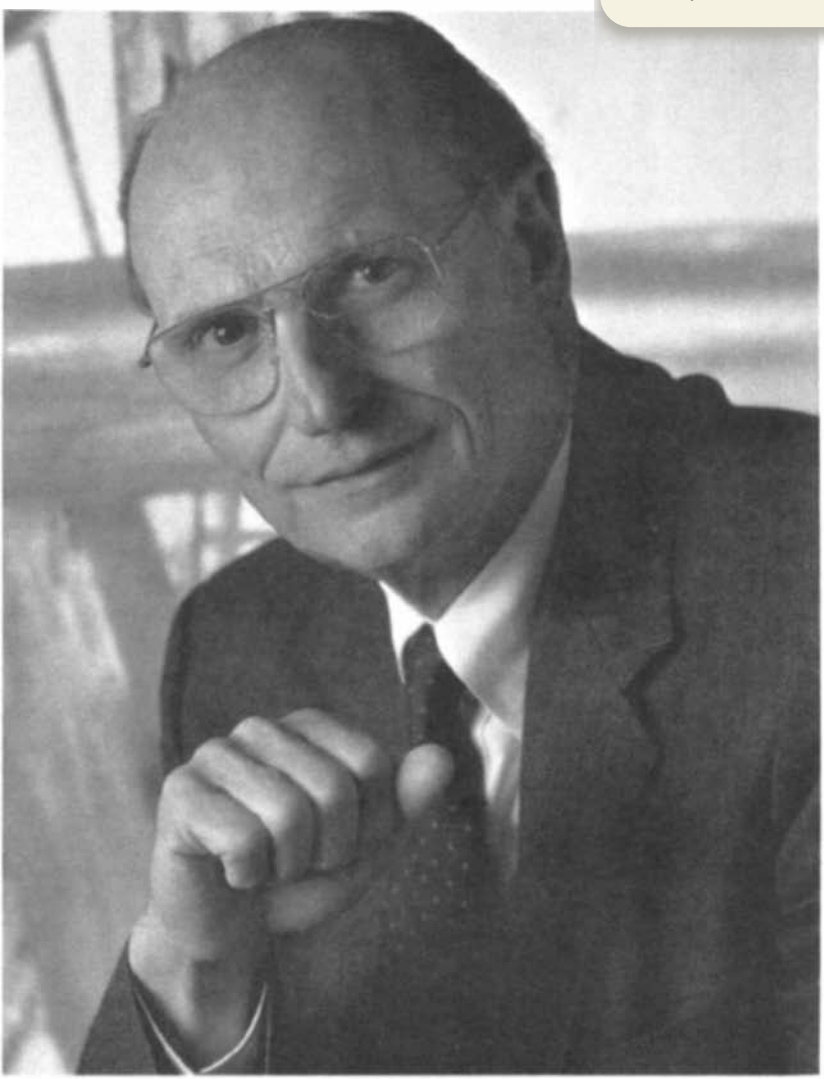

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FROM PEPTIDES TO PEPTIDASES: A Chronicle of Drug Discovery*

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INTRODUCTION

On one of his regular visits to the research laboratories of the Institute in Buenos Aires, Argentina, in the spring of 1960, the president of the Squibb Institute for Medical Research, Asger Langlykke, invited me and another member of the senior staff to continue our careers at the main laboratories in New Jersey. When I transferred later that summer I was assigned to the peptide synthesis group that had recently been set up under the leadership of Miklos Bodanszky. The idea of entering into a completely new research area in a group headed by an established investigator was most welcome, but I had some misgivings. Peptide synthesis has never been very popular among synthetic organic chemists, probably because of the repetitive nature of its synthetic procedures.

When I started working in Miklos' group, he was extremely generous not only with his advice concerning the techniques of peptide synthesis, but also in elaborating on his ideas about the future of peptides in pharmaceutical research. Since peptides are the natural messengers of the body, compounds of this type could be expected to lead to very specific drugs with few side effects. The large variety of structures possible with sequences as small as a tri- or tetrapeptide, and the significant changes in activity achievable by single amino-acid replacements in a small hormone, for example oxytocin or vasopressin, predicted that biological activities could be finely tuned. Despite the poor likelihood of obtaining oral activity with compounds bigger than a di- or tripeptide, and the short duration of action

*Dedicated to the memory of Emily F. Sabo (1926–1992), unique collaborator, unforgettable friend.

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after intravenous administration, the hope was always there that prolonged activity could be obtained by subcutaneous or intramuscular administration with a proper carrier, and a carrier found to provide oral activity.

