Adenine-1 recombination results

Serial no. of mutant	3	25	40	51	107	153	169	199	233	249
3	0 (*1.84*)	74 (*1.76*)	166 (*3.29*)	132 (*3.77*)	130 (*1.71*)	160 (*1.73*)	265 (*1.89*)	159 (*1.30*)	11.7 (*2.13*)	136 (*4.31*)
25		0.29 (*3.41*)	206 (*5.29*)	15.7 (*11.01*)	8.8 (*6.24*)	49.5 (*3.28*)	32.8 (*5.98*)	17.4 (*6.52*)	Comple-mentation	16.2 (*6.22*)
40			0.62 (*4.87*)	260 (*8.99*)	327 (*5.78*)	130 (*8.09*)	325 (*6.27*)	316 (*5.51*)	116 (*6.27*)	237 (*7.21*)
51				0 (*7.70*)	6.05 (*11.57*)	10.4 (*8.52*)	9.7 (*10.84*)	2.1 (*10.96*)	Comple-mentation	6.2 (*12.43*)
107					0 (*3.42*)	13.8 (*6.90*)	13.2 (*8.98*)	6.95 (*4.90*)	Comple-mentation	2.15 (*8.82*)
153						0 (*2.89*)	7.65 (*8.77*)	4.24 (*13.44*)	85 (*8.14*)	25.9 (*12.2*)
169							0 (*3.69*)	5.35 (*4.86*)	Comple-mentation	22.2 (*8.56*)
199								0 (*2.89*)	Comple-mentation	7.8 (*6.68*)
233									0 (*1.8*)	98 (*5.60*)
249										0 (*5.68*)

Adn$^+$ per 10^6 spores (weighted mean values, based on 2–5 estimates per cross). All mutants induced by UV. Example: 159 (*1.30*) = 159 adn$^+$ recombinants per 10^6 viable spores. $1.30 \cdot 10^6$ viable spores plated.

additional data[6,12], a probable order for the ten UV-induced adenine-1 mutants is 40...(233, 3)...(25, 107, 249, 51, 199, 169, 153) where those mutants in brackets are clustered together.

The recombination frequencies obtained are not strictly additive. Probably dilution and plating errors will have affected the estimates of adn$^+$ recombinants per 10^6 viable ascospores, and there may be present also errors resulting from the presence of low frequencies of diploid cells in the haploid strains which were crossed[12]. In this context it is interesting to compare the three sets of data available for some crosses between adenine-8 mutants[2,8,9]. HOLLIDAY[13] has recently postulated mechanisms by which non-additivity in intragenic recombination frequencies can occur.

Zusammenfassung. Zehn der von LEUPOLD isolierten UV-induzierten Mutanten beim Adenin-1-Gen von *Schizosaccharomyces pombe* sind in zehn verschiedenen Stellen im Gen lokalisiert.

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Deamino[1]-phenylalanine[2]-arginine[8]-vasopressin: A Peptide with Highly Selective Antidiuretic Activity

During the last ten years, the effect of slightly modifying the chemical structure of the neurohypophysial hormones on their principal biological activities has been rather extensively investigated[1,2].

Such studies have revealed that the suppression of the phenolic OH-group[1,3–12] in position 2 as well as the removal of the amino group[1,13–19] in position 1 leads to highly active compounds with interesting pharmacological profiles. Some of these compounds possess qualities which – from the therapeutic point of view – confer advantages over the naturally occurring neurohypophysial hormones.

We therefore investigated the influence of both these modifications together on human antidiuretic hormone by synthesizing and biologically testing deamino[1]-phenylalanine[2]-arginine[8]-vasopressin.

