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NEUROHYPOPHYSIAL PEPTIDES IN MEDICINE FROM PRAGUE AND SWEDISH LABORATORIES. PART I: HISTORY OF RESEARCH AND EARLY PRODUCTION OF DRUG FORMS

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Introductory comment

From the 1950s to the 1970s, the peptide hormones from the neurohypophysis, vasopressin and oxytocin, attracted the attention of many research groups worldwide. They were one of the salient research programmes at the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences (hereafter referred to as the IOCB), and the subject of interest of a substantial part of the scientific, clinical and pharmaceutical research in the then Czechoslovakia. Newly developed chemical, biophysical and pharmacological techniques enabled numerous laboratories in many other countries to investigate the relationships between the structure of the peptide molecules and their biological activities. Prague's contribution to this research is substantially linked to the IOCB and especially to the names of František Šorm and Josef Rudinger[1].

Historical note: Neurohypophysis, oxytocin and vasopressin: the first half of the 20th century

Clinical interest in neurohypophysial peptides is strikingly old, having aroused interest of clinicians as early as in the beginning of the 20th century. Until the 1950s, however, they were, like other hormonal preparations, available only in the form of extracts from biological materials, mostly from porcine and bovine pituitary glands obtained from slaughtered animals.

The uterotonic effects of pituitary extracts were first described in 1909 by H. H. Dale[2]. Their use in obstetrics, initially for the treatment of postpartum haemorrhage in cases of hypotonia/atony of the uterine myometrium or after caesarean section[3,4] and later to enhance uterine retraction for placental delivery[5], followed soon after the publication of Dale's communication in the Biochemical Journal. Clinically applied by way of parenteral administration were slightly acidic aqueous extracts of bovine neurohypophysis, produced by pharmaceutical companies under various commercial names – Pitocin, Pituitrin (Parke Davis & Co), Pituglandel (Hoffmann-La Roche), Infundibulin. Their standardization was defined by the United States Pharmacopoeia (USP): it allowed 16% of total contamination, the preparations should contain 10 international units of oxytocin[a], admitted was a low percentage of preservatives (0.5% of chlorobutanol).

Since the second decade of the 20th century, pituitary extracts also found occasional use in nephrology and angiology. The association of the neurohypophysis with *diabetes insipidus* was known from clinical experience[6,b]; the treatment of associated polyuria with pituitary extracts was first reported in 1913 by F. Farini[7] and R. von den Velden[8]. Later applications of these extracts in variceal bleeding as haemostatics, in anti-shock therapy and in local anaesthesia are cited in a review by K. Saameli[9].
However, therapeutics prepared by extraction of animal neurohypophyses contained not only peptide hormones but also many other components – potential allergens or pyrogenic substances – and it is surprising that adverse effects during parenteral administration are rarely found in the literature (see e.g.[8]). Nevertheless, these forms of peptide

* † 15.7.2021. For the obituary of Dr. Flegel see Drašar P.: Chem. Listy*115*, 575 (2021).

[a] The International Unit (IU) of oxytocin is redefined by as the uterotonic activity of 1.68 μg of the international standard of synthetic oxytocin NIBSC (*National Institute for Biological Standards and Control*). In conversion: 1 mg of the (new) international standard (ca. 10^{-6}mol of oxytocin) = 595 IU. The old standard (lyophilised bovine neurohypophysis) stated 500 IU. (https://www.nibsc.org/documents/ifu/76-575.pdf)

[b] The clinical cases described in the communications of the time were very probably neurogenic (central) forms of *diabetes insipidus* (*d.i. centralis*). The renal form (*d.i. renalis*), which is independent of plasma vasopressin concentration, is usually a consequence of mutation of the vasopressin receptor V_2R gene or its signalling pathway.

pharmaceuticals persisted as successful drugs at least until the mid-1950s[c].

