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Final Progress Report for DE-FG02-90ER61091

December 24, 1993 - February 28, 1994

Dr. Markus Schwaiger, the original PI on this grant, departed for the University of Munich on June 1, 1993. Donald M. Wieland assumed a caretaker role on this grant, the budget of which was essentially depleted at the time of Dr. Schwaiger's departure. In fact Dr. Wieland was left with overdrafts on the budget which he had to pay for out of his own discretionary monies. A Progress Report for the period from December 24, 1992 to December 23, 1993 was submitted previously (see attached progress report for this period). For the approximately 3-month extension period, no further work was done because essentially no money was left in the budget.

In the final year of this grant period, work focused on the use of the isolated perfused rat heart system to study the mechanism of neuronal storage of [^{11}C]epinephrine. This work was presented at the Society of Nuclear Medicine Meeting; reference to the published abstract is given below. A manuscript is in preparation on this work. The basic studies of [^{11}C]epinephrine that were supported by this grant have led to the successful use of this tracer in humans.

A compilation of all publications funded by this grant is presented below. Copies of any additional publications that result from this grant support - and there should be 3 or 4 more - will be sent to you.

A. Papers Published

1. Schwaiger M, Kalff V, Rosenspire K, Haka MS, Molina E, Hutchins GD, Deeb M, Wolfe E, Jr., Wieland DM: The noninvasive evaluation of the sympathetic nervous system in the human heart by PET. Circulation 1990;82:457-464.

2. Schwaiger M, Guibourg H, Rosenspire K, McClanahan T, Gallagher K, Hutchins G, Wieland DM: Effect of regional myocardial ischemia on sympathetic nervous system as assessed by F-18 metaraminol. J Nucl Med 1990;31:1353-1357.

3. Schwaiger M, Hutchins GD, Kalff V, Rosenspire K, Haka MS, Mallette S, Deeb GM, Abrams GD, Wieland DM: Evidence for regional catecholamine uptake and storage sites in the transplanted human heart by positron emission tomography. J Clin Invest 1991;87:1681-1690.

4. Wolpers HG, Nguyen N, Rosenspire K, Haka M, Wieland DM, Schwaiger M: C-11 hydroxyephedrine as marker for neuronal catecholamine retention in reperfused canine myocardium. Coronary Artery Disease 1991;2:923-929.

5. Shulkin BL, Wieland DM, Schwaiger M, Thompson NW, Francis IR, Haka MS, Rosenspire KC, Shapiro B, Sisson JC, Kuhl DE. PET scanning with hydroxyephedrine: A new approach to the localization of pheochromocytoma. J Nucl Med 1992; 33:1125-1131.

6. Allman KC, Stevens MJ, Wieland DM, Hutchins GD, Wolfe ER, Greene DA, Schwaiger M: Noninvasive assessment of cardiac diabetic neuropathy by carbon-11 hydroxyephedrine and positron emission tomography. J Am Coll Cardiol 1993; 22:1425-1432.

7. DeGrado TR, Hutchins GD, Toorongian SA, Wieland DM, Schwaiger M. Myocardial kinetics of carbon-11-meta-hydroxyephedrine: Retention mechanisms and effects of norepinephrine. J Nucl Med 1993; 34:1287-1293.

8. Calkins H, Lehmann MH, Allman K, Wieland D, Schwaiger M. Scintigraphic pattern of regional cardiac sympathetic innervation in patients with familial long QT syndrome using positron emission tomography. Circulation 1993; 87:1616-1621.

9. Allman KC, Wieland DM, Muzik O, Degrado TR, Wolfe ER, Schwaiger M. Carbon-11 hydroxyephedrine with positron emission tomography for serial assessment of cardiac adrenergic neuronal function after acute myocardial infarction in humans. J Am Coll Cardiol 1993; 22:368-375.

DISCLAIMER

10. Calkins H, Allman K, Bolling S, Kirsch M, Wieland D, Morady F, Schwaiger M. Correlation between scintigraphic evidence of regional sympathetic neuronal dysfunction and ventricular refractoriness in the human heart. Circulation 1993; 88:172-179.

