Chem. Listy *116*, 101−109 (2022) Review

NEUROHYPOPHYSIAL PEPTIDES IN MEDICINE FROM PRAGUE AND SWEDISH LABORATORIES: PART II: DESMOPRESSIN, TERLIPRESSIN, CARBETOCIN – PHARMACOLOGY AND CLINICAL APPLICATIONS

Vladimír Pliska[a], Antonín Pařízek[b], Martin Flegel*[c]

[a] Department of Biology, Eidgenössische Technische Hochschule (ETH), Wofgang-Pauli-Strasse 27, CH-8093 Zürich, [b] Department of Obstetrics and Gynaecology of the 1st Faculty of Medicine and General University Hospital in Prague, Perinatology Centre, Apolinářská 18, CZ-128 51 Praha 2, [c] Department of Natural Chemistry, Faculty of Food and Biochemical Technology, VŠCHT Praha, Technická 5, CZ-166 28 Praha 6
vladimir. pliska@biol.ethz.ch, parizek@porodnice.cz

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

https://doi.org/10.54779/chl20220101

Production of pharmaceuticals based on agreements between the Institute of Organic Chemistry and Biochemistry (Czechoslovak Academy of Sciences) and Ferring AB

In the course of the 1970s and 1980s, the Swedish pharmaceutical company Ferring AB Malmö obtained from the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences (IOCB) the rights to manufacture a number of neurohypophysial peptide analogues[1,2] and to distribute them on the world market outside the countries associated in the COMECON (abbreviation for the Council for Mutual Economic Assistance, the Eastern Bloc's economic organization from 1949 to 1991)[a]. Three of them have found use as successful drugs.

dDAVP: 1-deamino-8-D-arginine vasopressin (Desmopressin)

The design of this analogue, which started the cooperation between the IOCB and Ferring[1,2], was based on earlier known data about the outcomes of the deletion of the N-terminal amino group and the D-enantiomeric substitution at position 8 on the antidiuretic and vasopressor activity in the group of vasopressin-type peptides. 1-Deamino-L-arginine-vasopressin (dAVP) was described already in 1966 by Huguenin and Boissonnas (Sandoz S.A., Basel)[3] and later (1974) by Sawyer and co-workers[4]; both laboratories found identical antidiuretic (1300 and 1390 IU/mg) and vasopressor (370 IU/mg) activities[b], i.e., a significantly increased antidiuretic effect with the same vasopressor activity as compared to L-arginine-vasopressin. Substitution of D-arginine at position 8 of arginine-vasopressin resulted in a slight decrease in antidiuretic activity (from 332 to 257 IU/mg[5], or 114 IU/mg[6]), but a substantial decrease of vasopressor activity (between 1.1 and 4.1 IU/mg). It was assumed that the combination of the two structural modifications might offer an interesting peptide for replacement therapy of *diabetes insipidus*, a disorder causing an imbalance of body fluids[c] accompanied with a large volume of excreted urine. Despite the fact that the accumulation of structural modifications in an individual peptide chain does not commonly lead to the expected accumulation of activities associated with these modifications, the director of the IOCB, František Šorm, appointed Milan Zaoral's group to synthesize a peptide with a combination of both structural modifications[7]. The result was more than surprising. Already the first antidiuretic tests carried out by the pharmacological group of the IOCB showed that the antidiuretic activity was at least fifty times higher as compared to 8-L-arginine-vasopressin (AVP), while the vasopressor activity tested by the group of Ivan Krejčí from the Research Institute of Natural Drugs[d] was less than 3%. Standard pharmacological tests of antidiuretic activity are, however, not unambiguous and not sufficiently reliable with such large differences be-

* † 15. 7. 2021.

[a] After disbanding of the Soviet Union and the COMECON (see text) in the early 1990s, this restriction became irrelevant and Ferring expanded its activities worldwide.

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

[c] Clinical cases described in the cited communications were most probably central (neurohypophysial) forms of *diabetes insipidus* (*d.i. centralis*). The renal form (*d.i. renalis*), independent of plasma vasopressin concentration, usually arises out of a mutation of the vasopressin receptor V_2R gene or its cellular signalling pathway.

tween the standard and the tested peptide[8]: further laboratories reported values for dDAVP between 870 IU/mg[7] and about 50 000 IU/mg[9,10,e]. The lower-limit value, later quoted again[5], was cited by the authors of the first (preliminary) publication[6], without mentioning the substantially higher activities found in the then pending tests from the IOCB. These remarkable discrepancies can perhaps be partly explained by the sensitivity of dDAVP to even relatively small differences in the tests used, but mainly by the use of often quite different tests and, last but not least, by using inconsistent numerical descriptors of the activities. The antidiuretic activity data from the individual laboratories come from tests on anaesthetised rats hydrated with a single oral dose of isotonic hydration medium[11] (with 1–2% ethanol or without alcohol), or rats with continuously maintained hydration equivalent to 8–10% of the animal's body weight[12,13]. The antidiuretic effect is related to the total volume or weight of urine excreted (the value of the time integral of the diuretic curve), a quantity defined in pharmacological measurements as the AUC (area-under-the curve). In the case where activity is defined as a ratio to a given standard (here, AVP), the AUC of the two must be related to a defined time[14]. If this is not the case, the final data lacks a reliable comparative value. (This is the case, for example, for some of the data cited above[10]). Thus, in the case of non-parallel dose-effect relationships, the concept of relative specific activity is inadequate and only allows comparison of equipotent doses (doses of two substances having the same effect) at different levels of action.