Oxytocin, vasopressin and their analogues: research at the IOCB

A major breakthrough in the availability of pure peptide drug preparations occurred after the publication of the first synthesis of oxytocin[10,11] and vasopressin[12] in Vincent du Vigneaud's laboratory at Cornell University in New York. Almost simultaneously, chemists at the Basel-based Sandoz AG[13] and Josef Rudinger's laboratory in Prague[14] proposed alternative methods for synthesizing oxytocin; diagrams of then existing syntheses were summarized in the late 1960s by Boissonas and Guttmann[15]. Thus, by the end of the 1950s, secure methods of peptide synthesis "in solution" were available[d]. Corresponding pharmacological assays and physiological experimental procedures were also improved and adapted to test their biological activities. This completed the first stage of their research, primarily aimed at elucidating the dependence between the structure of the peptide chain and the spectrum of biological activities of hormones. The outcome were analogues of neurohypophysial hormones changed in individual chain positions, frequently with remarkable pharmacological properties. Moreover, from the phylogenetic point of view, it was of interest that neurohypophysial hormones originally identified in mammals occur in the various animal classes as their structural analogues. During the first half of the 1960s, the question arose as to which of these compounds might be clinically useful; a number of the analogues suggested such a possibility.

The clinical use of a new drug is indeed always connected with the question of its large-scale synthesis, the upscaling, which is a critical operational condition the production of pharmaceuticals. The laboratory synthesis procedures used in the 1950s and 1960s were demanding for peptide manufacturing and, moreover, uneconomical: on data in the older literature[10,14] the yields of products in each step of oxytocin synthesis are 30–85%; losses in a 9-step synthesis, relative to the starting products of the first step, could in average be estimated as high as 99% or more. However, the nowadays used solid-phase synthesis of shorter peptides on different supports combined with their purification by high-performance liquid chromatography (HPLC) has made the production considerably faster, simpler and more efficient.

Nevertheless, the first pharmaceutic forms of synthetic oxytocin were distributed by several European pharmaceutical companies already by the late 1950s. They contained oxytocin produced on an extended laboratory scale, often obviously in cooperation between these companies and research laboratories in various countries, e.g., as Oxytocin-Spofa (Prague), Syntocinon Sandoz (Basel), Oxytocin Ferring (Malmö, Kiel), and in the early 1960s Pituisan by Sanabo GmbH Wien[e].

The situation was even less favourable for the production of oxytocin analogues, despite the fact that some of them were considered very promising for clinical use. Their realistic therapeutic chances were, however, hard to judge in advance. Pharmacological tests on isolated tissues and on *in vivo* animals are usually dependent on extrinsic (ionic composition of the surrounding medium in the pharmacological text, pH, etc.) and intrinsic factors of the test system (hormonal status of the tissue concerned, etc.); this does not allow any reliable conclusions concerning their effects in clinical practice: solely clinical tests are decisive. This makes the introduction of a new drug substance and the outline of its dosage instructions a very long and complicated process. Only companies working within a 'Good Manufacturing Practice' system (GMP) can achieve it nowadays.

Before the end of the 20th century, pharmaceutical companies working according to the GMP rules were rare in Czechoslovakia. This may also explain the fact that only one new peptide uterotonic, Methyloxytocin Spofa, was produced there in gram quantities, although the Prague laboratories, especially at the IOCB, were among the world leaders in peptide design and synthesis. It was already known from earlier pharmacological studies that the

[c] A similar situation occurred in the first half of the 20th century with most, if not all, hormone preparations isolated by conventional biochemical techniques from animal tissues. The risk of dangerous consequences was considerable, with the threat that the development of an effective drug would be delayed for years. Perhaps the most dramatic situation arose in the case of insulin, as the first patient (14-year-old boy) with an unfavourable prognosis of diabetes (types I, II not yet identified) received in a critical situation an intravenous injection of a still imperfectly purified pancreatic extract prepared by F. Bantig and C. Best in December 1921. The attending physician at the Toronto General Hospital, Dr. W. Campbell, described it as "a thick brown muck". However, and fortunately, the hypoglycaemic effect was unexpectedly strong, no serious adverse reaction occurred, and therefore further research on insulin was not blocked (see Michael Bliss, "The Discovery of Insulin", The University of Chicago Press, 1982, p. 112).

[d] It is noteworthy that oxytocin manufactured – according to the procedure developed at the IOCB – by the Prague pharmaceutical company SPOFA n.p., obviously containing traces of diethyl ether after extraction of the residues of the protecting groups, was still in use for parenteral application in obstetrics in the 1970s.

