schliessende Hydrierung mit Pd-Mohr in Methanol/Eisessig/Wasser (6:1:1) lieferte H-Arg-Ser-Val-Gln-OtBu · Acetat. Nach Protonierung der freien Guanidinogruppe mit einem Äquivalent HCl lieferte eine stufenweise Kondensation von Dipeptideinheiten und nachfolgende Hydrogenolysen Bradykinin-Ser-Val-Gln-OtBu · HCl, das mit Cbo-Lys(BOC)-OH (DCCI/N-Hydroxysuccinimid) zum Tridecapeptid-Derivat und nach Hydrierung mit BOC-Met-Azid zum Di-BOC-Met-Lys-Bradykinin-Ser-Val-Gln-OtBu verlängert wurde: $Met_{0,83}$, $Lys_{1,02}$, $Arg_{2,18}$, $Pro_{3,34}$, $Gly_{1,05}$, $Phe_{1,97}$, $Ser_{1,58}$, $Val_{1,00}$, $Glu_{1,03}$; $[\alpha]_D^{23} = -129{,}5°$ ($c = 0{,}5$, H_2O). Nach Entfernung der Schutzgruppen mit 95%iger Trifluoressigsäure unter N_2 und Reinigung durch präparative Elektrophorese bei pH 2 wurde einheitliches PKFL (Ic) isoliert; Ausbeute 60%. $Met_{0,87}$, $Lys_{1,05}$, $Arg_{1,96}$, $Pro_{2,98}$, $Gly_{1,06}$, $Phe_{1,98}$, $Ser_{1,63}$, $Val_{1,01}$, $Glu_{1,00}$; $[\alpha]_D^{23} = -93°$ ($c = 0{,}5$, H_2O). Tert.-Butylester-Abspaltung von Bradykinin-Ser-Val-Gln-OtBu mit Trifluoressigsäure und Überführung in das Azetat lieferte freies Bradykinin-Ser-Val-Gln-OH (IIIc): $Arg_{1,85}$, $Pro_{3,10}$, $Gly_{0,91}$, $Phe_{1,95}$, $Ser_{1,37}$, $Val_{1,00}$, $Glu_{0,90}$; $[\alpha]_D^{23} = -89{,}5°$ ($c = 0{,}5$, H_2O).

Von den synthetisierten Verbindungen Ia–c, IIa, IIIb, c und IVb wurden direkt und nach Inkubation mit Trypsin, Crotalus-adamanteus-Gift, Pankreas-Kallikrein, Carboxypeptidase A die Aktivitätsäquivalente (γ Bradykinin/mg Peptid) am Meerschweinchenileum bestimmt, vgl. [8])[16].

Peptid	Trypsin	Crotalus-adamanteus-Gift	Pankreas-Kallikrein	CPA	Meerschweinchenileum (direkt)
Natürlich I	350	300	80	90	2
Ia	160	80	60		
Ib	–[a]	175	–[a]	–[a]	3
Ic	400	175	100	100	3
IIa	–[a]	40	–[a]	–[a]	3
IIIb	–[a]	500	–[a]	–[a]	1,5
IIIc	500	500	500	600	0,5
IVb	–[a]	450	–[a]	–[a]	0,4

[a] nicht getestet.

Summary. H-Met-Lys-bradykinin-Ser-Val-Gln-OH and H-Met-Lys-bradykinin-Ser-Val-Gln-Val-OH, sequences of bovine serum kininogen have been synthetized by different approaches. The natural and synthetic peptides were tested for their kinin liberating potency.