The goal of peptide research groups at that time, particularly those in the pharmaceutical companies, was to locate an endogenous biologically active peptide and then, if the biological activity was desirable, to produce analogs with improved features, e.g. separation of activities if there was more than one, retention of full activity in a smaller sequence to increase the chances for oral absorption, prolongation of the duration of action by replacing critical amino acids, etc. If the biological activity was undesirable, the key strategy was to synthesize analogs that would act as antagonists. Some groups, including ours, were also studying the methodology of peptide synthesis, but the main target was always the synthesis of novel and biologically active peptides.

My first assignment was the synthesis of the nonapeptide bradykinin. After four months of intense efforts I obtained a small purified sample of the final product, which I took with great apprehension to the department of pharmacology for biological testing. It was then that I first met Bernard Rubin, my collaborator on and off for the next thirty years. A few days later Bernie told me that my sample was one of the most active compounds he had ever tested. Fractions of a nanogram per ml were sufficient to produce maximal contraction of the rat uterus, confirming that I had indeed synthesized a nonapeptide with the amino acid sequence of bradykinin. These results dispelled any misgivings I had about working on the synthesis of peptides; even though synthetic chemists might not find variety in the synthetic procedures to obtain peptides, I realized that biological receptors were exquisitively sensitive to the variety of structural features present in them.

Following this successful synthesis we attempted to prepare analogs with antagonistic activity as potential antiinflammatory agents. Unfortunately, all the analogs I synthesized, and many more synthesized by other research groups in industry and academia in years to come, were full agonists with various degrees of potency and exhibited no antagonistic activity. Over twenty years would elapse before John M. Stewart and collaborators synthesized the first true antagonist of bradykinin.

In my search for novel endogenous peptides in the early sixties, I tried to isolate calcitonin, a recently discovered peptide, from the parathyroid gland. Despite months of intensive effort in collaboration with a veterinarian at the Toxicology Department, I was unsuccessful in duplicating the published results. It was later disclosed that calcitonin is produced not in the parathyroid gland but in special cells of the thyroid gland!

SECRETIN

After a brief excursion into the synthesis of cyclic peptide antibiotics, I joined the group effort to synthesize gastrointestinal hormones. We were collaborating with the group working under J. Erik Jorpes and Viktor Mutt at the Karolinska Institute in Sweden. Under this arrangement we secured information on the structure of the pancreatic hormone secretin, a twenty-seven amino acids peptide amide that stimulates the pancreas to secrete bicarbonate and water. Miklos Bodanszky tackled the synthesis of secretin with the strategy that he had perfected of stepwise synthesis of peptides, starting with the amino acid at the carboxyl terminal of the peptide chain using p-nitrophenyl esters. I was charged with using the alternate procedure of condensing four peptide fragments. Each fragment was in turn synthetized stepwise from the carboxyl terminal amino acid.

The synthesis of a peptide with twenty-seven amino acids was a major undertaking in the mid-sixties, and the head of the organic chemistry department, Frank L. Weisenborn, established a special task force for the synthesis of secretin, providing us with the temporary help of chemists recruited from other programs. This decision had momentous repercussions for my future career: Emily F. Sabo was assigned to work directly with me for the duration of the task force. Emily had been associated with Joseph (Gus) Fried in the pioneering synthesis of 9-halo steroids, a milestone in steroid antiinflammatory therapy, but was then working in an unrelated area. She was happy to move into peptide synthesis and soon became as accomplished in this field as she had been in steroid chemistry. After the completion of the task-force effort, she requested to remain in the peptide group, and with her inexhaustible energy and superb experimental technique went on to make substantial contributions to our research.

Both synthetic approaches, the stepwise and the fragment condensation, led to fully active synthetic secretin (1, 2), confirming the sequence of the natural hormone proposed by Jorpes & Mutt. The biological assay for this hormone was cumbersome and we established a collaboration with the laboratory of Morton I. Grossman at the Veterans Administration Center in Los Angeles. He was interested not only in exploring the physiological aspects of pancreatic secretion, but also the potential clinical utility of this hormone. Since secretin alkalinized the duodenal contents by stimulating the secretion of bicarbonate from the pancreas, he suggested it could be considered "Nature's antacid," and urged the initiation of clinical studies in the treatment of duodenal ulcer with our synthetic secretin.

By this time, 1967, Miklos had left Squibb and I had become the group leader. Since the Chemical Development group had been unable to

scale up the synthesis of secretin beyond the tetradecapeptide stage by the stepwise procedure, Emily and I undertook to complete it in our own laboratory using the fragment condensation approach. Starting with crude carboxyl terminal tetradecapeptide obtained from the Chemical Development group, we obtained 10 grams of purified synthetic secretin, an enormous amount by the extraction procedures from hog intestines then current. Unfortunately, the preliminary results of the clinical studies with synthetic secretin were not encouraging enough to justify further development.