[e] *Peptidlaboratorium der Firma Sanabo GmbH Wien XII*. Data of this company are missing. An associate of Professor Hans Tuppy, Hans Nesvadba, formerly of the *Organisch-chemischen Institut der Universität Wien,* was active in the company in a prominent position. According to a verbal communication to one of the authors of this text, the company was taken over by Sandoz AG (V. P., communication by B. Berde, Sandoz AG, Basel, 1968).

protection of the hydroxyl by an alkyl group on the aromatic ring of tyrosine at position 2 leads to a reduction in uterotonic activity while maintaining a certain degree of affinity for the oxytocin receptor, i.e., to an effect denoted as a shift from agonism to antagonism[16,17]. In general, older data on 2-*O*-methyltyrosine-oxytocin activity are rather unclear; more precise data in the agonist-acting peptide region report 0.05–0.1% oxytocin activity, zero (only antagonistic) vasopressor activity in the rat and 1.8% in the chicken. Published data on clinical properties (Spofa) are not very convincing in this respect[18]. Interesting from a clinical point of view, although not fully explicable physiologically, is the reported decrease of lactacidemia in prolonged labours. In deliveries in which uterine contractions were reinforced by oxytocin, a decrease of plasmatic lactic acid was not observed: the authors from the Institute for Mother and Child in Prague-Podolí[19] report lactate levels about 20 times lower when oxytocin is replaced by methyloxytocin. Anyway, this pharmaceutical did not find a widespread use in gynaecological and obstetric practice and was of rather local importance.

More interesting for clinical practice were analogues of vasopressin (mainly 8-lysine-vasopressin) synthesized in the laboratories of IOCB. These were mainly analogues of the "hormonogen" type[20,21] with prolonged actions, of which [$(Gly)_3$-Cys^1,Lys^8]vasopressin was later produced in Sweden as Glypressin Ferring® (Terlipressin INN) and in the Federal Republic of Germany as Haemopressin by Curatis Pharma GmbH and Meduna Arzneimittel GmbH. This, and occasionally similar hormonogens, were applied in cases where cardiovascular collapse was imminent: traumatic shock or oesophageal variceal bleeding. The literature provides a number of reports on their therapeutic use, but they scarcely competed with other cardiovascular drugs. On the other hand, another vasopressin analogue with a significant shift in the pharmacological spectrum towards antidiuretic activity, 1-deamino-8-D-arginine--vasopressin (dDAVP, Desmopressin INN), appeared to be very promising[22–24]. It was recognized already in the first clinical trials as a potential drug for replacement therapy of *diabetes insipidus* (cf.[b]). In smaller quantities, around 30 g per year, dDAVP was first produced at the IOCB and later, after 1976, in state-owned enterprise Léčiva-SPOFA in Prague. František Šorm, the then director of IOCB and the chairman of the Academy of Sciences, tried to introduce quickly its upscale production and hence to enable its broad clinical use. However, he was not very successful, mainly due to a rigid economic and legislative policy in former Czechoslovakia. It succeeded only after the Swedish contacts had been established.

The beginnings of the cooperation between the IOCB and Ferring Läkemedel AB Malmö

The first contact between the IOCB and representatives of Ferring Läkemedel AB Malmö took place in the autumn of 1967 at the symposium "Pharmacology of Hormonal Polypeptides" in Milan through the Danish neurophysiologist Niels A. Thorn, professor of physiology at the University of Copenhagen[f]. The symposium was focused on hormonal polypeptides and proteins[g] and was attended by Josef Rudinger, Karel Jošt and Vladimír Pliska from the IOCB. (Their summary communication was published in Advances of Experimental Medicine and Biology[25]). Niels Thorn introduced V. P. with the then head of the medical section of Ferring AB, Jan Mulder, who was also among the participants of the symposium. The purpose of their contact was not, however, the future collaboration of the company with the IOCB (and even less the later production of dDAVP as a pharmaceutic), but the long-planned joint project of Thorn and V. P., concerning the then unexplained transport of neurohypophysial hormones from nerve endings in the neurohypophysis to the peripheral circulation[26,27]. In the Ferring laboratories, L. Carlsson and I. Sjöholm[h] had shortly before synthesized oxytocin and 8-lysine-vasopressin specifically tritiated at the tyrosine in position 2 (cit.[28,29]). It was assumed that the isotope exchange proton – tritium in aqueous environments will be substantially reduced in oxytocin and vasopressin specifically labelled on the aromatic ring of tyrosine, as compared to the non-specifically tritiated products; this was later confirmed in a long-term stability study of tritiated vasopressin[30]. Thus, the use of specifically tritiated vasopressin in this project was considered as particularly suitable. Jan Mulder not only gave his approval for using this peptide in the suggested experiments but also guaranteed his full support. Part of the experimental work moved then from Copenhagen to the Ferring laboratories in Limhamn

[f] Niels Anker Thorn (1924–2014), Institute of Medical Physiology C, Panum Institute, University of Copenhagen, Denmark.