Fig. 1 shows the spectrum of published antidiuretic

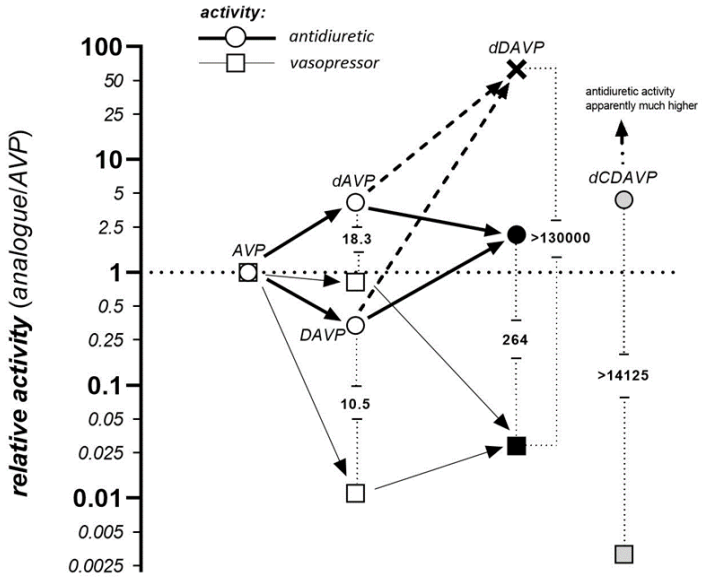

Fig. 1. **Schematic of a dDAVP design assuming accumulation of the desired peptide activities with isolated structural change.** Antidiuretic (circles) and vasopressor (squares) activities of arginine-vasopressin analogues (AVP), deamino-arginine-vasopressin (dAVP), D-arginine-vasopressin (DAVP), and the combined peptide deamino-D-arginine-vasopressin (dDAVP, black symbols). Differences between the two activities are indicated by vertical dotted lines, the numbers in between indicate the "*ad hoc* preference index" (PI, see formula in the text) for antidiuretic activity: the ratio antidiuretic/vasopressor activity multiplied by the relative antidiuretic activity (analog/AVP). The two antidiuretic activity data for dDAVP correspond to different estimates from different participating laboratories, often using different antidiuretic assays (see text); the lower value refers to the extrapolated (threshold) dose of dDAVP, the specific activity for higher doses[10] is not clearly defined (point × indicates the approximate upper limit of the data reported in the literature). The scheme suggests that in the case of dDAVP, the assumption of activities accumulation was largely satisfied. For comparison, dCDAVP activities are shown (gray symbols; see text). The antidiuretic activity (2200 IU) was tested in unanesthetized rat[21]; its value in the assay (rat under ethanol anaesthesia) is, according to the authors, very high and not measurable by the standard method. The predicted preference index of dCDAVP could therefore be much higher compared to dDAVP.

[d] Later the Research Institute for Pharmacy and Biochemistry (VUFB); the Endocrinology Laboratory of the Third Internal Clinic of the General University Hospital in Prague participated in the testing.

[e] Converted from data in the publications mentioned. Data from the VUFB for anaesthetised rats are based on the international standard of the time, pituitrin (bovine neurohypophysis extract), after conversion to ~31 560 IU/mg; in experiments on non-anaesthetised rats (standard: synthetic 8-lysine-vasopressin – LVP) ~25 000 to 50 000 IU/mg.

and vasopressor activities for each "intermediate" stage of dDAVP development, as well as a “preference index” (*PI*) for antidiuretic activity (numbers between vertical arrows), here defined ad hoc as the product of the ratio of antidiuretic (A_{ad}) to vasopressor (A_{vp}) activity and antidiuretic activity (the two activities relative to AVP), i.e.,

$$PI = \frac{A_{ad}}{A_{vp}} \cdot A_{ad} \equiv \frac{A_{ad}^2}{A_{vp}}$$

Its high values for the reported limits of antidiuretic activity document its preference among analogues potentially useful in the replacement therapy of *diabetes insipidus*.

As to the duration of the effects of dDAVP: the prolonged effect was one of the features that was foreseen and required already in the design of the analogue. The authors of the first clinical publication[10] report a 12.75 times slower decline of the antidiuretic response to dDAVP compared to the pituitary standard (i.e., AVP), which would indicate a high metabolic stability of the analogue in target tissues. However, their data (presented in Figure 1) indicate that they were not evaluated by a suitable kinetic analysis and that the reported high persistence parameter is not reliable. A comparison of the linear dose-response slopes suggests an approximate persistence index (I_p)[14] between 2.5 and 3.9.[9] The response (diuresis) half-life data in the following publication[9] allow a rough indication of the persistence index (using, for example, the Kimura and Yokoyama conversion[15]), but due to the choice of inadequate experimental conditions, they do not allow a detailed analysis. Nevertheless, the recalculation suggests that I_p is roughly equal to 2 , a value close to the assumption. Although the kinetic analysis of various series of neurohypophysial peptides was carried out very thoroughly in the work of Tomislav Barth and his group at the IOCB, dDAVP was not included in these studies. The increased persistence and the associated prolonged effect are probably due to the inhibition of the N-terminal peptide bond (cysteine-tyrosine) cleavage by aminopetidases, which in comparable cases leads to a two- to fourfold increase in the persistence index. The reported extreme persistence values in the cited publication[10] are therefore probably due to an overdose of this very potent antidiuretic peptide in the cited

United States Patent Office 3,497,491 Patented Feb. 24, 1970

1

3,497,491
1-DEAMINO-8-D-ARGININE VASOPRESSIN
Milan Zaoral, Ivan Vavra, Alena Machova, and Frantisek Sorm, Prague, Czechoslovakia, assignors to Ceskoslovenska Akademie Ved., Prague, Czechoslovakia
No Drawing. Filed Sept. 14, 1967, Ser. No. 667,679
Claims priority, application Czechoslovakia, Sept. 15, 1966, 5,996/66
Int. Cl. C07c 103/52; A61k 17/00; C07d 93/36
U.S. Cl. 260—112.5 1 Claim

ABSTRACT OF THE DISCLOSURE

Mep-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH_2
1 2 3 4 5 6 7 8 9 (I)

wherein Mep is β-mercaptopropionic acid (Mep) at position 1 and D-arginine is at position 8.