SINCALIDE

Simultaneously with their studies on secretin, Jorpes & Mutt were also involved in isolating and purifying another gastrointestinal hormone, cholecystokinin-pancreozymin. This hormone, which stimulates the contraction of the gall-bladder and the secretion of enzymes from the pancreas, is thirty-three amino acids long. From their enzymatic degradation studies, Jorpes & Mutt had evidence that carboxylterminal (C-terminal) fragments of this hormone contained substantial biological activity, unlike secretin. These C-terminal fragments, particularly the C-terminal octapeptide, are very similar to the C-terminal sequence of the gastric hormone gastrin. Jorpes & Mutt were sure about the amino acid sequence of the two enzymatic fragments, the C-terminal octa and dodecapeptides, but were uncertain as to the nature of the acidic substituent present on the tyrosine residue of these sequences, even though it was probably a sulfate residue as it is in one form of gastrin. We were well into the synthesis of these C-terminal fragments when a publication appeared (3) describing the isolation from the skin of an amphibian of a decapeptide, named caerulein, with an amino acid sequence similar to the C-terminal sequence of cholecystokinin. Caerulein has biological properties similar to the mammalian hormone, and studies with synthetic analogs had shown that full biological activity can only be achieved when the tyrosine residue present in its sequence is sulfated. With Emily we quickly completed the synthesis of the C-terminal-sulfated octa- and dodecapeptides with the amino acid sequences proposed by Jorpes & Mutt. The synthetic samples were shown to have the same biological activity and physicochemical properties of the natural fragments (4), and, like caerulein, required sulfation of the tyrosine residue for biological activity. The potency of the synthetic C-terminal octapeptide was almost 5 to 8 times that of the full hormone molecule!

Since the crude porcine cholecystokinin was already being used in diagnostic cholecystography, our medical group supported the development of the synthetic octapeptide as a diagnostic agent for the same purpose.

The large-scale synthesis of this peptide was carried out by the Department of Chemical Development, while the peptide synthesis research group was synthesizing analogs and derivatives to clarify the structure activity relationships, SAR, of this potent peptide. To support our synthetic work we again established a close collaboration with Bernie Rubin who had developed an *in situ* guinea pig gall-bladder assay that became essential not only for our research, but also for developing a suitable injectable formulation of the final product. This octapeptide was introduced into clinical studies under the generic name of sincalide, and eventually marketed under the trademark Kinevac®.

Our publications on synthetic secretin and cholecystokinin prompted considerable interest among gastrointestinal researchers, and we distributed a considerable amount of material to outside investigators, particularly in the case of the C-terminal octapeptide of cholecystokinin. Generally we did not become directly involved in those investigations. When enough evidence was accumulated for a potential role of cholecystokinin in appetite suppression, our medical department organized a clinical trial, with encouraging results. However, the need for parenteral administration discouraged pursuance of this application. A major goal in cholecystokinin research was the development of a radioimmunoassay using our synthetic octapeptide to prepare conjugated antigens and radiolabeled ligands. Despite great synthetic difficulties, several groups finally developed workable radioimmunoassays that were crucial to the discovery that the C-terminal octapeptide of cholecystokinin is an endogeneous neurotransmitter. After this unexpected finding, interest mounted in our synthetic octapeptide and remained high for over twenty years after we ourselves left this field of research.

TEPROTIDE

In 1967, Arnold D. Welch, chairman of the Department of Pharmacology at Yale, became president of The Squibb Institute and initiated a complete overhaul of research goals. Our research on peptides was considered worthy of continuation, but not neccessarily in the gastrointestinal field. Our successes in this area notwithstanding, company interest in the gastrointestinal field had dwindled. The problem of lack of oral activity and short duration of action of peptide after systemic administration had changed little from the early sixties, despite intensive experimentation with hormone analogs containing unnatural amino acids to provide resistance to peptidase attack. Personally, I had become interested in directly blocking the action of those peptidases, following the approach of active sited-directed irreversible inhibitors, but did not know to what particular peptidase to apply the concept. Biochemists had developed these tools to block the action of very

general peptidases such as trypsin and chymotrypsin, which did not seem to be suitable therapeutic targets at that time.