Methyl L-phenylalaninate was reacted with 2,4,5-trichlorphenyl N-benzyloxycarbonyl-L-phenylalaninate to

yield methyl N-benzyloxycarbonyl-L-phenylalanyl-L-phenylalaninate. After removal of the benzyloxycarbonyl group with hydrogen bromide in acetic acid, this dipeptide was condensed with 2,4,5-trichlorphenyl S-benzyl-β-mercaptopropionate to give methyl S-benzyl-β-mercaptopropionyl-L-phenylalanyl-L-phenylalaninate. This was converted via 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-phenylalanyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-G-tosyl-L-arginyl-glycinamide (m.p. 224°; $[\alpha]_D^{22} = -38°$ in dimethylformamide. Calculated for $C_{67}H_{84}O_{13}N_{14}S_3 \cdot 1\,H_2O$: C 57.2; H 6.2; O 15.9; N 13.8; S 6.8%. Found: C 57.1; H 6.0; O 16.0; N 13.7; S 6.9%). Removal of the protecting groups by treatment with sodium in liquid ammonia, followed by oxidation with hydrogen peroxide and counter-current distribution (K = 0.45) in the system sec-butanol/water/acetic acid (120:160:1) yielded deamino[1]-phenylalanine[2]-arginine[8]-vasopressin acetate, which was proved to be pure by various chromatographic and electrophoretic methods and gave the correct amino acid composition on hydrolysis. ($[\alpha]_D^{23} = -100°$ in 0.1 *N* acetic acid. Calculated for $C_{46}H_{64}O_{11}N_{14}S_2 \cdot 1\,CH_3COOH \cdot 2\,H_2O$: C 50.2; H 6.3; O 20.9; N 17.1; S 5.6%. Found: C 50.6; H 6.4; O 20.8; N 16.7; S 5.7%.)

In order to define the pharmacological profile of a new synthetic peptide of the neurohypophysial type, a battery of five tests is routinely used in our laboratories: The pressor and the antidiuretic potencies are assayed in rats: the former on the blood pressure of animals in urethane anaesthesia after pretreatment with an adrenergic blocking agent[20,21], the latter on the high level diuresis induced by water load and alcohol administration[22–24]. The so-called oxytocin-like activities are assayed on isolated uteri of oestrous rats[25], on the arterial blood pressure of roosters anaesthetized with phenobarbitone sodium[26,27] and on the mammary gland of lactating rabbits in urethane anaesthesia[28,29]. Deamino[1]-phenylalanine[2]-arginine[8]-vasopressin was submitted to two additional assays, designed to assess the effect on smooth muscle, namely the pressor effect on the blood pressure of spinal cats and the stimulating effect on motility of the rabbit ileum in vitro[3]. All activities were determined by comparison with a reference standard: the Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances[30].

The activities of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin, as determined in the above-mentioned tests, are summarized in the Table, which also indicates the corresponding potencies of deamino[1]-arginine[8]-vasopressin, phenylalanine[2]-arginine[8]-vasopressin and arginine-vasopressin for purposes of comparison.

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Pharmacological activities in international units per mg

Compound	Oxytocin-like activities			Vasopressin-like activities				Selectivity of the antidiuretic effect (A/B)
	Rat uterus (in vitro)	Chicken blood pressure	Rabbit mammary gland	Rat blood pressure (B)	Spinal cat blood pressure	Rabbit intestinal motility (in vitro)	Rat antidiuresis (A)	
Deamino[1]-Phe[2]-Arg[8]-vasopressin	*0.30 ± 0.09*	*less than 1, biphasic*	*~ 3.8*	*29 ± 7*	*30.8 ± 1.6*	*12.5 ± 2.7*	*800 ± 170*	*27.5:1*
Deamino[1]-Arg[8]-vasopressin	27 ± 4	150 ± 4	80 ± 30	370 ± 20	–	–	1300 ± 200	3.5:1
Phe[2]-Arg[8]-vasopressin	~ 0.2	< 1	3 ± 0.4	122 ± 13	–	–	~ 350	2.9:1
Arginine-vasopressin	~ 20	~ 60	~ 70	~ 400	–	–	~ 400	1:1

The antidiuretic activity of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin is qualitatively (phenotypically) similar to that of arginine-vasopressin. It differs from that of lysine-vasopressin in that it sets in more gradually and dies away more slowly (see, for example, [22]). The dose-response curves of arginine-vasopressin and deamino[1]-phenylalanine[2]-arginine[8]-vasopressin run parallel, so that the higher activity found for deamino[1]-phenylalanine[2]-arginine[8]-vasopressin may be considered to be generally valid and not merely confined to a particular dose range. The selectivity of the antidiuretic effect of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin, if contrasted with its vasoconstrictor activity, is, however, 27.5 times higher than that of arginine-vasopressin. The relatively weak effect on vascular smooth muscle is not restricted to one species, as the same low pressor activity was found both in spinal cats and in rats. Nor is this low activity on smooth muscle confined to vascular smooth muscle: its contractile action on intestinal smooth muscle, as evident from rabbit ileum assays, is weaker still.

