Furthermore, students of physiological rhythms need not restrict their attention to the circadian component by viewing only its presence or absence in the raw data. In a variance spectrum components which are quite irregular on inspection of the raw data can also be objectively quantified and bands in the higher-than-circadian domain of frequencies are actually of interest to a more complete analysis of circadian system physiology. Factors underlying shifts of variance from the circadian component into the domain of adjacent frequencies seem to be of particular interest[19–21].

Zusammenfassung. Der Sauerstoffverbrauch von *Periplaneta americana* (L.) wurde im Dauerlicht und im 24-stündigen Licht-Dunkelwechsel bei 30, 24 oder 18°C alle 20 min bestimmt. Im Varianzspektrum dieser Beobachtungsreihen lassen sich neben der ungefähren Tagesperiodik (Circadian-Periodik) auch höherfrequente, unregelmässigere und weniger prominente Rhythmen nachweisen.

A. G. RICHARDS and F. HALBERG

Departments of Pathology and Entomology, University of Minnesota (Minneapolis, U.S.A.), September 6, 1963.

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Vasopressin Analogues with Selective Pressor Activity

It is generally recognized that the basicity of the amino acid residue in position 8 of the molecule, i.e. in the penultimate position of the peptide side-chain, is of considerable importance for the specific biological properties of the vasopressins. This has been pointed out for both the pressor[1] and the antidiuretic[2] effect. In continuation of our studies on the influence of small structural modifications on the pharmacological properties of the neurohypophysial hormones[3,4], some new analogues modified in the position 8 have been synthesised and investigated biologically. Of these compounds, ornithine[8]-vasopressin, phenylalanine[2]-ornithine[8]-vasopressin, ornithine[8]-oxytocin and phenylalanine[2]-ornithine[8]-oxytocin proved to be particularly interesting. The present paper gives a short account of their synthesis[5] and main pharmacological properties.

Nα-CBO-Nδ-tosyl-L-ornithine was condensed with ethyl glycinate by the dicyclo-hexylcarbodiimide method to yield ethyl Nα-CBO-Nδ-tosyl-L-ornithyl-glycinate. After removal of the CBO protecting group, this dipeptide was condensed with N-CBO-L-proline by the same method. The product, ethyl N-CBO-L-prolyl-Nδ-tosyl-L-ornithyl-glycinate, was converted by amidification to the corresponding amide. Removal of the CBO protecting group and condensation with N-CBO-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-azide[6] afforded N-CBO-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide. After removal of the CBO protecting group this hexapeptide was condensed with *p*-nitrophenyl N-CBO-S-benzyl-L-cysteinyl-L-tyrosyl-L-phenylalaninate[7] to N-CBO-S-benzyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide, with *p*-nitrophenyl N-CBO-S-benzyl-L-cysteinyl-L-phenylalanyl-L-phenylalanate[7] to N-CBO-S-benzyl-L-cysteinyl-L-phenylalanyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide, with *p*-nitrophenyl N-tosyl-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucinate[8] to N-tosyl-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide and with *p*-nitrophenyl N-CBO-S-benzyl-L-cysteinyl-L-phenylalanyl-L-isoleucinate[7] to N-CBO-S-benzyl-L-cysteinyl-L-phenylalanyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide.

Removal of the protecting groups of the four above-mentioned nonapeptides by treatment with sodium in liquid ammonia, followed by oxidation with potassium ferricyanide, purification by counter-current distribution in the system sec-butanol/water/trifluoroacetic acid (120:160:1), conversion to the acetate and lyophilization, yielded Orn[8]-vasopressin, Phe[2]-Orn[8]-vasopressin, Orn[8]-oxytocin and Phe[2]-Orn[8]-oxytocin respectively. These four peptides were proved to be pure by different chromatographic and electrophoretic methods and they gave correct elementary analysis and amino acid composition on hydrolysis.

The main pharmacological effects of these peptides were determined by comparing them with the Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances[9]. Both the pressor and the antidiuretic potencies were assayed in rats: the former on the blood pressure of animals in urethane anaesthesia after pretreatment with an adrenergic blocking agent[10,11], the

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Pharmacological activities in international units per mg

Compound	Rat uterus (isolated)	Chicken blood pressure	Rabbit mammary gland	Rat blood pressure	Rat antidiuresis
Arg^8-vasopressin	~ 20	~ 60	~ 70	~ 400	~ 400
Phe^2-Arg^8-vasopressin	~ 0.2	< 1	3 ± 0.4	122 ± 13	~ 350
Lys^8-vasopressin	5 ± 0.5	40 ± 5	60 ± 10	270 ± 20	~ 250
Phe^2-Lys^8-vasopressin	~ 0.3	~ 0.15	~ 2.5	55 ± 7	20 ± 2
Orn^8-vasopressin	10 ± 2	21 ± 1	~ 50	360 ± 26	88 ± 17
Phe^2-Orn^8-vasopressin	~ 0.5	no depressor effect	~ 3	153 ± 11	16 ± 2
Orn^8-oxytocin	42 ± 5	90 ± 3	95 ± 6	103 ± 10	2.5 ± 0.3
Phe^2-Orn^8-oxytocin	~ 1	~ 3	7 ± 2	120 ± 10	0.55 ± 0.08
Oxytocin	450 ± 30	450 ± 30	450 ± 30	5 ± 1	5 ± 1

latter on the high level diuresis induced by water load and alcohol administration[12–14]. The so-called oxytocin-like activities were assayed on isolated uteri of oestrous rats[15], on the arterial pressure of roosters anaesthetized with phenobarbital sodium[16,17] and on the mammary gland of lactating rabbits in urethane anaesthesia[18,19].