Such was my frame of mind when, in early 1968, I was invited by the head of the Pharmacology Department, Zola Horovitz, to attend a meeting with their consultant, John Vane, to discuss recent results from his laboratory at the Royal College of Surgeons. These results indicated that peptides isolated in the crude extract of the venom of *Bothrops jararaca* could block the conversion of angiotensin I to angiotensin II and also the inactivation of bradykinin. Ferreira, Greene & Stewart had already isolated a pentapeptide from the same extracts, which they had synthesized and designated as BPP5a, and which had both biological activities. Other peptides were known to be present but their structure and biological properties were unknown. It was then suggested that our group should collaborate with David Cushman, a biochemist recently incorporated into the Department of Pharmacology, in the isolation, characterization, and eventual synthesis of those other peptidic inhibitors present in this venom.

This project attracted me for several reasons: it involved "natural product" research, an area in which I had been thoroughly schooled during my years of research with Venancio Deulofeu in Argentina; it gave me the chance to investigate a protease inhibitor in line with the company's therapeutic interest; and, finally, given the important contributions in the renin angiotensin field in made Argentina, it was an appropriate research endeavor for me emotionally. However, one important drawback was that we were in competition with a group with extensive experience in protein sequencing, whereas our expertise was in peptide synthesis.

We started with great enthusiasm at both ends of the project: David Cushman developing an assay for enzymatic activity, and Nina Williams and myself isolating active fractions from the crude extract of venom that we obtained in large quantities from the serpentarium at Butantan in Sao Paulo (Brazil). Nina had collaborated with Miklos Bodanszky in peptide synthesis but had to learn all the techniques of peptide isolation and sequencing from scratch, and she did so with zest. To facilitate learning, we first zeroed in on the purification of the inhibitory fraction present in the largest amount, which was an undecapeptide, even though it was not the most active. With this pure material in hand, we experimented with chemical and enzymatic degradation procedures, and finally put together a putative amino acid sequence. Emily synthesized the proposed peptide by the solid phase technique and confirmed its structure. Since the amino acid compositions of all inhibitory fractions were similar, knowing the amino acid sequence of this first one allowed us to proceed rapidly to clarify the sequence of the others, which Emily then synthesized to provide confirmatory

evidence. When we presented the results at the Second American Peptide Symposium in Cleveland, we had clearly beaten the competition!

As soon as synthetic material was available, pharmacological investigation began in earnest in Bernie Rubin's group. Isolated smooth muscle assays were used to measure antagonism of the contractile activity of angiotensin I and potentiation of the contractile activity of bradykinin. The high correlation of the two activities in the different inhibitory peptides convinced us that separating activities was not a reasonable goal. This conviction was strengthened by the finding by Erdös that angiotensin converting enzyme and kininase II are the same enzyme. Bernie's group also developed procedures to measure the in-vivo blockade of the pressor activity of angiotensin I, and the blood pressure-lowering effect on the two-kidney-one-clip model of hypertension in rats. These in vivo studies led us to conclude that the most effective inhibitor was the nonapeptide, later designated generically as teprotide. In spite of the peptidic nature of these inhibitors, their duration of action was considerable, up to several hours, but there was no indication of oral activity.

Publications on the chemistry (5) and pharmacology (6, 7) of this new type of blockers of the renin angiotensin system aroused widespread interest and strengthened support for clinical trials, in spite of the lack of oral activity. The first phase involved determining the dose ranges needed to inhibit the pressor effect of angiotensin I in normal volunteers, i.e. demonstrating that these inhibitors could block the renin angiotensin system in humans. The results indicated that small doses produced almost complete blockade and that the half time of recovery was several hours. The next phase of clinical studies involved hypertensive subjects who were generally selected among patients with clear indications of activation of the renin angiotensin system. Intravenous administration of the drug limited the scope of these studies.

While clinical studies were underway, we began to study the relationship between structure and activity in this new class of peptidic inhibitors to determine the minimal peptidic sequence with maximum inhibitory activity; this turned out to be the tripeptide Phe-Ala-Pro and its acylated derivatives. Unfortunately, this minimal inhibitory sequence was also a substrate for the converting enzyme and for other peptidases of the chymotrypsin family. We tried unsuccessfully to modify the labile peptide bond Phe-Ala to stabilize it towards enzymatic degradation while maintaining inhibitory activity. Years later, after our publications on captopril, Almquist and collaborators at the Stanford Research Institute synthesized very potent inhibitors by using this approach and replacing the peptide bond between phenylalanine and alanine (glycine in their compound) with a ketone bond. In spite of their enzymatic

stability, these and similar compounds synthesized later in our laboratories turned out to have poor oral bioavailability.