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Solid Phase Synthesis and Some Pharmacological Properties of 8-Glutamine-Oxytocin: a Possible Intermediate in the Evolution of the Neurohypophysial Hormones[1]

It has been suggested that 8-glutamine-oxytocin could be an intermediate in the evolution of the neurohypophysial hormones[2]. The isolation and characterization of an 8-glutamine substituted analog of oxytocin, glumitocin (4-Ser-8-Gln-oxytocin), from the elasmobranchs *Raia clavata*[3], *R. batis*, *R. fullonica* and *R. naevus*[4] gave further support to this hypothesis. Thus it had been suggested that an unidentified oxytocic principle, designated as EOP 1 (elasmobranch oxytocin-like principle 1)[5] which has recently been shown not to be glumitocin[6] might in fact be 8-glutamine-oxytocin[7]. To determine whether or not this might be the case, the synthesis of 8-glutamine-oxytocin was carried out by the Merrifield solid phase method[8] as applied to the synthesis of oxytocin[9] and glumitocin[10]. The synthetic product has been pharmacologically evaluated by methods previously described[5,11]. The results obtained together with the corresponding data for EOP 1 are presented in the Table.

The required protected nonapeptide amide intermediate was synthetized in a stepwise manner beginning with 3.0 g of t-butyloxycarbonylglycyl resin containing 0.591 mmole of glycine according to the general procedure of Merrifield[12], using the modification previously described[9,10]. 8 cycles of deprotection, neutralization and coupling were carried out with appropriate Boc-amino acids[13] producing the protected nonapeptide esterified to the resin. Boc-amino acids with protected side chains were S-Bzl-Cys, and O-Bzl-Tyr. The final cysteine residue was added as the N-Carbobenzoxy-S-Benzyl (N-Z-S-Bzl) derivative. All coupling reactions to form peptide bonds were mediated by dicyclohexylcarbodiimide[14] in methylene chloride except those involving the carboxyl groups of Asn and Gln, which were allowed to react in dimethylformamide (DMF) as their nitrophenyl esters[15].

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Pharmacological activities[a] and activity ratios[b] with standard errors of 8-Gln-Oxytocin and EOP 1[c]

	8-Glutamine-oxytocin	EOP 1
Rat uterus (RUsMg) (no Mg in bath)	58	
Rat uterus (RUcMg) (0.5 m*M* Mg in bath)	122	
RUcMg/RUsMg = R_{Mg}	2.1 ± 0.05	1.8–3
Rat vasopressor (RVP)	34	
RVP/RUsMg = R_{VP}	0.58 ± 0.02	< 0.02
Rat antidiuretic (RAD)	5.9	
RAD/RUsMg) = R_{AD}	0.10 ± 0.02	0.007
Isolated bullfrog bladder water permeability (FB)	1270	
FB/RUsMg = R_{FB}	22 ± 3	2.4–4.2
Rabbit milk-ejecting (ME)	256	
ME/RUsMg = R_{ME}	4.4 ± 0.2	1.5 ± 0.05

[a] Expressed in units/mg. [b] Biological assays were carried out and activity ratios were calculated as described in [11]. [c] See [5–7].