The compound of the invention has a high and specific antidiuretic action.

BACKGROUND OF THE INVENTION

Compounds exhibiting a high and specific antidiuretic activity are important in the therapy of diabetes insipidus. Such compounds are well known and represent structural modifications of the vasopressin molecule. The most suitable properties have been observed in the case of 1-deamino-Phe²-Arg⁸-vasopressin (cf. the table below).

SUMMARY OF THE INVENTION

It is an object of the present invention to provide for an antidiuretic that has a higher degree of activity and is more specific in its action than the just named vasopressin compound.

This is accomplished by a polypeptide of ' mula I

2

which latter is then oxidized, preferably with potassium ferricyanide in an aqueous solution at pH 6.5–7 to give the polypeptide of Formula I.

The protected octapeptide derivative of Formula II can also be obtained by other procedures such as are conventional in the preparation of polypeptides. The compound II may be thus prepared by a stepwise synthesis starting with the amino terminal group as well as the carboxylic terminal group, or by coupling of polypeptides containing the partial amino acid sequences of the final polypeptide of Formula I. The temporary protection of the amino groups, mercapto (sulfhydryl) groups and of the guanidine group may be performed by means of substituents that are conventional in the synthetic chemistry of polypeptides. E.g., a t-butyloxycarbonyl, o-nitrophenylsulfenyl or trityl group can be used for blocking of the amino groups, a trityl, benzoyl, or benzylthiomethyl group for the protection of the sulfhydryl function, and a tosyl or benzyloxycarbonyl group for the protection of the guanidine group.

The protective groups are then removed in the usual manner in one stage or successively. Oxidation of the reduced form of the polypeptide of Formula III to the cyclic polypeptide of Formula I may be accomplished by methods known per se, for instance, in an aqueous solution or in a mixture of water and solvents miscible with water.

Preferably, the following method is used. Condensation of β-benzylmercaptopropionyl chloride with tyrosine methyl ester leads to the so far unreported β-benzylmercaptopropionyl-L-tyrosine methyl ester. By the action of hydrazine hydrate, the latter compound is converted to the so far likewise unreported hydrazide and is then coupled with L-phenylalanine methyl ester. The resulting dipeptide ester derivative is condensed, again in the form of the azide, with L-glutaminyl-L-asparaginyl- methyl -

Fig. 2. **U.S. patent protecting dDAVP as a substance and the original process for its synthesis, filed 1967, claiming a priority of 1966**

experiments and an inadequate interpretation of the time-response data.

However, the therapeutic potential of dDAVP was soon recognized and the substance was covered by a U.S. patent[16] to the Czechoslovak Academy of Sciences (US 3,497,491; priority date Sep. 15, 1966 ; Fig. 2). Potential further patents could therefore only cover the manufacturing process or new medical uses. In this respect, Ferring already possessed the necessary infrastructure to quickly introduce dDAVP into clinical practice; the patent was granted on Feb. 24, 1970, and the license terms were therefore advantageous for both parties – IOCB and Ferring. The license agreement between the Czechoslovak Academy of Sciences (signature on the agreement: K. Friml, Secretary General), Polytechna[f] (two illegible signatures) and Ferring (S. Matarasso, Managing Director; J. Mulder, Research Director) was concluded in Prague in 1971.

The clinical use of dDAVP was then extended in the second half of the 1970s to haematological applications, based on studies of the haemostatic effect of vasoactive peptides. Cash et al.[17] (Regional Blood Transfusion Centre, Royal Infirmary, Edinburgh) reported an increased release of plasminogen activator and of coagulation factor VIII after intravenous administration of neurohypophysial hormones of the vasopressin type, including dDAVP. They later widened this study to include other interesting analogues[18] (remarkably high haemostatic activity was found for the 6-carba analogue of dDAVP, while 8-arginine-vasotocin was completely ineffective). The use of dDAVP to increase factor VIII concentrations in patients with milder forms of haemophilia A, von Willebrand-Jürgens syndrome and platelet dysfunction was then described by the Italian haematologist Pier Mannuccio Mannucci of the University of Milan and his colleagues[19,20]. The prevalence of these disorders of haemostasis, mostly hereditary, is apparently higher than that of *diabetes insipidus*; dDAVP (Desmopressin INN; Minirin® Ferring) has thus found a wider application in acute minor spontaneous bleedings and in minor surgical interventions (for example in dentistry)[g]. All this very substantially increased the importance of the licence that Ferring had obtained by the agreement with the IOCB.

The case of dDAVP also demonstrates the frequent differences in the completeness of pharmacological and clinical data. While the clinical documentation of dDAVP is extensive and detailed, reliable pharmacological data are scarce. Not fully explained are the aforementioned discrepancies in activity data between different laboratories, the lack of pharmacokinetic data and, moreover, the evidence that dDAVP represents an optimal choice for clinical purposes in a group of analogues with very similar activity spectra. For example, published data suggest that for the same therapeutic applications, the later synthesized carba-6-analogue of dDAVP, 1-deamino-8-D-arginine-6--carba-vasopressin[21] (dCDAVP, Figure 1), could be such a preferred drug. However, in view of the success that dDAVP has enjoyed in clinical use, this academic criticism is not entirely relevant.