The failure of these approaches prompted the suggestion that we test compounds randomly selected from our files of chemicals prepared in other research programs. David Cushman transferred the enzymatic assay to an outside contracting lab, and in collaboration with Bernie Rubin, retested all the compounds found to be active in this preliminary screening. Although some compounds that were structurally completely unrelated to peptides had some inhibitory activity in the isolated enzyme assay, none showed the "classical" pattern defined for teprotide in the isolated guinea pig ileum assay developed by Bernie. Only one compound in this assay, with the structural features of a zinc chelator, behaved as a true ACE inhibitor, e.g. blocked the contractile activity of angiotensin I and potentiated the contractile activity of bradykinin.

Clinical studies with teprotide continued meanwhile, but in a low key. The lack of oral activity only justified marketing of this agent for hypertensive emergencies, or, as proposed by John Laragh, as a diagnostic agent to determine involvement of the renin angiotensin system in essential hypertensive patients. Neither alternative was considered of sufficient commercial importance to justify the costs of clinical development. Some of these preliminary clinical studies had a significant influence on the future development of ACE inhibitors since they showed beneficial cardiodynamic effects in heart failure, and lowering of blood pressure in hypertensive patients with normal plasma renin levels.

CAPTOPRIL

In 1973, the peptide synthesis group, already reduced to a minimal strength of two Ph.Ds and three B.S.s, was subsumed into the new antibiotics group under my supervision. My responsibilities were subsequently widened to include synthetic antibiotics and topical steroids, groups that were directly supervised by Cristopher M. Cimarusti. This decision was purely pragmatic; researchers were needed in other areas, and the likelihood of finding an orally active cardiovascular, antiinflammatory or antiinfective agent in the peptide field was minimal. Our experience with natural products, and the fact that some of the new antibiotics then under investigation were cyclic peptides justified merging the peptide group into the new antibiotics group. David Cushman and Bernie Rubin were reassigned to other programs, and the ACE inhibitors project was formally discontinued.

In March 1974, I was totally involved in supervising research in antibiotics and steroids when I received through Zola Horovitz a note that had been

sent to him by David Cushman. Zola Horovitz was then Associate Director in charge of all the biological research, and David and Bernie were part of his division. David had received through a publication-alerting service a card with a reference to a paper by Byers & Wolfenden on carboxypeptidase inhibitors (8). The card showed the structure of benzyl succinic acid and indicated that it was a very potent inhibitor of carboxypeptidase A (Ki: 0.093 μg/ml). David added the comment that this compound, or one similar, might be an inhibitor of ACE, and sent it to Zola who forwarded it to me. David and I agreed to meet in my office to discuss the paper. Soon thereafter I told Zola that David and I decided that Emily should make two compounds to test: succinyl-L-proline and fumaryl-L-proline.

Our interest in the Byers & Wolfenden paper stemmed from the fact that the biochemical studies conducted by the discoverer of ACE, Skeggs, and by David and his coworker, Hong Son Cheung, had clearly shown that ACE was an exopeptidase that cleaved dipeptides from the carboxyl terminal of a peptide chain. Like carboxypeptidase A, which also cleaves aromatic amino acids from the carboxyl terminal of peptides, ACE contains a zinc atom at the active site. Given this analogy, the concept put forward by Byers & Wolfenden that benzyl succinic acid was a potent inhibitor because it combined the features of the two products of the enzymatic action (bi-product analog), could, in our view, be extended *mutatis mutandi* to ACE. Using this idea and what we had learned from our studies on peptidic inhibitors of ACE, we proposed that the compound most likely to be an inhibitor of ACE would be α-methyl succinyl-L-proline. For the earliest possible test of the hypothesis we decided to make the simplest prototype: succinyl-L-proline.

Emily quickly synthesized this proline derivative but could not obtain it in crystalline form and had to characterize it as a dicyclohexylammonium salt. The enzymatic inhibition assay gave an I_{50} of 135 μg/ml, a disappointing result considering the 0.093 μg/ml of benzyl succinic acid against carboxypeptidase A and even compared to the 1 μg/ml of teprotide against ACE. Bernie tested a sample of the salt in his isolated smooth muscle screen but the dicyclohexylamine interfered with the test. We then provided him with a sample of the oily free acid and the result raised our hopes. Succinyl-L-proline was indeed a poor antagonist of the contractile effect of angiotensin I and had no action against the contractile activity of either angiotensin II or acetylcholine. But . . . it potentiated the contractile activity of bradykinin! A long road lay ahead of us to reach the level of activity of teprotide, but we firmly believe we were on the right track to a new class of inhibitors.

