CMLS, Cell. Mol. Life Sci. 62 (2005) 1–3
1420-682X/05/010001-03
DOI 10.1007/s00018-004-4437-9

CMLS Cellular and Molecular Life Sciences

Memories of a Senior Scientist

Some highlights of a 47 year career in research

Ferid Murad
M. D., Ph. D. The John S. Dunn Professor and Chair of Integrative Biology and Pharmacology and Director of the Institute of Medicine at the University of Texas-Houston Health Science Center

The University of Texas-Houston Health Science Center, Medical School, 6431 Fannin MSB 4.098, Houston, Texas 77030 (USA), Fax: +1 713 500 0790, e-mail: Ferid.Murad@uth.tmc.edu

Received 1 October 2004; accepted 21 October 2004

My first exposure to research was with a senior elective research project in college. I wanted to find out how fish without parathyroid glands regulated their calcium and bone metabolism. Although I made little progress with the project, I became intrigued with the process of discovery. I was so influenced that I decided to enter a seven year curriculum for both M.D. and Ph.D. degrees at Western Reserve University, the first such program in the U.S., to study both medicine and pharmacological/biochemical research.

I was fortunate to work in the laboratories of Earl Sutherland and Theodore Rall shortly after they discovered cyclic AMP. Within 6 to 12 months in the lab, I found that beta adrenergic agonists activated adenylyl cyclase from heart, liver and other tissues and that muscarinic agonists inhibited the enzyme. The beta adrenergic agonists were blocked by beta adrenergic antagonists and not alpha adrenergic antagonists while the muscarinic cholinergic agents were blocked by atropine. My first publication was in the Journal of Biological Chemistry in 1962 [1] and was one of several classical papers in the field at the time.

I discontinued research for two years during my clinical residency training in internal medicine at Massachusetts General Hospital. However, during this period I read journals and abstracts from the FASEB meetings. I developed a notebook with a number of research ideas and experiments to be done. I began to work on some of these ideas when I joined Martha Vaughan's laboratory at the NIH after my residency. As I was completing my three years of fellowship training at NIH, cyclic GMP was emerging as another possible second messenger regulated by several hormones and drugs. I turned more and more of my interests to cyclic GMP as I left NIH to join the faculty at the University of Virginia with a primary appointment in the Department of Medicine and a joint appointment in the Department of Pharmacology.

The cyclic AMP field was becoming more and more popular while the new cyclic GMP field had attracted few

scientists. I thought as a new young faculty member developing an independent research program that perhaps there was a greater chance of success with less competition working with cyclic GMP. Most of our work with cyclic GMP resulted in novel important information in the field and we rapidly became quite productive.

Within several years we found that most tissues possessed multiple isoforms of guanylyl cyclase with different properties. As we examined the biochemical properties of the isoforms, we accidentally found that azide, nitrite and hydroxylamine activated the soluble isoform of guanylyl cyclase [2]. This was an important finding since several hormones which increased cyclic GMP levels in heart and vascular preparations (acetylcholine and prostaglandins) failed to activate the enzyme in cell-free preparations. Our goals were 1) to determine the molecular mechanisms of hormonal activation of guanylyl cyclase and 2) find a biological function of cyclic GMP. To determine the molecular mechanism of hormone activation required an effect of hormones in cell extracts which didn't occur. We were determined to figure out how azide, nitrite and hydroxylamine activated the enzyme both in intact tissue and tissue extracts. When these agents increased cyclic GMP levels in various tissues including smooth muscle strips, it became apparent that the first biological effect of cyclic GMP to be discovered was smooth muscle relaxation (tracheal and gastrointestinal smooth muscle). [3] We then learned that other smooth muscle relaxants such as nitroglycerin and nitroprusside were also activators of guanylyl cyclase. After several years our observations all began to make sense in that nitric oxide was the active principal of this growing list of "nitrovasodilators" as we came to call these nitric oxide prodrugs [4]. What an exciting moment in December 1976 to find that nitric oxide gas generated chemically in the fume hood could activate soluble guanylyl cyclase and increase cyclic GMP levels in numerous tissues [5, 6]. It was unprecedented that a gas and free radical considered to be a pollutant and toxic substance could active an enzyme at nanomolar concentrations. Of course, there were many disinterested skeptics of our work. We realized much, but not all, of the significance of our work and showed for the first time the molecular mechanism of action of nitroglycerin, a drug used clinically for more than a century. We also believed that nitric oxide would be an intracellular second messenger to mediate the effects of some hormones [7]. Because of the low concentrations of nitric oxide to produce the effects, it took about eight years to develop the technology to prove our hypothesis.

There were other exciting moments in the laboratory when we discovered that heat stable enterotoxin (STa) from some strains of E. coli that produced diarrhea also activated the particulate isoform of guanylyl cyclase (GC-C) [8]. This work led other laboratories to discover endogenous guanylin peptides that also activate GC-C. After the discovery of atrial natruretic factor (atriopeptins) which was a vasodilator and renal natriuretic, we thought that this new peptide hormone may also activate guanylyl cyclase. Indeed, the atriopeptins activated other particulate isoforms of guanylyl cyclase (GC-A and GC-B) [9]. In fact, the particulate isoforms of guanylyl cyclase copurify with the receptors for these peptides [10] since the particulate guanylyl cyclase possess an outer membrane receptor domain, a transmembrane span, an inner protein kinase-like domain and the inner catalytic domain. The endogenous peptide ligands for some of the other particulate isoforms of guanylyl cyclase have not yet been identified.