Triglycyl-8-lysine-vasopressin (Terlipressin)

The initial phase of the IOCB's license agreements with Ferring included, besides dDAVP, also a group of analogues of neurohypophysial hormones with an extended peptide chain on the N^{α}-terminal amino group, for which the name "hormonogens" was adopted[22]. Their synthesis was motivated by the notion that the predominant inactivation process of oxytocin and vasopressin is the cleavage of the peptide bond between 1-hemicystine and 2-tyrosine by aminopeptides, presumably by a leucine-aminopeptide-type enzyme[23]. An extension of the N-terminus by an amino acid (cleavable by aminopeptidases) or a short peptide chain was expected to result in a gradual (and temporary) release of the active hormone and thus to elongate the corresponding biological effect *in vivo*[24]. This putative mechanism was gradually confirmed by the synthesis of a series of 8-lysine-vasopressin[22,25–27] and oxytocin[28,29] analogues with significantly prolonged antidiuretic, vasopressor and uterotonic effects, with persistence index I_p between >1 (for easily cleavable amino acid substituents – leucine, tyrosine) and 5. N^{α}-$(Gly)_3$-[Cys^1,Lys^8]vasopressin, whose standard biological activities in the rat are low (antidiuretic 2.7, vasopressor 2.1 IU/μmol, i.e., about 0.5% of lysine-vasopressin activities), but the persistence index is high ($I_p \approx 5$)[22], suggested its possible clinical use as a vasoconstrictor spasmolytic drug increasing peripheral vascular resistance in patients with imminent haemorrhagic shock. The prolonged therapeutic effect is particularly advantageous in acute conditions of oesophageal and other gastrointestinal bleedings[30], traumatic and septic shock, in cirrhotic patients with portal hypertension[31] and with the so-called hepatorenal syndrome[32]. (A brief review of clinical applications was published in 2004 by Kam et al.[33]).

Analogues of 8-lysine-vasopressin acylated by gly-

[f] Polytechna (today: Polytechna Consulting, Inc.) was a state-owned foreign trade enterprise that also arranged for the exchange of medical services, including manpower, abroad. One of the authors (A. P.) draws attention to the reports of fellow doctors who, through Polytechna's mediation, went to work, for example, in Malta or North Africa: Polytechna appropriated a large part of their earnings. On the other hand, there was a suspicion that those who went abroad through Polytechna were suspected of collaborating with government and party espionage units (the Communist Party of Czechoslovakia and the Czechoslovak intelligence service STB).

[g] By way of comment: dDAVP has also broken into contemporary English fiction, albeit episodically; see Barbara Vine: *The Blood Doctor*, Viking – Penguin Random House, London 2002.

cine peptides on the N-terminal amino group were originally protected by Czechoslovak patent CS 4,399/64 (filed August 1, 1964), later by patents in other countries including the United States (US 3,558,590). The patents cover, in very general terms, lysine-vasopressin N-acylated derivatives of "short glycine-containing peptides", i.e., a large group of peptides, including intermediates of their synthesis; diglycyl and triglycyl analogues are cited as examples. Ferring obtained a licence for their production in the first phase of the collaboration with the IOCB and registered the second phase one of them, triglycyl-lysine--vasopressin, under the name Terlipressin (INN; Glypressin®). Numerous publications after 1974 suggest its rapid spread in various clinics, albeit in a limited range of applications.

Under consideration was also the potential use of triglycyl-vasopressin as an early abortion drug. This hormonogen peptide has a low but markedly prolonged pressor, and a low uterotonic effect. The peptide was thought to cause contraction of uterine smooth muscle during the first trimester of pregnancy, resulting in foetal death. Leaving aside the ethical aspect of testing such hypotheses, physiological research faces almost insurmountable difficulties: the course of decidualization and the structure of the placenta are fundamentally different in individual mammals and therefore the evidence obtained in animal experiments cannot be, in this specific case, used in human medicine. To our knowledge, no further steps have been taken in this direction.

Carbetocin: use in veterinary and human obstetrics

Motivation for synthesis and pharmacological conclusions

Carbetocin (INN)[h], deaminocarba[1]-2-*O*-methyltyrosine-oxytocin, now a widespread uterotonic, was design as a potential oxytocin antagonist and synthesized in the laboratory of Karel Jošt before 1971. The corresponding patent application was filed in Czechoslovakia in February 1971; the Czechoslovak patent (CS 149'028 B1)[34] was granted in June 1973. Its subject matter was not the substance itself – that was not permissible under the Czechoslovak patent law at that time – but the process of its preparation. A detailed description of the synthesis used at that time, together with data on biological activities, was presented in 1974 in a publication on the physicochemical properties of carba-analogues of oxytocin[35]. The reported biological activities of carbetocin (mentioned rather peripherally) did not attract particular attention from a pharmacological point of view: the uterotonic activity in the rat uterus experiment was about 3.5% (*ex vivo*) and 7% (*in vivo*) of that of oxytocin; no significant *ex vivo* antagonism was initially demonstrated. However, antagonism was documented in later *ex vivo* experiments and on isolated rat myometrial cell membranes[36]. The same study demonstrated that carbetocin is a partial agonist of oxytocin (maximal uterine contraction *ex vivo* reached 50% of the contraction initiated by oxytocin), presumably as a result of partial inhibition of the oxytocin receptors by the peptide itself and/or by its successively formed metabolites (Table I, Fig. 3). Thus, the *in vivo* inhibition of oxytocin and the associated

Table I
Carbetocin and selected inhibitors of uterotonic activity of oxytocin

	Uterotonic response (rat): response descriptors					Elimination half-life [min]		
	activity [IU/mg]		pD_2	partial agonism E_{max} rel. to oxytocin	antagonism pA_2 [a]	women		female rat [d]
	in vivo	*ex vivo*				Nonpregnant [b]	*post partum* [c] (24 – 48 h)	
Oxytocin	470	485	8.28	1		5.5–6.1		2.5
Carbetocin	45	17.1	7.32	0.52	8.21	41–42.7	60	8.8
Metabolite I[e]					7.81			
Metabolite II[e]					8.01			
Atosiban					8.29			
Ac-Tyr(Me)-OXT[f]					7.03			

[a] Antagonistic activity towards oxytocin *in vitro* (pA_2: see text, note[i]). The lower part of the table includes other potential inhibitors mentioned in the text, including metabolites of carbetocin. [b] Plasma clearance (Sweeney et al. 1990, cit.[48]). [c] Clearance derived from the uterotonic effect (Hunter et al. 1992, cit.[49]). [d] Clearance derived from the uterotonic effect (Barth et al. 1980, cit.[37]). [e] Identified cleavage products of carbetocin[36]. [f] N^{α}-acetyl-2-*O*-methyltyrosine-oxytocin[40].