In the following months I felt completely justified in having Emily spend all her time on the synthesis of modifications of succinyl proline, even

though we were both assigned to antiinfective agents. From those efforts the only compound worthy of mention was α-D-methyl succinyl-L-proline. This was the real target of our design since it was the full analog of L-alanyl-L-proline one of the products of the enzymatic hydrolysis of ACE on the very efficient substrate Benzoyul-Phe-Ala-Pro. After considerable effort, Emily obtained a purified sample of the desired isomer, portions of which we sent to David and Bernie for testing. Both the isolated enzyme and the smooth muscle assays showed it to be ten times more potent than the prototype succinyl proline and still preserved the specificity of being an antagonist of angiotensin I and a potentiator of bradykinin on the isolated guinea pig ileum. These results were in full agreement with the prediction of our hypothetical model. If we once thought that we were on the right path, now we knew it!

When chemists and pharmacologists collaborate, typically the pharmacologists want larger amounts of the most active compound to confirm and expand the evidence for biological activity, while the chemists want to make new and better analogs before the scale-up. Fortunately, Emily came to the rescue; she convinced me that she could do both simultaneously, and so Bernie got his gram while we continued to make samples of new analogs. Bernie quickly showed that α-D-methylsuccinyl-L-proline blocks the angiotensin I pressor effect in the rat when given i.v., and by using very large doses could even see oral activity. At this point David, Bernie and I felt that we should "go public", i.e. make a presentation before the Internal Medicine Area Team coordinating the research efforts on cardiovascular drugs. We convinced them that we were on the right track and gained support for a small increase in the number of researchers. It so happened that Emily and I reached the end of this particular road without any additional help.

To clarify why our subsequent efforts developed the way they did, I should come back to our reading of the Byers & Wolfenden paper. From the very beginning David and I felt that instead of postulating a bi-product analog concept, the high inhibitory activity of benzyl-succinic acid would be simpler to explain if one of the carboxyls was assumed to bind to the enzyme as the C-terminal carboxyl of the substrate and the other to the zinc atom present at the active site. Byers & Wolfenden had also contemplated this hypothesis but dismissed it as unlikely. In the earlier stages of the design of ACE inhibitors it mattered little whether we followed one interpretation or the other, but the further we proceeded into the modification of succinyl-L-proline, the more constrained we felt by the interpretation of these inhibitors as biproduct analogs. I began to design and have Emily synthesize compounds in which the carboxylic group of the succinyl moiety was replaced by other groups that could also perform as ligands to the zinc.

The enzymatic and tissue assays of some of these new prototypes were encouraging, but made no significant breakthrough.

As soon as we started to explore non-carboxyl zinc ligands, the possibility of using a sulfhydryl group was high on my list of priorities. However, I hesitated to start Emily on this task because my previous experience with cysteine-containing peptides relied on protecting groups that were cumbersome to apply and to remove. One day, however, I received a bulletin from a biochemical products company offering a new reagent, propiothiolactone, to introduce sulfhydryl groups on proteins. Upon reaction with the ϵ-amino groups of lysine moieties this reagent forms 3-mercaptopropionyl derivatives of the starting protein. Since our target compound as a prototype of a new class of ACE inhibitors was 3-mercaptopropanoyl-L-proline, this reagent offered a way for us to prepare it. Using an ester of L-proline and this new reagent, Emily obtained first an ester of 3-mercaptopropanoyl-L-proline, and finally the desired free acid, which we submitted to David and Bernie for testing as a preweighed oily residue. When they brought us the results we realized that here was the breakthrough we were waiting for. It was three thousand times more potent than our first prototype succinyl-L-proline, and as active as teprotide on the enzymatic and smooth muscle assays, and with the same pattern of specificity in the latter. Soon thereafter Bernie showed that 3-mercaptopropanoyl-L-proline blocked the pressor effect of angiotensin I in the rat by the intravenous and the oral routes, with similar doses by either route, indicating a fairly good oral absorption.

Once again Emily rushed to prepare a large amount of this prototype for indepth pharmacological evaluation and to synthesize the appropriate isomer of the corresponding α-methyl analog, which she eventually managed to do after devising a procedure for fractionating the mixture of diastereomers. This compound would later receive the generic name of captopril. In smooth muscle assays in vitro it was ten times more active than teprotide, and in vivo, almost as efficacious when given orally as when given intravenously, with considerably longer duration of action by the former route. The pharmacological profile of captopril was so outstanding that from the very beginning it was designated as the clinical candidate. By 1976, the first clinical trial for inhibition of the pressor effect of angiotensin I took place in normal volunteers in Lausanne, Switzerland. Oral doses of 1 to 10 mg of captopril were shown to block the pressor effect of angiotensin I and very significant levels of blockade were observed for several hours at the higher doses.