It is also noteworthy that, whereas arginine-vasopressin possesses considerable uterotonic, avian depressor and milk-ejecting potencies, deamino[1]-phenylalanine[2]-arginine[8]-vasopressin shows hardly any oxytocin-like activities. Its oxytocin-like effect on the chicken blood pressure is atypical and indeed in this test it antagonizes oxytocin to a certain extent. To obtain 50% inhibition, it is necessary to inject 20 to 60 times more deamino[1]-phenylalanine[2]-arginine[8]-vasopressin than oxytocin.

The high degree of selectivity of the antidiuretic action of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin compared with its vasoconstrictor effect could hardly have been predicted: Although the omission of the phenolic group was known to increase the selectivity of the antidiuretic effect of arginine-vasopressin, this modification has been observed to have the opposite effect, i.e. to increase the selectivity of the pressor activity in the case of lysine-vasopressin, lysine-vasotocin, arginine-vasotocin, ornithine[8]-vasopressin and ornithine[8]-oxytocin. Suppression of the terminal amino group enhances antidiuretic selectivity in the case of arginine-vasopressin, lysine-vasopressin, ornithine-vasopressin, ornithine-oxytocin, oxytocin, phenylalanine[2]-ornithine-vasopressin, phenylalanine[2]-ornithine-oxytocin and phenylalanine[2]-oxytocin, albeit to a lesser extent than in the case of deamino[1]-phenylalanine[2]-arginine[8]-vasopressin. It seems therefore that the nature of the shift in pharmacological profile brought about by a relatively minor chemical modification depends not only on the alteration itself but also on the general structure of the molecule. It is also evident from these results that our present knowledge concerning the receptor sites involved in the antidiuretic and pressor activities of the neurohypophysial hormones is still rather incomplete and that predictions on structure/activity relationships can only be made within narrow limits.

Zusammenfassung. Deamino[1]-Phenylalanin[2]-Arginin[8]-Vasopressin, dessen Synthese und pharmakologische Haupteigenschaften beschrieben werden, zeichnet sich durch eine hohe antidiuretische Wirkung aus. Dieser Effekt ist demjenigen des menschlichen antidiuretischen Hormons qualitativ ähnlich, jedoch wesentlich selektiver.

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Induction of Antibody Response in Lymphoid Cells in vivo and in vitro by RNA-Immuno-Carrier from Immune Serum

In previous papers the presence of a RNA carrier of the antibody template in sera of immunized rabbits (RNA-immuno-carrier or RNA-IC) was reported: this RNA-IC extracted from the serum of immunized animals is able to elicit in normal animals a precocious antibody response to the same antigens used for immunizing the animal source of RNA-IC[1-5].

Since it is known that the second phase of the antibody production occurs in the lymphoid tissues[6], we tested, in the present investigations, the influence of RNA-IC from the serum of rabbits immunized with guinea-pig RBC on lymphoid cells in vivo and in vitro. In consideration of the importance of the thymus in the immune processes we tested also the action of the RNA-IC on thymic cells: in fact the problems concerning the thymus are several and very interesting because, although it is commonly assumed that this organ is of great importance for the normal development of the lymphatic tissue and for inducing the formation of immuno-competent cells, it is still doubtful whether the thymocites are able, by themselves, to produce antibodies[7-10].

Production of RNA-IC. Selected rabbits weighing about 2 kg, fed on a standard diet, were immunized by 6–8 intravenous injections of guinea-pig RBC at 4–6 day intervals (the average antibody titer obtained was 1:3200). The RNA-IC from the immune sera was extracted by the phenol method described by CHARGAFF[11] and subjected

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