[h] Generic under the name Duratocin®.

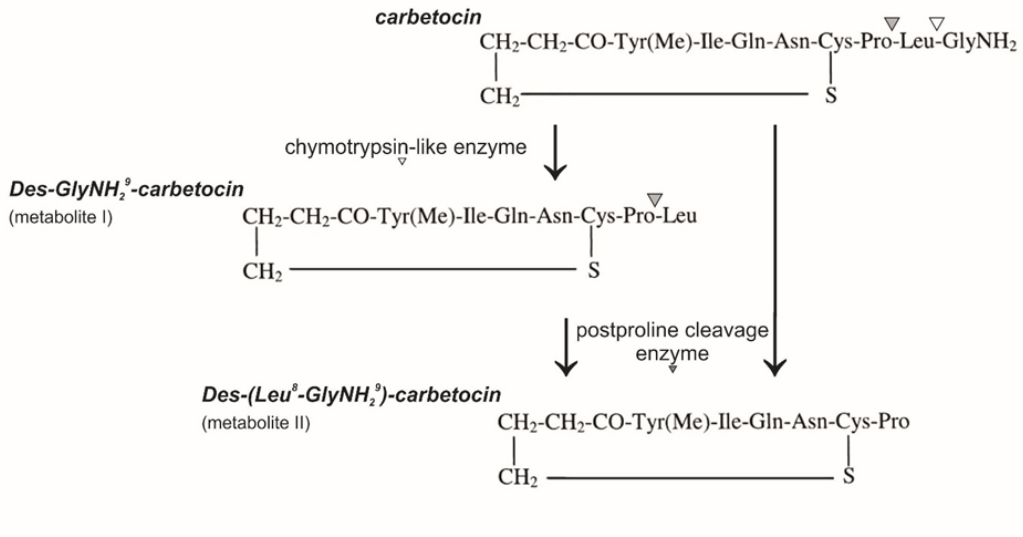

Fig. 3. **Potential metabolites of carbetocin identified *in vitro* and their antagonistic binding (pA_2) at the oxytocin receptor** (rat)[36]. Assumed is parallel cleavage of the C-terminal peptide bond leucine-glycinamide by a "chymotrypsin-like enzyme"[50–52](white triangle; metabolite I), and by an enzyme hydrolyzing the Pro7-Leu8 bond ("postproline cleavage enzyme"[53,54], black triangle; metabolite II) in the first step, followed by cleavage of metabolite I to the final metabolite II in the next step.

tocolytic effect could not have been assumed; the peptide exhibited approximately an uterotonic activity ten times lower than oxytocin (see Table I). The half-life of the uterotonic effect *in vivo* in the rat is several times longer than that of oxytocin[37] (the ratio of the elimination rate constants is 3.5), probably mainly due to the deletion of the N-terminal amino group (pharmacological data are summarized in Table I). In summary, when compared with oxytocin, carbetocin appeared to be a long-acting agonist with a weaker uterotonic effect. At that time, however, antagonistically acting oxytocin analogues were one of the priority projects of the IOCB[38], and this interest also motivated the synthesis of carbetocin. A few remarks:

The antagonism of the uterotonic effects of oxytocin was, in the second half of the 1960s, mainly investigated by Josef Rudinger group at the IOCB and in the group of Ivan Krejčí at the Research Institute for Pharmacy and Biochemistry (VUFB) in Prague. Their studies were summarized in a number of publications[23,38,39]. The interest in oxytocin antagonists was also stimulated by the presumed possibility of their use in clinical cases of threatening miscarriage (*abortus imminens*), and continued in Prague after Josef Rudinger's emigration to Switzerland in 1968. In the late 1960s, Karel Jošt synthesized 1-N^{α}-acetylcysteine-2-*O*--methyltyrosine-oxytocin[40], one of the first inhibitors of the uterotonic action of oxytocin; it was protected by patents in more than two dozen countries (e.g, US 3,752,799, priority date June 4, 1969; cit.[41]). Prior to the publication, Jan Mulder had expressed interest in this potential tocolytic and, together with one of us (V. P.), were in October 1969 preparing documentation for its filing in Sweden. The course of the licensing negotiations with the IOCB is unknown to us; it is likely that a license was granted but that a further development of the analogue did not proceed. It is possible that the next steps in clinical trials were postponed in the expectation that the newly synthesized compounds would possibly have more favourable antagonistic properties than the existing peptide with a relatively low inhibitory constant – on rat uterus *ex vivo* pA_2 of 7.03 was reported[42,i]. Certainly, the next steps in this direction were also influenced by the sudden death of Jan Mulder in April 1976. The intensive development of new antagonists in Malmö continued after the arrival of a new director of research, Hans Vilhardt, largely under the guidance of the pharmacologist Per Melin. In the second half of the 1980s this effort resulted in the 1-deamino-8-ornithine-vasopressin analogue with additional changes at position 2 (D-*O*-ethyl-tyrosine) and 4 (threonine)[43] which gradually became a standard inhibitor in experimental pharmacology and physiology of neurohypophysial peptides (pA_2 = 8.29) and, as Atosiban (INN), a widespread clinical tocolytic[44,j].