The activities of Orn^8-vasopressin, Phe^2-Orn^8-vasopressin, Orn^8-oxytocin and Phe^2-Orn^8-oxytocin, as determined in the above-mentioned tests, are summarized in the Table which also contains, for comparison, the corresponding potencies of Arg^8-vasopressin, Lys^8-vasopressin, Phe^2-Arg^8-vasopressin, Phe^2-Lys^8-vasopressin and oxytocin (from 3). All figures refer to International Units per mg peptide (free base).

It is evident that Orn^8-vasopressin is a highly active synthetic peptide of the neurohypophysial type. Its most prominent feature is its pressor activity which is considerably greater than that of Lys^8-vasopressin and not far removed from that of Arg^8-vasopressin. In contrast to its pronounced vasoconstrictor effect, the antidiuretic potency of Orn^8-vasopressin is weak: only one-fourth of its pressor activity. The renal effect of Orn^8-vasopressin is therefore much less pronounced than that of Lys^8-vasopressin and Arg^8-vasopressin.

In its oxytocin-like properties, Orn^8-vasopressin follows the pattern of the vasopressins occurring in nature: the effect on the myoepithelial elements of the mammary gland is its most prominent oxytocin-like activity, its avian vasodilator (blood pressure lowering) potency is weaker and its uterine contracting activity weaker still. The overall oxytocin-like activity of Orn^8-vasopressin is less pronounced than that of Lys^8-vasopressin and Arg^8-vasopressin.

The basicity of the ornithine residue is in the same range as that of the lysine residue and less pronounced than that of the arginine residue. The side-chain in position 8 is shorter in the case of Orn^8-vasopressin than in the case of the two naturally occurring vasopressins. This change in the shape of the molecule clearly does not impair its ability to combine with the constrictor receptors of vascular smooth muscle, but it weakens the power to combine with the receptors in the distal parts of the nephron.

The pharmacological properties of Phe^2-Orn^8-vasopressin (Desoxy-Orn^8-vasopressin) indicate that the replacement of the tyrosine residue in position 2 of Orn^8-vasopressin by a phenylalanine residue—i.e. the removal of a phenolic OH-group—attenuates all the biological activities. Indeed, the avian depressor action is lost altogether. Of the two vasopressin-like effects, that on the blood pressure is less strongly affected than that on the diuresis. The ratio between these two potencies is thus increased from 4:1 (Orn^8-vasopressin) to 10:1 (Phe^2-Orn^8-vasopressin).

A qualitatively similar although quantitatively less pronounced dissociation of these two types of activity was observed[20] when the same structural modification was carried out in position 2 of lysine-vasopressin resulting in Phe^2-Lys^8-vasopressin (Desoxy-Lys^8-vasopressin). However, the elimination of the phenolic hydroxyl-group in position 2 of arginine-vasopressin yields Phe^2-Arg^8-vasopressin (Desoxy-Arg^8-vasopressin), whose antidiuretic activity is relatively greater than its pressor activity. The pharmacological properties of Phe^2-Orn^8-vasopressin could, therefore, hardly have been predicted.

It is particularly interesting that the ornithine^8-analogue of oxytocin (Orn^8-oxytocin = Orn^8-vasotocin = Ile^3-Orn^8-vasopressin) shows an even stronger dissociation of the pressor and antidiuretic activities, their ratio being 40:1. When the tyrosine residue of this peptide is replaced by a phenylalanine residue (Phe^2-Orn^8-oxytocin = Desoxy-Orn^8-oxytocin = Desoxy-Orn^8-vasotocin = Phe^2-Ile^3-Orn^8-vasopressin), the so-called oxytocin-like activities are further diminished (as was already observed[20] in the case of Phe^2-Lys^8-vasopressin and Phe^2-Orn^8-vasopressin), and the ratio between the pressor and the antidiuretic potencies increases still further, reaching nearly 220:1.

Zusammenfassung. Die Synthese und die pharmakologischen Haupteigenschaften von Orn^8-Vasopressin, Phe^2-Orn^8-Vasopressin, Orn^8-Oxytocin und Phe^2-Orn^8-Oxytocin werden beschrieben. Die pressorische Aktivität dieser Peptide zeigt eine in der Reihenfolge zunehmende Selektivität.

B. Berde, R. A. Boissonnas,
R. L. Huguenin, and E. Stürmer

Medizinisch-biologische und Pharmazeutisch-chemische Forschungslaboratorien der Sandoz AG, Basel (Switzerland), 30. September 1963.

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