[g] *International Symposium on the Pharmacology of Hormonal Polypeptides*, held in Milan, Italy, September 14–16, 1967. (Publication: Proceedings of an International Symposium on the Pharmacology of Hormonal Polypeptides, held in Milan, Italy, September 14–16, 1967. Editors: Nathan Back, Luciano Martini, Rodolfo Paoletti. Plenum Press, 1968).

[h] Lars Aake Ingemar Carlsson was a senior peptide chemist at Ferring's corporate laboratories, Ingvar Gösta Holger Sjöholm, later a professor at Uppsala University, worked at the Royal Pharmaceutical Institute (Kungliga Farmaceutiska Institutet) in Stockholm. For the synthesis, they used in the first step the L-3 iodotyrosine-lysine-vasopressin synthesized by them, in which they substituted iodine with tritium through exposition to tritium gas in an alkaline environment. Thus, they obtained a tritiated peptide with a then impressive specific radioactivity of 2–3 Ci/mmol (74–111 GBq/mmol), avoided the then commonly used direct exposure of the final product – i.e. oxytocin or 8-lysine-vasopressin – with tritium (Wilzbach method) and largely prevented a non-specific tritiation at different positions of the peptide chain.

near Malmö, on the other side of the Öresund, where the company's headquarter and production facilities were located at that time. In addition to J. Mulder, the main contributors to the project were in Malmö the head of the chemical laboratories, Lars Aake Ingemar Carlsson, his assistant Jan-Åke Sköldböck, who synthesised and purified oxytocin for the production of dosage forms, and the pharmacologist Per Melin, who worked on neurohypophysial hormones (his name is closely associated with the later successful drugs Atosiban and Carbetocin). This list also includes Ferring's chief economist Sam Matarasso and, above all, Ferring's founder, president and at that time major shareholder Frederick Paulsen Sr and his wife Eva Paulsen.

In discussions with V. P. was also mentioned the newly synthesized dDAVP analogue from the laboratory of Milan Zaoral at the IOCB and its remarkable spectrum of activities with a strong shift towards antidiuretic activity and with almost complete absence of vasoconstrictor activity. Despite the relatively low prevalence of the central form of *diabetes insipidus*, dDAVP attracted the attention of the scientists from Ferring since it could have been regarded as an absolutely preferable drug for its replacement therapy, especially as the so far used Pitressin (from various manufacturers) showed unpleasant to dangerous side effects associated with vasoconstrictor activity. The analogue aroused understandable deep interest, especially in J. Mulder and F. Paulsen. This interest was reported by V. P. after his return to the IOCB director František Šorm. In the meantime, the latter seems to have made another attempt to promote the production of dDAVP in Czechoslovakia; after its failure, F. Šorm asked V. P. to mediate a contact with Ferring. J. Mulder came to Prague for preliminary discussions on the terms of cooperation or later licensing, which at this stage in early 1969 was agreed within a few hours. The first agreement was documented in writing and signed by F. Šorm, J. Mulder and V. P. and submitted to the legal department of the Czechoslovak Academy of Sciences for approval[i]. Its validity was confirmed later that afternoon and J. Mulder returned to Sweden with a preliminary written agreement that brought unexpected success and profit to Ferring, advantageous contacts to the chemists of both parties, and also financial benefits to the IOCB.

A few remarks on the Ferring company at the early time of the cooperation with the IOCB

At the beginning of the 1970s, when the contacts between the two institutions began, Ferring AB was a rather small pharmaceutical company, since 1954 in Malmö-Limhamn[j]. In the year when the cooperation with the IOCB began, the company's entire operations were concentrated in a villa near the Öresund coast; an expansion to other properties in the area and their large extension took place after that date. The company was headed by the aforementioned founder Dr. med. Frederik Paulsen (1909–1997; originally Friedrich, born in Dagebüll, Nordfriesland, Schleswig-Holstein)[31] (Fig. 1). He was in every respect a rare and highly cultured personality. His interests included not only his original scientific field, the endocrine pharmacology: he was equally interested in the history and the language of his native county, the North Frisian Islands (the name of the *Faroe* island and its inhabitants gives the name of the Ferring society), world history, literature and linguistics. He observed contemporary social developments and the political issues associated with them through the eyes of a liberal progressive observer. Obviously, as a director of the company he had a responsibility for its economic benefit, but the scientific side was just as important to him. He also regarded the cooperation with the Czech side from this point of view, even though he did not actively participate in the negotiations with the IOCB.