11. Chakraborty PK, Gildersleeve DL, Jewett DM, Toorongian SA, Kilbourn MR, Schwaiger M, Wieland DM. High yield synthesis of high specific activity R-(-)-[^{11}C]epinephrine for routine PET studies in humans. Nucl Med Biol 1993; 20:939-944.

12. DeGrado TR, Mulholland GK, Wieland DM, Schwaiger M. Evaluation of (-) [^{18}F]fluoroethoxybenzovesamicol as a new PET tracer of cholinergic neurons of the heart. Nucl Med Biol 1994; 21:189-195.

13. Melon PG, Nguyen N, DeGrado TR, Mangner TJ, Wieland DM, Schwaiger M. Imaging of cardiac neuronal function after cocaine exposure using carbon-11 hydroxyephedrine and positron emission tomography. J Am Coll Cardiol 1994; 23:1693-1699.

B. Articles submitted for publication

1. Melon PG, Boyd CJ, McVey S, Mangner TJ, Wieland DM, Schwaiger M. Effects of active chronic cocaine use on cardiac sympathetic neuronal function assessed by C-11 hydroxyephedrine and positron emission tomography. Submitted to J Am Coll Cardiol, 1994.

Manuscripts in preparation

1. Nguyen N, DeGrado T, Chakraborty P, Wieland, D, Stafford K, Schwaiger M. Evaluation of C-11 epinephrine in the isolated working rat heart. See Abstract #36.

2. Schwaiger M, Wieland DM, Muzik O, Chakraborty P, Nguyen N, Stafford K. Comparison of C-11 epinephrine and C-11 HED for evaluation of sympathetic neurons of the human heart. See Abstract #37.

3. Corbett JR, Raffel D, del Rosario RB, Gildersleeve DL, Chiao P-C, Schwaiger M, Wieland DM. Initial clinical experience with carbon-11 phenylephrine: A new marker of cardiac sympathetic neuronal function and metabolism.

C. Published Abstracts

1. Allman K, Wolfe E, Sitomer J, Hutchins G, Wieland D, Schwaiger M: C-11 hydroxyephedrine assessment of regional myocardial sympathetic neuronal function following acute myocardial infarction in man. J Nucl Med 1991;32(5):1040.

2. Calkins H, Bolling SF, Allman K, Kirsh MM, Wieland D, Morady F, Schwaiger M. Correlation of regional C-11 HED retention and electrophysiologic mapping in the human heart. Circulation 1991;84(4):414.

3. Allman K, Hutchins G, Wolfe E, Allman C, Wieland D, Schwaiger M. C-11 hydroxyephedrine myocardial retention following acute myocardial infarction. Circulation 1991;84(4):423.

4. Haka MS, Jung Y-W, Rosenspire KC, Wieland DM. Synthesis of [C-11]-para-hydroxyephedrine (PHED) -- Comparison with the heart agent MHED. J Nucl Med 1990; 30: p 738.

5. Rosenspire KC, Pisani TL, Haka MS, Schwaiger M, Wieland DM. Metabolic studies of the PET neuronal heart agent [C-11]-meta-hydroxyephedrine (MHED). J Nucl Med 1990; 30: p 831

6. Hutchins GD, Schwaiger M, Rosenspire KC, Wieland DM. Evaluation of sympathetic innervation in the human heart using [C-11]hydroxyephedrine: A distribution volume approach. J Nucl Med 1990; 31: p 725.

7. Schwaiger M, Hutchins G, Rosenspire K, Haka M, Abrahms GD, Kuhl DE, Wieland DM. Myocardial C-11 hydroxyephedrine (HED) kinetics early and late following cardiac transplantation. Society of Nuclear Medicine, June 19-22, 1990.

8. Schwaiger M, Hutchins G, Rosenspire K, Haka M, Wieland DM. Quantitative evaluation of the sympathetic nervous system by PET in patients with cardiomyopathy. J Nucl Med 1990; 31: p 792.

9. Wolpers HG, Nguyen N, Rosenspire K, Haka M, Wieland DM, Schwaiger M. Comparison of C-11 HED and I-131 MIBG for the assessment of ischemic neuronal injury in the canine heart. J Nucl Med 1990; 31: p 725

10. Allman K, Hutchins G, Wolfe E, Allman C, Wieland D, Schwaiger M. C-11 Hydroxyephedrine myocardial retention following acute myocardial infarction. J Nucl Med 1991; 32:994.

