travel		3,000	3,180	3,370
Total Direct		$201,165	$183,450	$194,350
Indirect Costs @21%		36,365	38,525	40,814
Total		$237,530	$221,975	$235,164

The percent effort for each investigator is the approximate effort that will be devoted to this project if it is funded. Raja Sekhar will mainly be involved in cGMP analog synthesis and purification in collaboration with Wells. Ken Grimes will carry out the experiments using intact coronary artery slices and do many of the kinase, binding and PDE assays. Jeff Smith will be studying cGMP analog effects on smooth muscle cGMP-dependent protein kinase. Corbin and Francis will coordinate all aspects of the project. It is expected that the three scientific members will be in frequent consultation concerning results of the experiments. The Supplies request seems modest in view of the chemicals and materials needed for this project. The Travel request is for one meeting per investigator. The annual increases in each category are six percent.

*The graphics system that has been used previously is no longer available because of loss of the departmental support. A system is needed to do the modeling work required for this project. The graphics system will not be subjected to the 21% indirect costs.