The first medical director of the research was until his tragic death (30 April 1976) the Dutch Dr. med. Jan Louis Mulder, who, as already mentioned, established the cooperation with the IOCB from the Swedish side. Thanks to his organizational skills and contacts in European clinics, he quickly obtained permissions for clinical trials. The fact that dDAVP was available early and widely in medical practice was mainly due to his effort. J. Mulder also took in late 1969 the first steps towards clinical trials in Sweden of one of the first potent oxytocin antagonists from the IOCB laboratories, 1-N^{α}-acetylcysteine-2-*O*-methyltyrosine-oxytocin[32,33]. Successful application of tocolytically acting oxytocin analogues (Atosiban) arose after his death. He was succeeded as medical director by Hans Vilhardt (1940–2015) of the Institute of Medical Physiology at the University of Copenhagen (later professor there)[k].

[i] The document mentioned above was only a preliminary version of a later legally documented licence agreement and is not available in the archives of the IOCB today (or perhaps the competent authorities are presently not interested in publishing the course of these negotiations).
In this context, it seems necessary to mention the entirely erroneous account of the history of the beginning of the cooperation between the IOCB and Ferring in the 1999 publication "Ahead of his time" (ref.[31], pp. 31–32). The author (Birgit Amon) does not cite the source of the incorrect or even absurd information; an example of its casualness is the claim that the design of the dDAVP molecule came from Niels Thorn!

[j] In 1956, the Kiel branch of Ferring GmbH was opened, headed by Frederik's brother, Dr. Otto Paulsen. From the beginning, it focused on the production and marketing of pharmaceuticals, especially for the German market.

[k] By way of comment (V. P.): after Hans Vilhardt's return to the University of Copenhagen, F. Paulsen Sr considered inviting Tomislav Barth (1938-2007) from the IOCB to this post. However, at that time (early 1980s), there were justified doubts that anyone from the then Czechoslovakia (and from other countries under Soviet influence) could take over such a position. Nonetheless, this is an evidence of the close relationship between Ferring and the IOCB at that time.

Fig. 1. **Photographs, available to the authors, of people associated with the cooperation between the Institute of Organic Chemistry and Biochemistry in Prague and Ferring Läkemedel AB, Malmö**

H. Vilhard's successor was then Jan Ivan Thorell (*1934), Professor of Clinical Chemistry at Lund University; he later went on to a senior research position at Pharmacia Diagnostics AB (Uppsala). The last of this line, still appointed by Frederik Paulsen Sr, was Iain C. A. F. Robinson (*1949) of the National Institute for Medical Research, Mill Hill, London.

The leading positions in the company's research were held by two persons, the peptide chemist Lars Aake Ingemar Carlsson and the pharmacologist Per Melin[1]. The for-

[1] At present, the authors have not been in a position to obtain their full biographical details. Lars Carlsson died – perhaps in the late 1990s – of diabetes, Per Melin retired after 1999 and moved to the U.K.

mer has already been mentioned several times here as a prominent synthetic chemist who cared little for publicity among his European colleagues, publishing rather as a co-author; his domain were meticulous challenging syntheses in the laboratory which he considered with respect to the upscale production of peptide drugs. His approaches were appreciated by many young peptide chemists, and hence, an innovative group of peptide synthesisers was established in Malmö. Among others, the Polish chemist Jerzy Trojnar (*1943) joined them, who later took over L. Carlsson's position (he was then active at Ferring Research Institute Ltd., San Diego, CA[m] as one of the directors from 2003–2008).

Per Melin was at the forefront of the development of pharmacological testing and research at Ferring. His publishing activity is extensive and he was a welcomed partner in numerous research and clinical projects. He was instrumental in introducing the tocolytic drug Atosiban into clinical practice and in its use as an inhibitor of oxytocin receptors in pharmacological and physiological studies[34,35].