11. Rosenspire KC, Van Dort ME, Haka MS, Jung Y-W, Gildersleeve DL. Search for a non-metabolizable PET tracer for heart neuronal imaging. 9th International Symposium on Radiopharmaceutical Chemistry, April 5-10, 1992, Paris, France. J Labeled Cmpd Radiopharm 1993; 32:

12. DeGrado TR, Toorongian SA, Hutchins GD, Wieland DM, Schwaiger M. Characterization of C-11 m-hydroxyephedrine (HED) kinetics in isolated rat heart. J Nucl Med 1992; 33: p 194.

13. Allman KC, Stevens MJ, Wieland DM, Hutchins GD, DeGrado TR, Schwaiger M. Neuronal imaging with PET for the quantitative characteriztion of cardiac diabetic neuropathy. American Heart Association 65th Annual Meeting, November 16-19, 1992, New Orleans, LA.

14. Melon PG, DeGrado TR, Nguyen N, Mangner TJ, Hutchins GD, Wieland DM, Schwaiger M. Quantitative evaluation of cardiac sympathetic neuronal function following cocaine exposure. American Heart Association 65th Annual Meeting, November 16-19, 1992, New Orleans, LA.

15. Wieland DM, Chakraborty PK, Gildersleeve DL, Sherman PS, Caraher JM, Nguyen NTB. Comparison of true and false neurotransmitters in the heart: Evaluation of R-(-)-[C-11]epinephrine in animals. J Nucl Med 1993; 34:123P.

16. Nguyen NTB, DeGrado TR, Chakraborty P, Stafford K, Wieland D, Schwaiger M. Evaluation of C-11 epinephrine in isolated working rat heart. J Nucl Med 1993; 34:45P.

17. Schwaiger M, Wieland D, Muzik O, Chakraborty P, Nguyen N, Stafford K, Gildersleeve D. Comparison of C-11 epinephrine and C-11 HED for evaluation of sympathetic neurons of the heart. J Nucl Med 1993; 34:13P.

18. Van Dort ME, Chakraborty PK, Caraher J, Gildersleeve DG, Sherman PS, Wieland DM. Search for kinetic richness: Evaluation of stereoisomers of [C-11]HED. J Nucl Med 1994; 35:98P.

19. Corbett JR, Chiao P-C, del Rosario R, Gildersleeve DL, Tluczek L, Schwaiger M, Wieland DM. Mapping neuronal enzyme function of the human heart with C-11 phenylephrine. J Nucl Med 1994; 35:109P.

20. Raffel DM, Corbett JR, Schwaiger M, Wieland DM. Mechanism-based strategies for mapping heart sympathetic nerve function. Presented at the VIII Bottstein Colloquium/Workshop of COST-B3-Action on "New Radiotracers and Methods of Quality Assurance for Nuclear Medicine Application". Oct. 6-7, 1994. Paul Scherrer Institute, Villigen, Switzerland.

21. Raffel DM, del Rosario RB, Tluczek L, Wieland DM. Kinetic properties of C-11 phenylephrine in isolated rate heart: effects of di-deuterium substitution, age, MAO inhibition and reserpine. Submitted to Society of Nuclear Medicine 42nd Annual Meeting, Minneapolis, MN, 1995.

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Schedule of Current and Future Research Activities.

During the first year of funding, C-11 hydroxy ephedrine has been introduced as the first clinically usable norepinephrine analogue. Studies in normal volunteers and patients with various cardiac disorders indicated the feasibility of this tracer for further evaluation. Simultaneously, animal studies have been used to assess the use of these radiopharmaceuticals in ischemic injury in order to define neuronal damage (see enclosed manuscript).

Current research focuses on the comparison of C-11 hydroxyephedrine with other neurotransmitters such as C-11 epinephrine and C-11 threo-hydroxyephedrine. Epinephrine is primarily stored in vesicles of the nerve terminal, while threo-hydroxyephedrine is only substrate to uptake I mechanism. Such a combination of radiotracers may allow the dissection of uptake I mechanism as well as vesicular storage.