Apparently, the undetectability of antagonism in carbetocin after its synthesis was a disappointment to the Prague peptide group, and even its agonistic effects did not promise significant advantages over other uterotonics. This would explain why this analogue, or its original synthesis,

[i] pA_2: negative decadic logarithm of the molar antagonist concentration halving the response *E* to a corresponding agonist concentration, *E/2*.
[j] Tractocile®, *Ferring Pharmaceuticals* A/S.

was not patented in countries whose pharmaceutical industry was in a position to ensure its production in a short time. Thus, its patent coverage was not very consistent and many companies have carbetocin in their assortment. Conditions under which Ferring took over carbetocin into its production programme in such a situation (presumably again under a licence from the Czechoslovak Academy of Sciences) could not been ascertained by the authors of this communication.

Carbetocin as a veterinary drug

The first clinical use of carbetocin in veterinary medicine dates back to the second half of the 1970s. Probably at the instigation of Karel Jošt, the Research Institute for Biofactors and Veterinary Drugs in Pohoří-Chotouň near Jílový (Czech Republic; Bohumil Ševčík was its director) took over the veterinary clinical development of carbetocin and its introduction into veterinary practice. The institute was in June 1994 acquired by Fatro S.p.A., Bologna. The dossier for the FDA (U.S. Food and Drug Administration[k]) in the form of a "Drug Master File" (DMF)[l] for the drug substance (Depotocin®) was compiled in the Prague company PolyPeptide Laboratories s.r.o. for the company Veyx-Pharma GmbH, Sachwarzenborn (Hessen). The stated aim was to facilitate and manage parturitions in cows and sows, but above all to achieve synchronised parturitions in farms where female animals were artificially inseminated at the same term. It was also aimed at a reduction of the veterinarian's assistance in deliveries – characteristic aims of the collective livestock farming in the then Eastern Bloc countries. However, it turned out that while the birth of progenies could be widely synchronised, the delivery of the placenta was independent and had a different course. Nevertheless, carbetocin became a commonly used agent in veterinary medicine for induction and management of labour; it appeared to be generally milder than oxytocin just by reducing the number of labour and postnatal complications. An emphasised advantage was the safer management of labour in cows[45] and especially in (multiparous) pigs[46,m], absence of tetanic uterine contraction (common with oxytocin), avoidance of postpartum hypoxia/asphyxia in piglets and acceleration of postpartum uterine involution in cows[47].

It should be noted here that the research carried out at said institute has not been published *in extenso*. The first publications from other laboratories date from 1977–1979. However, research in veterinary medicine later facilitated the introduction of carbetocin in human medicine, especially in obstetric practice.

Carbetocin, human clinical application

In human medicine, carbetocin has become in the last two decades a globally used drug for the prevention and treatment of obstetric haemorrhage, the most common and dangerous complication of childbirth from the mother's point of view.

The physiological obstruction of postpartum haemorrhage follows after delivery of the foetus (in the third stage of labour) due to a redistribution in the blood circulation and a simultaneous activation of haemostatic factors. This final part of labour is clinically timed: it begins after the delivery of the foetus (the expulsion phase: the foetus ceases to be a foetus and becomes a newborn) and ends with the delivery of the placenta (placental phase). As the placenta separates from the uterine wall, haemostasis is controlled by a combination of two mechanisms:

– retraction of the smooth muscle of the uterus (myometrium). This causes a compression of the vasculature, which supplies, besides the myometrium, also the placenta. The result is the mechanical haemostasis;
– haemostatic tissue factors from the uterine mucosa in pregnancy (decidua[n]), an inhibitor plasminogen activator of type 1, systemic coagulation factors (fibrinogen, etc.), which all participate on blood clotting.

In pathological cases, a malfunction of at least one of the four factors involved (also referred to as the 4T-rule: tonus – trauma – tissue – thrombin) results in severe, even life-threatening bleeding, the *peripartum/postpartum haemorrhage* (PPH). This is one of the five most common causes of death in women related to pregnancy, childbirth and puerperium (thromboembolic disease, hypertensive disease, cardiopathy, sepsis, and PPH). PPH mortality comes about in both high and low per-capita-income countries, although the absolute risk of death from peripartum haemorrhage in high-income countries is many times lower.

To reduce the risk of PPH in all vaginal deliveries at placental phase, or after foetal delivery at caesarean section, intravenously or intramuscularly administered oxytocin was the drug of choice. Since the second half of the 1990s, carbetocin has been increasingly used in clinical medicine for the same purposes. The mechanism of action of the two peptides is the same; they increase uterine contraction (retraction) in the placental stage and thus counteract uterine hypotonia or even atony of the uterus, which is the cause of up to 80% of peripartum life-threatening haemorrhage (by definition, this is a blood loss of more

[k] U.S. Food and Drug Administration: state authority approving the use of pharmaceuticals and foodstuffs in the U.S.A.
[l] https://de.wikipedia.org/wiki/Drug_Master_File
[m] The author of this communication *is not the same* person as J. H. Cort (Joseph Henry Cort) mentioned in our foregoing reviews[1,2]: Nicholas Cort, PhD, DVM, Department of Obstetrics & Gynaecology, College of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, is listed as Senior Research Scientist at Ferring Pharmaceuticals. It is not known to us whether he is related to J. H. Cort.
[n] https://www.wikiskripta.eu/w/Decidua

than 1500 ml of maternal blood). Compared to oxytocin, a distinct advantage of carbetocin as a uterotonic in this critical obstetric situation is its significantly longer elimination half-life (approximately 8 times in non-pregnant women[48], 10 to 20 times 24–48 hours *post partum*[49]). It is administered only intravenously, at a rate of 100 mg/min; no serious side effects have so far been described when using this protocol.