Research on neurohypophysial peptides in Czechoslovakia after 1968

The year 1968 was a landmark in Czechoslovakia from many historical perspectives. After the invasion of the country by the armies of the Warsaw Pact states, it was also critical year for the peptide research and development at the IOCB. Several leading researchers left after the Russian occupation the country. The influence of IOCB's founder and director, F. Šorm, who deeply disagreed with the Russian occupation, was suppressed and gradually diminished. Despite the continued scientific quality of the Institute and its members, research conditions became very difficult, particularly due to politically motivated restrictions in the contacts with the world scientific community, the lack of imported chemicals and, above all, by official attempts at bureaucratic management of research.

The laboratory of Josef Rudinger (1924–1975) was split into two groups after his departure to Switzerland in 1968. One of them was headed by Karel Bláha, whose scientific activity in collaboration with Ivo Frič was rather focused on problems of physicochemical and spectral chiroptical properties of peptides. Research on neurohypophysial hormones and especially on oxytocin analogues was continued by Karel Jošt and Karel Poduška, together with Vladimír Gut. The biochemical and biological properties of the substances were further investigated in the group of Tomislav Barth, the successor of one of the authors (V.P.). At the Research Institute of Pharmacy and Biochemistry (VÚFB) in Prague, the research on peptides continued in the laboratories of Ivan Krejčí and Evžen Kasafírek, J. Rudinger's pupil (he died tragically in May 2001 (cit.[36])[n]).

The second peptides group was headed by Milan Zaoral. His main interest in that time were vasopressin analogues, especially the effect of enantiomeric substitution of the L/D arginine side chain of the vasopressin structure in position 8, investigated in detail already before 1968. As mentioned above, the analogues 1-deamino-8-D--arginine-vasopressin (dDAVP, Desmopressin) and 1-triglycyl-8-lysine-vasopressin (Terlipressin) were then sold under licence to Ferring AB, which obtained the right to manufacture these substances for Western European countries and for the USA.

Production of peptide drugs in Czechoslovakia in the 1970s and 1980s

In Czechoslovakia, peptides were traditionally produced in gram quantities in the Léčiva n.p. (n.p. stands for "national enterprise"i.e., state-owned enterprise) within the SPOFA group (Czech abbreviation for United Pharmaceutical Plants). Although the management of SPOFA and Léčiva had been cooperating with the IOCB since the 1950s, the peptides represented there only a small production volume and a small profit. This was matched by the low interest of the Czech pharmaceutical industry in this type of highly qualified chemistry. After the conclusion of the licence agreement with Ferring, the uptake in peptide production at Léčiva increased somewhat and as a result, the peptide laboratories at the Léčiva plant in Prague-Komořany (municipal district of Prague 12) were gradually expanded and reconstructed; however, the new peptide department at Léčiva was not opened before January 1976. Analogues of neurohypophysial hormones, which were already produced in relatively large volumes under the licence by Ferring, were produced at Léčiva in much smaller quantities for the socialist countries of Eastern Europe and for the Soviet Union. Nevertheless, the profit share from the production of peptides was still very low in the overall production volume of Léčiva.

Some peptides, approved as medicinal substances, were synthesized for Léčiva n.p. in the pilot plant department of the IOCB. It should be mentioned that in the 1970s, the GMP system was not yet in place even in the pharmaceutical production plants (see above). The adequately, but from the point of view of GMP, still insufficiently equipped pilot plant of the IOCB could thus still be used for the production of pharmaceuticals. In particular, peptide purification was a major problem in the pharmaceutical industry. The first batches of the drug desmopressin were therefore produced in the pilot plant of IOCB and purified directly in M. Zaoral's laboratory. 20 to 30 g of desmopressin were then supplied for the production of the

[m] The Institute closed in 2016.
[n] http://www.csbmb.cz/bulletin/2002_2.pdf

pharmaceutical form of desmopressin under the name Adiuretin SD (nasal drops) in a production branch of Léčiva n.p. in Měcholupy near Prague.

The oxytocin analogue 2-*O*-methyltyrosine-oxytocin (methyloxytocin) was purified in the peptide department in K. Bláha's group only in small quantities (about 5 g) by the counter-current distribution introduced in 1944 by L. C. Craig[37]. The raw methyloxytocin was produced directly at Léčiva in Prague Komořany by a process developed at the IOCB. The IOCB thus helped in the production of peptides until almost the mid-1980s.