What came after has been described as "The Saga of Captopril," perhaps because, as in all sagas, there were moments of both tremendous exultation and profound despair. George B. Mackaness, the director of the Trudeau Institute, became the new president of The Squibb Institute in 1976, and

in association with Zola P. Horovitz, Patrick A. Diassi, G. Richard Keim, and James R. Knill guided the development efforts on captopril during those difficult years. We were first in the clinical exploration of this novel type of agent and learned almost everything by trial and error. The studies with teprotide provided little help since they had been restricted to a very small number of patients and intravenous administration had served to limit the dose. The rapid escalation of the oral dose and the extensive use of captopril in all types of complicated hypertension, which characterized the early stages of captopril clinical development, led to clear indications of efficacy but also to a significant number of side effects. It took several years of extensive clinical experience in uncomplicated hypertensive patients to demonstrate that low doses of captopril were safe and efficacious in controlling blood pressure in 50–60% of patients and that the addition of diuretics and not increased dosage was the procedure to attain blood pressure control in more than 90% of all hypertensive patients.

Many clinical observations made during the early stages of clinical research, cardiovascular or otherwise, that could not be explained by the then current understanding of the function of the renin angiotensin system, were attributed to nonspecific effects of the sulfhydryl group present in captopril. Later experience derived from animal experimentation and the availability of ACE inhibitors of different structure demonstrated that most of the effects of captopril on the renal and cardiovascular systems are due to its inhibition of ACE. Some noncardiovascular side effects observed in a small number of patients (e.g. rashes) are probably due to the thiol nature of captopril, but others (cough, angioneurotic edema) are common to all ACE inhibitors. Differences between captopril and the so-called non-sulfhydryl inhibitors have subsequently been reported in the literature, but none has been documented well enough to ascertain their molecular mechanism.

In April 1981, captopril was approved for the treatment of hypertensive patients who had not responded satisfactorily to multidrug therapy, and in October 1982, for the treatment of heart failure. Approval for the treatment of all degrees of hypertension was granted in July 1985. In April 1991, the results of the SAVE (Survival And Ventricular Enlargement) trial demonstrated that initiation of captopril therapy in the early days following a heart attack can significantly improve long-term survival and reduce the chances of the patient developing heart failure or suffering a recurrent heart attack.

AFTER CAPTOPRIL

For those of us not involved in the clinical studies the synthesis of captopril marked the beginning of an extensive and multifaceted research effort that

was to last approximately ten years, and involve many scientists. On the pharmacological front, Michael J. Antonaccio, who was to head the Department of Pharmacology soon after the synthesis of captopril, in collaboration with Bernie Rubin and a number of eager and able pharmacologists, explored the full profile of pharmacological activities of captopril and its successors. As soon as the first disclosures on captopril were announced on April 1977 (9), a continuous flow of requests for samples began and as a consequence, pharmacological studies of captopril in both academic and industrial laboratories escalated. These studies helped to elucidate the functioning of the renin angiotensin system under normal and pathological conditions. By 1988 the list of titles of all papers published on captopril was three hundred pages long!

The chemistry and biochemistry of ACE inhibitors also received widespread attention in the years after captopril. After seeing the extraordinary increase in activity obtained in going from the succinyl to the mercaptopropanoyl derivatives, we asked ourselves whether we were dealing with a truly specific enzyme inhibitor or a very potent metal chelator. David showed that after inhibition enzymatic activity could be fully recovered by dializing away the inhibitor, evidently without removal of the zinc. He also showed that captopril is not an inhibitor of other zinc-containing proteases. Emily and I, now with the collaboration of several eager medicinal chemists, dissected the captopril molecule and showed that practically every functional group with the appropriate stereochemistry was needed to obtain the optimal inhibitory activity displayed by captopril. These results indicated that multiple interactions were established between captopril and the active site of ACE (10, 11).

Following the approach that had guided us in the design of captopril, we synthesized sulfhydryl-containing inhibitors of carboxypeptidase A and B (12). Those compounds not only showed very potent inhibitory activity against their respective enzyme, but were inactive for the other enzyme and also against ACE. Other groups have subsequentgly synthesized potent and specific sulfhydryl-containing inhibitors against other metalopeptidases, e.g. collagenase, thermolysin, neutral endopeptidase, and aminopeptidase. Using a sulfhydryl-containing inhibitor designed specifically for thermolysin, Matthews and his collaborators at the University of Oregon demonstrated that crystals of the enzyme-inhibitor complex showed the sulfur atom coordinated with the zinc atom at the active site, confirming our original assumption (13). The same group showed that in the complex with a carboxyl-containing inhibitor, the carboxylate group also binds to the active site with the carboxyl group acting as a ligand of the zinc. These studies proved that the process of inhibitor design that we had developed for ACE inhibitors could be extended to many other metallo enzymes.