As a result of incomplete patent protection of carbetocin as a substance, since the beginning of the 21st century several companies have been producing veterinary drugs based on carbetocin (Depotocin®:Veyx Pharma, Germany; WDT, Germany; Veyx-Pharma, Netherlands and Tettnang, Baden-Württemberg, BRD; Decomoton®: Calier Polska; Laboratorios Calier SA, Barcelona; etc.)[o]. Ferring AB, however, remains the most important distributor of its dosage forms (under various trade names[p]) for human medicine.

Carbetocin, designed and synthesized for the first time in the laboratory of Karel Jošt at the IOCB, has thus become an effective, frequently life-saving drug in critical obstetric conditions, thanks to the close cooperation with the Swedish company Ferring AB.

The authors are indebted to Dr. Paul Pliska for reviewing the English version of the manuscript – especially its parts relating to the technical and legal patent circumstances.

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.*

REFERENCES

1. Pliska V., Pařízek A., Flegel M.: Chem. Listy *116*, 20 (2021).
2. Pliska V., Pařízek A., Flegel M.: Chem. Listy *113*, 675 (2019).
3. Huguenin R. L., Boissonnas R. A.: Helv. Chim. Acta *49*, 695 (1966).
4. Sawyer W. H., Acosta M., Balaspiri L., Judd J., Manning M.: Endocrinology *94*, 1106 (1974).
5. Manning M., Balaspiri L., Moehring J., Haidar J. H. S. W.: J. Med. Chem. *19*, 842 (1976).
6. Zaoral M., Kolc J., Šorm F.: Collect. Czech. Chem. Commun. *32*, 1242 (1967).
7. Zaoral M., Kolc J., Šorm F.: Collect. Czech. Chem. Commun. *32*, 1250 (1967).
8. Pliška V., Krejčí I.: Arch. Int. Pharmacodyn. *161*, 289 (1966).
9. Vávra I., Machová A., Krejčí I.: J. Pharmacol. Exp. Ther. *188*, 241 (1974).
10. Vávra I., Machová A., Holeček V., Cort J. H., Zaoral M., Šorm F.: The Lancet *291*, 948 (1968).
11. Burn J. H.: Quart. J. Pharm. Pharmacol. *4*, 517 (1931).
12. Pliška V., Rychlík I.: Acta Endocrinol. *54*, 129 (1967).
13. Sawyer W. H.: Endocrinology *63*, 694 (1958).
14. Pliška V.: Arzneim.-Forsch./Drug Res. *16*, 886 (1966).
15. Kimura T., Yokoyama R.: Tohoku J. Exp. Med. *109*, 281 (1973).
16. Zaoral M., Vavra I., Machova A., Sorm F.: U.S. Patent: US 3,497,491 (1970).
17. Cash J. D., Gader A. M., da Costa J.: Br. J. Haematol. *27*, 363 (1974).
18. Prowse C. V., Sas G., Gader A. M. A., Cort J. H., Cash J. D.: Br. J. Haematol. *41*, 437 (1979).
19. Mannucci P. M., Ruggeri Z. M., Pareti F. I., Capitanio A.: The Lancet *310*, 1171 (1977).
20. Mannucci P. M., Ruggeri Z. M., Pareti F. I., Capitanio A.: The Lancet *309*, 869 (1977).
21. Jošt K., Procházka Z., Cort J. H., Barth T., Škopková J., Prusík Z., Šorm F.: Collect. Czech. Chem. Commun. *39*, 2835 (1974).
22. Kynčl J., Řežábek K., Kasafirek E., Pliška V., Rudinger J.: Eur. J. Pharmacol. *28*, 294 (1974).
23. Rudinger J., Pliška V., Krejčí I.: Rec. Prog. Hormone Res. *28*, 131 (1972).
24. Pliška V., Chard T., Rudinger J., Forsling M. L.: Acta Endocrinol. *81*, 474 (1976).
25. Kasafirek E., Rábek V., Rudinger J., Šorm F.: Collect. Czech. Chem. Commun. *31*, 4581 (1966).
26. Zaoral M., Pliška V., Řežábek K., Šorm F.: Coll. Czech. Chem. Commun. *28*, 747 (1963).
27. Zaoral M., Šorm F.: Collect. Czech. Chem. Commun. *30*, 2812 (1965).
28. Jošt K., Rudinger J., Šorm F.: Collect. Czech. Chem. Commun. *28*, 2021 (1963).
29. Beranková-Ksandrová Z., Bisset G. W., Jošt K., Krejčí I., Pliska V., Rudinger J., Rychlík I., Šorm F.: Brit. J. Pharmacol. *26*, 615 (1966).
30. Cort J. H., Hammer J., Ulrych M., Píša Z., Douša T., Rudinger J.: The Lancet *284*, 840 (1964).
31. Rabøl A., Juhl E., Schmidt A., Winkler K.: Digestion *14*, 285 (1976).

[o] Company overview: https://www.drugs.com/international/carbetocin.html
[p] Lonactene®, Duratocin®, Pabal®, Duratobal®.

