Desamino-arginine-vasopressin, an Analogue of Arginine-vasopressin with High Antidiuretic Activity

Investigations into the basic pharmacological qualities of a large number of synthetic peptides related to the neurohypophysial hormones showed that relatively small modifications of the structures occurring in nature can affect the biological activities to a varying extent[1,2]. Among the modifications which were carried out on the N-terminal end of the molecules, the elimination of the N-terminal amino group of oxytocin has been shown to have no deleterious effect on the activities of this hormone[3,4]. Most oxytocin-like properties are even enhanced by this modification[5-7]. Removal of the N-terminal amino group of lysine-vasopressin produces no change in its antidiuretic activity, but causes a loss of half its pressor effect[8].

We have investigated the effect of the same modification on arginine-vasopressin.

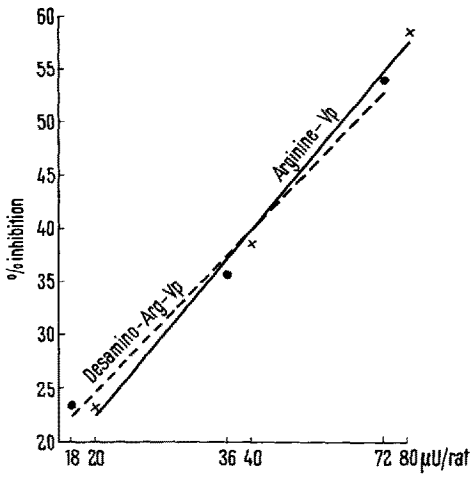
Methyl L-phenylalaninate was reacted with 2,4,5-trichlorphenyl O,N-bis-benzyloxycarbonyl-L-tyrosinate to yield methyl O,N-bis-benzyloxycarbonyl-L-tyrosyl-L-phenylalaninate. After removal of the benzyloxycarbonyl groups by hydrogen bromide in acetic acid, this dipeptide was condensed with 2,4,5-trichlorphenyl S-benzyl-β-mercaptopropionate to methyl S-benzyl-β-mercaptopropionyl-L-tyrosyl-L-phenylalaninate. This was converted over the hydrazide to the corresponding azide, which was reacted with L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-G-tosyl-L-arginyl-glycinamide to give S-benzyl-β-mercaptopropionyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-G-tosyl-L-arginyl-glycinamide (m.p. 196°; $[\alpha]_D^{23} = -36.5°$ in dimethylformamide. Calculated for $C_{87}H_{84}O_{14}N_{14}S_3 \cdot 1H_2O$: C 56.5; H 6.1; O 16.9; N 13.8; S 6.8%. Found: 56.5; H 6.2; O 17.1; N 14.1; S 6.7%). Removal of the protecting groups by treatment with sodium in liquid ammonia, followed by oxidation with hydrogen peroxide and counter-current distribution (K = 0.33) in the system sec-butanol/water/acetic acid (120:160:1) yielded desamino[1]-arginine-vasopressin diacetate, which was proved to be pure by different chromatographic and electrophoretic methods and gave the correct amino acid composition on hydrolysis. ($[\alpha]_D^{23} = -92.5°$ in 0.1*N* acetic acid. Calculated for $C_{46}H_{64}O_{12}N_{14}S_2 \cdot 2CH_3COOH$: C 50.5; H 6.1; O 21.5; N 16.5; S 5.4%. Found: C 50.2; H 6.2; O 21.6; N 16.2; S 5.6%.)

In order to assess the main pharmacological activities of the new peptide, it was compared with the 3rd International Standard for Oxytocic, Vasopressor and Antidiuretic Substances[9] in five tests. Two so-called vasopressin-like activities were measured: the pressor potency in rats under urethane anaesthesia after pretreatment with an adrenergic blocking agent[10,11] and the antidiuretic activity in rats with a high level of urine secretion induced by oral administration of water and alcohol[12-14]. The so-called oxytocin-like activities were assayed on isolated uteri of oestrous rats[15], on the arterial blood pressure of roosters anaesthetized with phenobarbital sodium[16,17] and on the mammary gland of lactating rabbits under urethane anaesthesia[18,19].

The activities of desamino[1]-arginine-vasopressin, as determined in the above mentioned tests, are summarized in the Table, which also contains for comparison the corresponding potencies of arginine-vasopressin. All figures refer to International Units per mg peptide (free base). It is evident from these values, that of the oxytocin-like

Pharmacological activities in IU per mg

Compound	Oxytocin-like activities			Vasopressin-like activities	
	Rat uterus (isolated)	Chicken blood pressure	Rabbit mammary gland	Rat blood pressure	Rat antidiuresis
Desamino[1]-arginine-vasopressin	27 ± 4	150 ± 4	80 ± 30	370 ± 20	1300 ± 200
Arginine-vasopressin	~ 20	~ 60	~ 70	~ 400	~ 400



Dose-response curves of the antidiuretic activity of arginine-vasopressin (3rd International Standard = ——) and desamino[1]-arginine-vasopressin (= ----) in the rat. Each value (x = arginine-vasopressin; • = desamino[1]-arginine-vasopressin) is the average of 10 doses. The difference in the slopes is statistically not significant.