Following the coupling of the final residue, the resin was removed from the synthesis vessel, washed and dried in vacuo. Ammonolytic cleavage of the protected nonapeptide resin (2.0 g) was carried out as previously described[9] to give the protected nonapeptide amide Z-Cys (Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Gln-Gly(NH_2) as a white amorphous powder, weight 250 mg; mp 256 to 258°C, $[\alpha]_D^{21}$-33.5° (c, 1, dimethylformamide). Anal. calcd. for $C_{71}O_{15}N_{13}H_{89}S_2$: C, 59.70; H, 6.24; N, 12.75. Found: C, 59.53; H, 6.43; N, 12.65. The yield of the protected nonapeptide amide based on the amount of glycine originally esterified to the resin was 50%. Amino acid analysis[16] gave: Asp, 1.00; Glu, 2.00; Pro. 1.00; Gly, 1.00; Ile, 0.94; Tyr, 0.81; Bzl-Cys, 2.1; NH_3, 4.0. Debenzylation of the protected nonapeptide was performed with sodium and liquid ammonia[17] following the general procedure used in the synthesis of deamino oxytocin[18]. A glass tube containing fresh sodium was dipped intermittently into a solution of the protected nonapeptide (100 g) in anhydrous refluxing liquid ammonia (300 ml). The final light blue color was discharged after 15 sec by the addition of 3 drops of dry glacial acetic acid. The resulting dithiol was oxidized at pH 6.5 with an aqueous solution of 0.011 *M* potassium ferricyanide[18] (5 ml) and the solution was lyophilized. The 8-Gln-oxytocin was purified by gel filtration[19] as follows: the lyophilizate was dissolved in 3 ml of 50% acetic acid and applied to a Sephadex G-15 (40–120 μ) column (1.2 × 110 cm) which was pre-equilibrated with 50% acetic acid. The column was eluted with the same solvent at a flow rate of 10 ml/h and 2 ml fractions were collected by an automatic fraction collector. The peptide material, as measured by both UV-absorption at 280 nm and by the Folin-Lowry method[20], emerged from the column as 2 peaks clearly separated from inorganic salts. The second peak, which contained the majority of the peptide material was located between fractions 30–36 with the maximum color value at fraction 33, whereas the chloride was detected between fractions 60–70. The fractions corresponding to the major peptide peak were pooled, diluted with twice the volume of water and the resulting solution was lyophilized. The lyophilized, desalted material was dissolved in 2 ml of 0.2 *N* acetic acid and subjected to gel filtration on a Sephadex G-15 (40–120 μ) column (1.2 by 110 cm) that had been pre-equilibrated with 0.2 *N* acetic acid. The column was eluted with 0.2 *N* acetic acid at a flow rate of 10 ml/h and 2 ml fractions were collected. A plot of Folin-Lowry color values of the fractions showed a single symmetrical peak with a maximum at fraction 43. The fractions corresponding to this peak were pooled and lyophilized to give a white powder; weight 38 mg, $[\alpha]_D^{22.0}$-16° (C, 0.5, 1 *N* acetic acid). Anal. calcd. for $C_{42}O_{13}N_{13}H_{63}S_2$: C, 49.36; H, 6.17; N, 17.83.Found: C, 49.24; H, 6.21; N, 17.68. Amino Acid Analysis gave: Asp. 1.00; Pro 1.05; Gly, 0.94; Cys, 1.89; Ile, 0.97; Tyr 0.92; NH_3, 4.18.

Examination of aliquots (100 μg) by thin-layer chromatography on silica gel H and by paper chromatography (ascending) on Whatman No. 1 paper in the solvent system butanol-acetic acid-water (4:1:5)[21] using ninhydrin and platinum reagent[22] for detection revealed only one component with an Rf of 0.24 in both instances. Likewise only one component in the direction of the cathode was observed when paper electrophoresis of a further aliquot (100 μg) in 2 pyridine acetate buffers of pH 3.5 and 6.5 was carried out using the same detecting reagents. The overall yield of pure product was 26.8% based on the initial glycine incorporation on the resin.

The pharmacological properties of synthetic 8-glutamine-oxytocin (Table) clearly differentiate it from EOP 1. This is most evident by a comparison of their activities on the bullfrog bladder and on rat vasopressor and antidiuretic assays. It can be concluded, therefore, that EOP1 is not 8-glutamine-oxytocin. However, this does not rule out the possibility that 8-glutamine-oxytocin does occur naturally. It may very well be present in a hitherto unexamined vertebrate. Should this indeed be the case, the knowledge of its pharmacological properties, as outlined in the present communication, should lead to its rapid detection and characterization when the appropriate tissue extracts become available.

Zusammenfassung. Die erstmalige Synthese des Peptidhormons 8-Glutamin-Oxytocin wird beschrieben. Das synthetische Peptid ist mit einem anderen Peptid des Dornhaies von noch ungeklärter Struktur nicht identisch.

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[13] The abbreviations used for amino acids and protecting groups are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, J. biol. Chem. *241*, 2491 (1966); Biochemistry *5*, 1445, 2485 (1966).

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