32. Döhler K. D., Meyer M.: Best Pract. Res., Clin. Anaesthesiol. *22*, 335 (2008).
33. Kam P. C. A., Williams S., Yoong F. F. Y.: Anaesthesia *59*, 993 (2004).
34. Jošt K., Barth T., Krejčí I., Šorm F.: Czechoslovak Patent 149'028 B1 (1973).
35. Frič I., Kodíček M., Jošt K., Bláha K.: Collect. Czech. Chem. Commun. *39*, 1271 (1974).
36. Engstrøm T., Barth T., Melin P., Vilhardt H.: Eur. J. Pharmacol. *355*, 203 (1998).
37. Barth T., Slaninová J., Lebl M., Jošt K.: Collect. Czech. Chem. Commun. *45*, 3045 (1980).
38. Rudinger J., Krejčí I., in the book: *Neurohypophysial Hormones and Similar Polypeptides* (Berde B., ed.), p. 748. Springer Verlag, Berlin 1968.
39. Krejčí I., Poláček I., Rudinger J.: Br. J. Pharmacol. Chemotherapy *30*, 506 (1967).
40. Jošt K., Šorm F.: Collect. Czech. Chem. Commun. *36*, 297 (1971).
41. Jost K., Pliska V., Krejci I., Sorm F.: U.S. Patent 3,752,799 (1973).
42. Krejčí I., Kupková B., Barth T., Jošt K.: Physiologia Bohemoslovaca *22*, 315 (1973).
43. Melin P., Trojnar J., Johansson B., Vilhardt H., Åkerlund M.: J. Endocr. *111*, 125 (1986).
44. Åkerlund M., Carlsson A. M., Melin P., Trojnar J.: Acta Obstet. Gynecol. Scand. *64*, 499 (1985).
45. Věžník Z., Holub A., Zralý Z., Kummer V., Holčák V., Jošt K., Cort J. H.: Am. J. Vet. Res. *40*, 425 (1979).
46. Cort N., Einarsson S., Viring S.: Am. J. Vet. Res. *40*, 430 (1979).
47. Bajcsy Á. C., Szenci O., van der Weijden G. C., Doornenbal A., Maassen F., Bartyik J., Taverne M. A. M.: Theriogenology *65*, 400 (2006).
48. Sweeney G., Holbrook A. M., Levine M., Yip M., Alfredsson K., Cappi S., Ohlin M., Schulz P., Wassenaar W.: Current Therapeutic Research *47*, 528 (1990).
49. Hunter D. J. S., Schulz P., Wassernaar W.: Clin. Pharmacol. Ther. *52*, 60 (1992).
50. Barth T., Pliška V., Rychlík I.: Collect. Czech. Chem. Commun. *32*, 1058 (1967).
51. Barth T., Hütter H. J., Pliska V., Šorm F.: Experientia *25*, 646 (1969).
52. Glass J. D., Dubois B. M., Schwartz I. L., Walter R.: Endocrinology *87*, 730 (1970).
53. Walter R., Schlank H., Glass J. D., Schwartz I. L., Kerenyi T. D.: Science *173*, 827 (1971).
54. Yoshimoto T., Orlowski R. C., Walter R.: Biochemistry *16*, 2942 (1977).

Abstract

Licence agreements between the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague (IOCB), and the pharmaceutical company Ferring AB, Malmö, enabled the Swedish company to produce and commercialize worldwide a number of neurohypophysial peptides designed at the IOCB. Several of them found therapeutic applications.

dDAVP: 1-deamino-8-D-arginine-vasopressin was designed in one of the IOCB peptide laboratories (M. Zaoral and F. Šorm) in 1967. It displayed an extremely high antidiuretic activity (various tests indicate a 2- to 50-fold increase, as compared to arginine vasopressin) and a very low pressor activity. The peptide (covered by the U.S. Patent No. 3,497,491, priority 1966) has been used as a preferred drug in the substitution therapy of the central form of *diabetes insipidus* (Minirin®, today as Desmopressin INN). Besides, as later discovered (Mannucci et al. 1977), dDAVP increases the plasma concentration of the blood-clotting factor VIII. This fact extended its clinical use as haemostatics in cases of milder forms of haemophilia A, von Willebrand-Jürgens syndrome and some thrombocyte dysfunctions. Despite the clinical success of dDAVP, a closer look reveals certain inadequacies in the presently available pharmacological data: several reports declare activity values and the prolongation effect (index of persistence) in very broad ranges.

Triglycyl-8-lysine-vasopressin (Terlipressin), a peptide with the lysine vasopressin chain extended at the N-terminal by a triglycine residue, acts mainly as a prodrug (releasing lysine vasopressin after aminopeptidase splitting at the N^{α}group). The analogue belongs to the so-called "synthetic hormonogens"; individual peptides carrying various acylating groups were synthesized in the mid-sixties at the IOCB and legally protected by U.S. Patent No. 3,558,590 (priority 1964). It was a part of the license agreements mentioned above. The activities of triglycyl-8--lysine-vasopressin (both antidiuretic and vasopressor) are about 100 times lower than those of lysine vasopressin, but its persistence is 5 times longer. As such, it is occasionally used in emergency medicine in cases of esophageal (and other gastroenteral) bleeding, traumatic or septic shock, in cirrhotic patients and patients with portal hypertension. Its use as an early abortion drug was discussed but not pursued.

Carbetocin (deaminocarba^{1}-2-*O*-methyltyrosine-oxytocin) was synthesized in the laboratory of Karel Jošt at the IOCB before 1971; its synthesis was covered by a Czechoslovak patent (CS-149'028 B1) filed 1971 (at that time, Czechoslovak patent law did not provide for the patentability of substances as such) and first published in a biophysical communication by Frič *et al*., 1974). As a part of the licence agreement, it was included in the production program of Ferring AB, but marketed later also by several other pharmaceutical companies due to an incomplete patent protection. The peptide is a moderately active uterotonic partial agonist and as such has been utilized in veterinary obstetrics for delivery induction in cows and (multiparous) pigs: its milder and better-controlled uterotonic action was found preferential as compared to oxytocin so far used for these purposes. In the last two decades, carbetocin has been commonly used also in the human obstetrics, especially to prevent the peri- and post-partum haemorrhage, from the maternal side the most frequent and

most severe delivery complication. It became a life-saving drug in emergency obstetrics.

● Pliska V., Pařízek A., Flegel M.: Chem. Listy *116*, 101–109 (2022).
● https://doi.org/10.54779/chl20220101