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activities of desamino[1]-arginine-vasopressin, only that on the chicken blood pressure is higher than the corresponding potency of arginine-vasopressin. The suppression of the N-terminal amino group of arginine-vasopressin does not equally affect the so-called vasopressin-like activities. The pressor activity is practically unchanged, whereas the antidiuretic potency is approximately tripled. With an antidiuretic activity of 1300 IU/mg, desamino-arginine-vasopressin is the most active compound so far found in the field of the neurohypophysial peptides. The dose-response curves of arginine-vasopressin (International Standard) and desamino[1]-arginine-vasopressin shown in the Figure were obtained in 6 rats by repeating each dose ten times in a fully randomized sequence. Statistical analysis showed that the calculated dose-response curves can be regarded as parallel. Consequently the high antidiuretic activity of desamino[1]-arginine-vasopressin is not confined to a particular dose range, but can be considered as generally valid.

Zusammenfassung. Die Synthese und die pharmakologischen Haupteigenschaften von Desamino[1]-Arginin-Vasopressin werden beschrieben. Dieses Peptid ist durch eine hohe (1300 IE/mg) und relativ selektive antidiuretische Wirksamkeit ausgezeichnet.

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Gonadotrophic Activity in Hypothalamic Extracts

It has been published repeatedly that some hypothalamic extracts can release the pituitary hormones. Several authors claimed success in obtaining a hypothalamic extract with LH releasing activity[1-4]. The depletion of ovarian ascorbic acid, the direct visualization of the tubal ova, the appearance of recent corpora lutea in the androgenized sterile female rat, were used as a test of the LH release. The extracts used by these authors were prepared from rat or sheep brains.

Guillemin[4] reported that the administration of hypothalamic extracts to sterile rats produced corpora lutea, and concluded from this and other evidence that these extracts contained an LH releasing factor. For the general work of our laboratory, it was important to check this conclusion in the androgenized sterile hypophysectomized rats.

Sheep hypothalamic extracts were prepared, following exactly the indication given by Guillemin. Sterile rats were obtained by means of injecting them with 200 μg of testosterone propionate on the 4th day after birth.

Animals were used when adult; some of them were hypophysectomized by way of parapharingeal or transauricular technique. Extracts were injected intravenously immediately after they were prepared. When hypophysectomized sterile rats were used, extracts were injected after the operation, as soon as the animal had recovered. Injected animals were killed 48–72 h after the injection. Immediately before the injection of the extract one of the ovaries was removed, weighed, fixed in Helly solution and kept as control of the action of the extracts. Weight and appearance of the corpora lutea were taken as indicators of gonadotrophic activity.

In the first experiment 6 sterile rats and 8 hypophysectomized sterile rats were injected with hypothalamic extracts. Corpora lutea were found in the ovaries of all the sterile rats and five out of eight hypophysectomized sterile rats showed also corpora lutea after the injection of hypothalamic extracts. In all the injected animals the weight of the remaining ovary increased. This experiment showed clearly that the production of corpora lutea cannot be attributed to an LH releasing factor but to an intrinsic gonadotrophic activity of the hypothalamic extracts (Figure 1).

A second experiment was undertaken in order to verify whether the gonadotrophic activity was found throughout the whole hypothalamus or concentrated in the region of the median eminence.

Eight sterile female rats were injected with extracts prepared from the region of the median eminence (Figure 2) and eight other rats with extracts prepared with the remaining hypothalamus.

Every animal of the group injected with the extracts from the median eminence showed corpora lutea in their ovaries, whereas the presence of corpora lutea among the animals of the group injected with the 'remaining' hypothalamus was shown by only one of them. This experiment pointed out that the gonadotrophic activity of the hypothalamic extracts was higher in the region of the median eminence than in the remaining hypothalamus. It is convenient to remember that the neurohypophyseal tissue which constitutes the median eminence and the

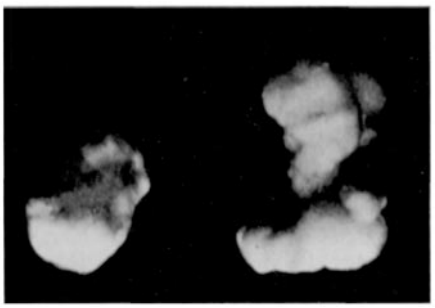

Fig. 1. Ovaries of a sterile hypophysectomized rat. Left: the ovary removed before injection of the extract. Right: the remaining ovary 48 h after injection of hypothalamic extract.

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