In parallel to the refinement of presynaptic tracers for the sympathetic nervous system, we are developing radiopharmaceuticals to delineate the adrenergic receptors in the heart. The combined evaluation of pre- and postsynaptic nerve function will improve our ability to identify abnormalities. We are currently developing a new radiosynthesis of the hydrophillic adrenergic receptor antagonist C-11 CGP-12177 which has been used by others for the visualization of adrenergic receptors in the heart.

In addition, we are participating in the development of radiopharmaceuticals for the delineation of presynaptic cholinergic nerve terminals. Derivatives of benzovesamicol have been labeled in our institution and are currently under investigation. The most promising agent is F-18 benzovesamicol (FEBOBV) which allows the visualization of parasympathetic nerve terminals in the canine heart as demonstrated by preliminary PET data.

1. Scientific Issues Currently Being Addressed and Their Significance.

1) Sympathetic presynaptic tracers:
Any radiopharmaceutical which functions as a false neurotransmitter will compete with endogenous neurotransmitters for uptake or storage sites. Since the levels of endogenous neurotransmitters may vary from patient to patient, it is important to elucidate the sensitivity of a given tracer approach to varying levels of naturally occurring neurotransmitters. Therefore, we are currently evaluating the competitive relationship of norepinephrine and hydroxyephedrine for the uptake in the sympathetic nerve terminal.

C-11 hydroxyephedrine provides a useful biological probe to assess the effect of various drugs on the function of the sympathetic nerve terminal. We are currently evaluating the effect of intravenous cocaine on uptake one mechanism of the sympathetic nerve terminal. Cocaine is known to produce pronounced cardiac toxic effects. These effects are linked to the inhibition of norepinephrine uptake caused by cocaine. Animal studies in combination with C-11 hydroxyephedrine are being performed at baseline and following cocaine exposure to quantitatively assess the acute and chronic effects of cocaine. Preliminary data indicate that cocaine leads to a 75% reduction of hydroxyephedrine uptake acutely. However, there appears to be a prolonged effect of this drug on the heart at 2 hours as well as at 24 hours after drug exposure. This study exemplifies the unique potential of PET to noninvasively study the pharmacokinetics of drugs such as cocaine. The advantage of this approach is that it cannot only be applied in animal models but also extended to the investigation of pharmacokinetics in the human heart (see enclosed abstract).

2) Parasympathetic nervous system: the development of tracers for the cholinergic nerve terminal includes feasibility studies to investigate the possibility of using positron emission tomography to map the parasympathetic innervation of the heart. Preliminary studies with F-18 benzovesamicol indicate that tracer retention in the atria is about 2-3 times higher than in the left ventricle, consistent with the known distribution of parasympathetic nerve terminals (see enclosed abstract). However, questions remain with regard to the nonspecific binding of this radiopharmaceutical and its kinetics in myocardial tissue. Therefore, animal studies are being performed using dynamic PET scanning to define the kinetics of these radiopharmaceuticals to study their suitability for further clinical studies. These studies are done in cooperation with other projects in radiochemistry dealing with the central nervous system.

This cooperation has allowed us to study a relatively large number of radiotracers in a short time period and therefore optimize the selection of radiotracers for subsequent clinical use.

2. Experimental and Theoretical Approach Taken, Techniques Used and Resources Applied.

The Division of Nuclear Medicine at the University of Michigan provides a wide variety of approaches to test new radiopharmaceuticals. For this project, we are employing autoradiography, isolated heart preparation, animal PET scanner and clinical studies in normal volunteers and patients with cardiac transplants who serve as a model for cardiac denervation.

The isolated heart preparation has been used to specifically assess the kinetics of tracer uptake and retention in the rat heart. Current work uses this model to assess the effect of varying levels of norepinephrine concentration in the perfusate on the uptake and clearance kinetics of C-11 hydroxyephedrine. These studies reveal that the clearance of hydroxy ephedrine from the myocardium is affected by increasing levels of norepinephrine. The goal of these studies is to develop correction factors for varying norepinephrine plasma levels in order to correct tracer kinetic analysis of clinical studies for the effect of endogenous competition. However, further validation is required before such an approach can be implemented.