After the preliminary reports of the clinical efficacy of captopril, a frantic effort began in our own chemistry department and in those of practically all other pharmaceutical companies to prepare new and patentable analogs. The goal was to obtain ACE inhibitors with potency similar to captopril and no sulfhydryl group, or compounds with longer duration of action, or both. In our laboratories these studies were characterized as the "search for the son of captopril." This title should more appropriately be given to the analog of captopril prepared by John Krapcho, which eventually received the generic name of zofenopril (14, 15). Extensive pharmacological and clinical studies were conducted with this new ACE inhibitor that showed very good antihypertensive and antiischemic activities, but its clinical development was eventually halted. The second ACE inhibitor introduced by our company into clinical practice and eventually marketed, was a completely novel chemical entity, fosinopril, developed by Edward W. Petrillo Jr. and his collaborators (16). The design of this compound followed a process similar to that used for captopril although its zinc-binding moiety is neither a carboxyl nor a sulfydryl group but rather a phosphinic acid group. To achieve good oral absorption a new type of ester of this acidic group had to be developed.

In June 1980, Patchett presented the results of Merck scientists efforts in the design of new ACE inhibitors at the national meeting of medicinal chemistry in Troy, New York (17). Two compounds were disclosed that were eventually marketed for the treatment of hypertension and heart failure, enalapril and lysinopril. The starting point for their studies was again the paper of Byers & Wolfenden, supplemented by the disclosures on captopril and analogs that we had made from 1977 on. Their goal had clearly been to avoid compounds containing a sulfhydryl group, and they had succeeded by manipulating the succinyl group of the prototype succinyl proline to obtain compounds that retain the carboxyl group as a zinc ligand and have intrinsic activity equal or superior to that of captopril. After their work most other pharmaceutical companies began to develop modifications of these two agents, some of which were eventually introduced into the market.

CODA

Even from the perspective of the 1990s, the answer to our question in the early 1960s of whether peptides can be made into orally active drugs, is still the same: not easily. Our experience with captopril indicates that success is more likely if we change the focus from peptides to the agents that control peptide metabolism, e.g. peptidases. Peptidase inhibition can not only antagonize the action of a peptide but can also produce an agonistic activity by blocking degradatation and thereby increasing the endogeneous

levels of a peptide, e.g. bradykinin. However, this approach through peptidase inhibition may not always be feasible, and in these cases the problem still remains. More sophisticated approaches to natural product screening in the past few years have provided compounds that can interact with peptide receptors without being peptides. This goal has also been achieved by pure synthetic procedures starting from a lead obtained by screening of chemical libraries (e.g. angiotensin antagonists). We are always assuming that nonpeptidic compounds can be made orally absorbable by appropriate chemical modification.

These screening approaches appear less likely to succeed if what is needed is an agonist of a peptide receptor. Recent advances of molecular biology techniques have provided a wealth of peptides and small proteins of great potential significance in medicine as agonists on their respective receptors. In many cases these peptides or proteins are being used clinically, but by the parenteral route. Approaches to the packaging of peptides and small proteins for oral absorption have been extensively described, but none has reached clinical practice. Intranasal administration of peptides was indeed introduced into clinical practice some years ago, but this route has limitations.

By 1981, my administrative responsibilities had increased to the point where it became impractical for me to maintain a day-to-day laboratory collaboration with Emily, and she continued her research in association with other senior members of the department of organic chemistry. I still participated actively in medicinal chemistry research, and eventually, as vice president for cardiovascular research, supervised the functioning of the chemical and biological sides of drug discovery in that area. Again and again I have come to appreciate that there is no successful research project in drug discovery that does not involve the close collaboration of biologists and chemists. We often try to enforce this collaboration through the specifics of administrative organizations, but it has always remained a matter of personal chemistry. I am firmly convinced that the formula for successful research is: listening, thinking, and doing. But listening not only means attending to experimental results, but also to the interpretation of those results by other people. The open dialogue between researchers from different disciplines is a key component of the drug-discovery process. Frequently, this dialogue has to be expanded to reach academic laboratories to reap richer rewards. The close interaction between industrial and academic research is probably one of the main driving forces behind the stupendous contributions of the pharmaceutical industry to the treatment of human diseases during the past fifty years.

The challenges of managing a complicated research effort in drug discovery, with its intricate mixture of personal relations and science, can

indeed be very rewarding. Nevertheless, I always felt that these rewards do not compare with the excitement to be derived from bench research, particularly when one has the good fortune I had of being associated with bright and enthusiastic investigators.

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