Later developments

The relationship between the researchers in Prague and the Ferring AB laboratories in Malmö was very productive from the late 1960s to the mid-1970s and led to the development of therapeutically interesting analogues of neurohypophysial peptides. After the conclusion of the agreement mentioned above, the IOCB became a partner for the scientific environment of the Swedish company, which was successively joined by peptide scientists from other countries in addition to the traditional Scandinavian university contacts (Uppsala, Lund), often mediated by Prague peptide researchers. On the other hand, from the Swedish side came impulses which in the later 1960s initiated the start of a more modern approach to peptide production in Prague.

At that time, however, the autocratic policy in Czechoslovakia did not favour scientific development – unless it led to short-term economic benefits – and, through administrative and financial measures, made contacts between scientists from Western countries and European countries under Soviet influence difficult, or rather impossible. After 1969, the management of the IOCB lost the possibility of free and independent cooperation with Western countries; personal contacts with colleagues from the Ferring laboratories were very limited at the end of the seventh decade. Under such circumstances, Ferring's research and production activities gradually focused on cooperation with other European and overseas laboratories.

In such a situation, a small international group of external scientists called the "Ferring Colloquium" was formed in January 1981 on the initiative of Frederik Paulsen Sr, with the intention of following developments in the field of peptide pharmaceuticals and thus supporting the research community in Malmö[o]. Joint publications by authors from this Colloquium and researchers from Ferring were common until the group ceased its activities in October 1986. At the suggestion of the Colloquium, Ferring (and later the PolyPeptide Group) sponsored the "Josef Rudinger Memorial Award" for the European Peptide Society, an award given for significant contributions to peptide chemistry, coupled with a plenary lecture at the regular European Peptide Symposia (in two-year cycles)[p]. In 1983, the Colloquium organised in Stockholm an international symposium on "Biogenetics of neurohormonal peptides", then published in Academic Press in 1985[q]. Ferring then established closer, and sometimes formal contacts with a number of members of the Colloquium: Ulf Ragnarsson (Biomedical Center, University of Uppsala) was a consultant to the company from 1980–1987; members of his laboratory (Lars-Eric Larsson, Bengt Sandberg) collaborated in projects on neurohypophysial hormones; collaboration with Michael Szelke led to the establishment of a research branch of Ferring in England. Close scientific contacts existed with the institutes of other members of the Colloquium, two of whom took temporary leadership of the Ferring laboratories in Malmö (Hans Vilhardt, Iain C. A. F. Robinson). The Colloquium ceased its activities, despite the disapproval of Frederik Paulsen Sr, due to disagreements with the incoming new leadership of Ferring. In 1988, Frederik's youngest son Frederik Dag Arfst Paulsen (*1950) took over the management of the family enterprise .

Under the leadership of F. Paulsen Jun, fundamental changes were made in the structure of the company, which

[o] Permanent members (original location): Rolf Håkanson (University of Lund), Vladimir Pliska (ETH Zürich), Ulf Ragnarsson (University of Uppsala), Iain C.A.F. Robinson (National Institute of Medical Research, London), Michael Szelke (Royal Postgraduate Medical School, Hammersmith Hospital, London), Jan I. Thorell (Ferring AB, Malmö), Hans Vilhardt (University of Copenhagen), Karlheinz Voigt (Universität Ulm, later Philipps-Universität Marburg, Institut für Physiologie und Pathophysiologie), Wolfgang Wuttke (Max-Plank-Institut für Biophysikalische Chemie, Göttingen). It is noteworthy that Czech researchers were not included in this group, although their presence would have been very welcome, especially the collaboration with T. Barth. However, there were doubts whether their regular participation in meetings in Western countries would meet with understanding from the Czechoslovak authorities and therefore their almost regular absence would have to be reckoned with. The management of the company also had legitimate concerns that if their travels were permitted, they would be questioned on their return by the Czechoslovak authorities about confidential internal company matters.

[p] *The Josef Rudinger Memorial Lecture Award* is presented "in commemoration of Josef Rudinger's role in the foundation of the European Peptide Symposia and of diverse contributions he made to peptide chemistry". The Award is presented during the European Peptide Symposia and is sponsored by PolyPeptide Group Prague.