The animal PET scanner is used to define the feasibility of new radiopharmaceuticals for imaging purposes. In addition, dynamic scanning is used to define regional tracer kinetics in large animals such as dogs. We are currently using the dedicated animal scanner for the evaluation of hydroxyephedrine kinetics prior to and after cocaine exposure. Regional time-activity curves are generated and fitted with a tracer kinetic model resulting in tracer tissue distribution volume estimates.

The animal scanner is also used to study the myocardial kinetics of FEOBV. Studies are performed at baseline and following the intravenous application of vesamicol to identify specific and nonspecific binding. In addition, models are developed to generate regional parasympathetic denervation. One approach currently being evaluated is the phenol application in the AV groove of the left ventricle which has been shown to not only disrupt sympathetic but also parasympathetic innervation of the heart. Such animal studies are necessary to define the imaging protocols applied in human studies and to develop tracer kinetic models providing quantitative estimates of parasympathetic function. In addition, assays are developed in our institution to define the density of parasympathetic innervation (CHAT), which are used as ancillary information validating the tracer approach.

Finally, the University of Michigan has two whole-body PET scanners for clinical use. Following the validation in animal studies the radiotracer will be first injected in normal volunteers and the regional tracer kinetics evaluated. Following these studies, the tracer will be employed in patients with cardiac transplantation or diabetic neuropathy in order to define nonspecific binding in patients with cardiac denervation. Such studies are important to define species differences and provide control data in the healthy human heart prior to clinical application.

3. Project Output

Bibliography of publications emanating from this project

A. Papers Published

1. Schwaiger M, Kalff V, Rosenspire K, Haka MS, Molina E, Hutchins GD, Deeb M, Wolfe E, Jr., Wieland DM: The noninvasive evaluation of the sympathetic nervous system in the human heart by PET. Circulation 1990;82:457-464.

2. Rosenspire KC, Haka MS, Jewett DM, Van Dort M, Gildersleeve DL, Schwaiger M, Wieland DM: Synthesis and preliminary evaluation of [^{11}C]meta-hydroxyephedrine: A false transmitter agent for heart neuronal imaging. J Nucl Med 1990;31:1328-1334.

3. Schwaiger M, Guibourg H, Rosenspire K, McClanahan T, Gallagher K, Hutchins G, Wieland DM: Effect of regional myocardial ischemia on sympathetic nervous system as assessed by F-18 metaraminol. J Nucl Med 1990;31:1353-1357.

4. Schwaiger M, Hutchins GD, Kalff V, Rosenspire K, Haka MS, Mallette S, Deeb GM, Abrams GD, Wieland DM: Evidence for regional catecholamine uptake and storage sites in the transplanted human heart by positron emission tomography. J Clin Invest 1991;87:1681-1690.

5. Wolpers HG, Nguyen N, Rosenspire K, Haka M, Wieland DM, Schwaiger M: C-11 hydroxyephedrine as marker for neuronal catecholamine retention in reperfused canine myocardium. Coronary Artery Disease 1991;2:923-929.

B. Articles Accepted for Publication:

6. Shulkin BL, Wieland DM, Schwaiger M, Thompson NW, Francis IR, Haka MS, Rosenspire KC, Shapiro B, Sisson JC, Kuhl DE. PET scanning with hydroxyephedrine: A new approach to the localization of pheochromocytoma. J Nucl Med.

7. Melon P, Schwaiger M. Imaging of metabolism and autonomic innervation of the heart by PET. Europ J Nucl Med.

C. Published Abstracts

1. Allman K, Wolfe E, Sitomer J, Hutchins G, Wieland D, Schwaiger M: C-11 hydroxyephedrine assessment of regional myocardial sympathetic neuronal function following acute myocardial infarction in man. J Nucl Med 1991;32(5):1040.

2. Calkins H, Bolling SF, Allman K, Kirsh MM, Wieland D, Morady F, Schwaiger M. Correlation of regional C-11 HED retention and electrophysiologic mapping in the human heart. Circulation 1991;84(4):414.

3. Allman K, Hutchins G, Wolfe E, Allman C, Wieland D, Schwaiger M. C-11 hydroxyephedrine myocardial retention following acute myocardial infarction. Circulation 1991;84(4):423.