Since our original publications with nitric oxide in 1977, more than 55,000 publications have appeared describing various aspects and effects of nitric oxide in a diverse area of biology. Numerous biotechnology companies have also been created. Attempts are being made by many companies and academic laboratories to develop diagnostic and therapeutic agents for a broad spectrum of disorders that are novel nitric oxide prodrugs (donors), nitric oxide synthase inhibitors, guanylyl cyclase activators and inhibitors, phosphodesterase inhibitors, nitric oxide and peroxynitrite scavengers, etc. Inhaled nitric oxide at controlled low concentrations is used for pulmonary hypertension in infants, congenital heart disease and inhibition of platelet aggregation in cardiopulmonary bypass surgery.

It is not common for a scientist to witness their fundamental research have such a broad application in biology and also result in the development of numerous therapeutic agents for a variety of disorders. While I entered medicine and research for these reasons, it is generally uncommon to see your work have such an influence in biological research and patient care. I understand that the field of nitric oxide research has benefited millions of people. While much is known about nitric oxide in biology and therapeutic applications, I believe that a great deal of information is lacking and that much research is yet to be done.

The ability of my laboratory to continue to contribute important information in both basic and translational research remains a very high priority for us. There is nothing more exciting than obtaining the answer to an important experiment that permits major pieces of the puzzle and information to come together that will have an important impact in biological research and hopefully medical application.

In spite of the 55,000 publications in the field of nitric oxide research, numerous important questions remain to be answered. I suspect that we have only seen 20% of the iceberg so far. Thus, I continue to remain excited and optimistic about research discoveries and their applications [11]. My laboratory continues to remain a very heteroge-

nous group of scientists with training in biochemistry, molecular biology, genetics, pharmacology, physiology and clinical medicine. We all learn from each other as we search for answers to pressing questions. We believe that our current work with guanylyl cyclase structure, mutants, constructs for possible gene therapy and knock outs can provide us and others with answers to understanding important questions and model systems.

It is also a joy to work with talented young people who are curious and ask lots of questions, some of which are critical to our work and plans. I have trained more than 100 young scientists in my lab who have become an extended family and many now have senior positions around the world. It is also exciting to see them become very productive and successful. Since Earl Sutherland, my mentor as a student, and his mentor Carl Cori both obtained the Nobel Prize, I tell my trainees that at least one of them needs to be invited to Stockholm in order not to break the genetic lineage.

1 Murad F., Chi Y. M., Rall T. W., Sutherland E. W. (1962) The effect of catecholamines and choline esters on the formation of adenosine 3′, 5′-phosphate by preparations from cardiac muscle and liver. J. Biol. Chem. **237:** 1233–1238

2 Kimura H., Mittal C. K., Murad F. (1975) Activation of guanylate cyclase from rat liver and other tissues with sodium azide. J. Biol. Chem. **250:** 8016–8022

3 Katsuki S., Arnold W. P., Murad F. (1977) Effect of sodium nitroprusside, nitroglycerin and sodium azide on levels of cyclic nucleotides and mechanical activity of various tissues. J. Cyclic Nucl. Res. **3:** 239–247

4 Murad F., Mittal C. K., Arnold W. P., Braughler J. M. (1978) Effect of nitrocompound smooth muscle relaxants and other materials on cyclic GMP metabolism. Proc. of the 7th International Congress of Pharmacology, Paris, France, July, 1978, in Advances in Pharmacology and Therapeutics, Vol. 3 Ions, 6 Cyclic Nucleotides, Cholinergy, Ed., J. C. Stocklet, Pergamon Press, New York, pp. 123–132

5 Katsuki S., Arnold W., Mittal C. K., Murad F. (1977) Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. J. Cyclic Nucl. Res. **3:** 23–35

6 Arnold W. P., Mittal C. K., Katsuki S., Murad F. (1977) Nitric oxide activates guanylate cyclase and increases guanosine 3′, 5′-monophosphate levels in various tissue preparations. Proc. Nat. Acad. Sci. USA **74:** 3203-3207

7 Murad F., Mittal C. K., Arnold W. P., Katsuki S., Kimura H. (1978) Guanylate cyclase: Activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin. Adv. Cyclic Nucl. Res. **9:** 145–158

8 Hughes J., Murad F., Chang B., Guerrant R. (1978) The role of cyclic GMP in the mechanism of action of the heat-stable enterotoxin of E. Coli. Nature **271:** 755–756

9 Waldman S. A., Rapoport R. M., Murad F. (1984) Atrial natriuretic factor selectively activates particulate guanylate cyclase and elevates cyclic GMP in rat tissues. J. Biol. Chem. **259:** 14332–14334

10 Kuno T., Andresen J. W., Kamisaki Y., Waldman S. A., Chang L. Y., Saheki S., Leitman D. C., Nakane M., Murad F. (1986) Co-purification of an atrial natriuretic factor receptor and particulate guanylate cyclase from rat lung. J. Biol. Chem. **261:** 5817–5823

11 Murad F. (1999) Discovery of some of the biological effects of nitric oxide and its role in cellular signaling. Nobel Lecture, Bioscience Reports **19**, 133–154, 1999; Les Prix Nobel, The Nobe/l Prizes, 1998, pp. 273–307