[q] The Ferring Symposium on "Biogenetics of Neurohormonal Peptides", held in Stockholm, Sweden, July 1–2, 1983; Biogenetics of Neurohormonal Peptides (eds R. Håkanson, J. Thorell), Academic Press, London 1985.

were very substantially related to the cooperation with the Czech side. Within the framework of Ferring's "joint venture" with the Léčiva n.p. Praha, the Prague Polypeptide Institute spol. s.r.o. in 1990–1992, and later Ferring-Léčiva A.S. were established. Part of the employees of Léčiva n.p. Prague were gradually transferred into the new companies and augmented by other peptide chemists. The production facilities for the production of basic peptides for pharmaceutical preparations remained at first in the Léčiva laboratories in Prague-Komořany; later the company under the name Polypeptide Laboratories Prague moved to laboratories in Prague-Hostivař, where the company rented the premises of the former Institute for Research, Production and Use of Radioisotopes.

After 1997, the newly built pharmaceutical plant of the company "Ferring Léčiva" in Jesenice near Prague was designated for the production of pharmaceutic forms. According to company information (https://www.ferring.cz/), the Czech branch was then split into two separate units in 2008:

– production plant Ferring-Léčiva a.s. for the production of peptide dosage forms and other drugs of interest to the Ferring Group,
– marketing organization Ferring Pharmaceuticals CZ s.r.o.

The more than fifty-year relationship between peptide researchers in Prague and the originally small Swedish company Ferring AB, which over time – and certainly also thanks to the Czech partners – developed into a major global pharmaceutical company, continued under new historical conditions on an entirely different personal and institutional level.

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On the current occasion: *The pharmaceutical company FERRING Pharmaceuticals CZ s.r.o. donated through the Vita et Futura Endowment Fund, by way of the Czech Red Cross, large quantities of Carbetocin to help women in childbed in war-affected areas of Ukraine. Carbetocin is in obstetrics one of the life-saving drugs for treatment of perinatal bleedings; it is one of the peptides that resulted from the cooperation between Prague and Swedish researchers, as mentioned in this report. As a result of the aggressive Russian invasion, medical care in Ukraine is presently struggling with great difficulties. Our thanks for this efficient help go to the organizations mentioned above, to the director of Ferring in Prague Ing. Branislav Kotlárik, to the President of the Czech Red Cross Dr. Marek Jukl and to all persons who participated in this humanitarian action.*

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Abstract

From the fifties to the seventies of the last century, the neurohypophyseal peptides oxytocin and vasopressin constituted one of the main research areas at the Institute of Organic Chemistry and Biochemistry in Prague (IOCB). A significant contribution to this area is associated with the names of František Šorm, director of the said institute, and Josef Rudinger, head of the institute's peptide laboratory. At that time, newly developed research tools enabled to synthesize structural analogues of these hormones in numerous laboratories worldwide and hence to investigate the structure-activity relationships within this peptide group. Contributions of single peptide-chain positions to the respective biological activities were identified which opened a possibility to rationalize a design of peptides with a combination of changes in several positions. Several clinically interesting peptides were synthesized in the late 1960s at the IOCB and employed as therapeutics: [$(Gly)_3$-Cys^1,Lys^8]-vasopressin (Glypressin Ferring®, Terlipressin INN), 1-deamino-8-D-arginine vasopressin (Desmopressin INN, dDAVP), and later the uterotonics carbetocin (INN), widely used in obstetrics to prevent postpartum haemorrhage. Since the industrial production of peptide therapeutics was scarcely possible under the conditions of socialist economy in Czechoslovakia as well as in other countries under the Soviet influence, F. Šorm agreed to use the already established scientific contacts of IOCB with the Swedish pharmaceutical company Ferring AB and to transfer the production licences to Sweden. The license agreements were signed in 1969 and led to a quick spread of dDAVP in the substitution therapy of the central form of *diabetes insipidus* and, moreover, contributed to a fast upsurge of the Ferring company. Somewhat later, Glypressin was produced as a therapeutic with a prolonged action in cases of cardiovascular collapse. Contacts between Prague peptide chemists and the Ferring company lasted on a rather informal base until the end of the 1980s. After the fall of the totalitarian regime in Czechoslovakia in 1990, Ferring started a joint-venture collaboration with the newly organized Czech company Léčiva st.p. Praha in a newly established group Prague Polypeptide Institute spol. s. r.o. (later Ferring-Léčiva A.S.). A substantial part of the peptide-production capacities was then transferred to new buildings in Prague.

Keywords: neurohypophyseal hormones, oxytocin, vasopressin, deamino-D-arginine vasopressin, DDAVP, Glypressin, Terlipressin, Carbetocin, Ferring company, peptide drugs

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