Significance of Amino Acid Residues in Position 8 of Vasopressin on Contraction in Rat Blood Vessels[1] (37186)

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It is generally believed that the *length* of the amino acid side chain in position 8 of the vasopressin hormones (Fig. 1) plays little or no role in the rat pressor activity of these hormones while the degree of basicity of the amino acid moiety in position 8 of these neurohypophyseal peptides is thought to be the primary structural determinant of the constrictor potency of these molecules (1–4). Recent findings, however, on a variety of isolated canine blood vessels question such a tenet and suggest that: (i) side chain length may be quite important in determining contractile action on arterial smooth muscle; and (ii) maximal basicity alone in position 8 of the vasopressin hormones does not, usually, in itself result in maximal contractile activity (5). Overall, these latter *in vitro* studies suggest that optimum contractile activity at the effector vascular smooth muscle cells, as well as hormone–receptor affinity, may be the result of an optimum combination of a certain degree of basicity and length of the amino acid side chain in position 8.

Presumably, the blood pressure responses obtained with rat pressor assays are reflections of the constrictor actions of the vasopressin hormones on arterioles. But, to my knowledge, no *direct,* quantitative structure–activity data are, as yet, available for either the vascular smooth muscle effector cells of rat arteries or arterioles. The latter information is critical in view of the recent, aforementioned *in vitro* observations on canine blood vessels. The present experiments, using isolated rat aortas as well as direct, quantitative *in vivo* microscopy on rat mesenteric arterioles, were therefore undertaken to gain insight into the relationship between the chemical structure of the amino acid moiety in position 8 and contractile activity of the vasopressin hormones on the effector vascular smooth muscle cells.

Methods. Thoracic aortas were obtained from male rats (Wistar strain, 200–370 g), cut helically into vascular strips (1.3–1.5 mm in width by 25 mm in length) and set up isometrically *in vitro* under a resting tension of 1.5 g, essentially similar to that described previously for rabbit thoracic aorta (6). All vascular strips were equilibrated for 2 hr in muscle chambers containing Krebs–Ringer bicarbonate solution, the composition of which has been given previously (7). Magnesium (1.2 m*M*) was utilized since this concentration is essential for optimum neurohypophyseal hormone action on these isolated blood vessels [(8), unpublished data]. The Krebs–Ringer bicarbonate solution was oxygenated continuously with a 95% O_2–5% CO_2 mixture and kept at 37° (pH 7.2–7.5). Complete, cumulative log dose–response curves, similar to that described previously (5), were obtained for five different synthetic vasopressin analogues (9). The results for these experiments are expressed in percentage of maximal [8-arginine]-vasopressin contractile responses since the latter is the native rat pituitary hormone.

In vivo quantitative microscopic observations were carried out on rat mesenteric arterioles by means of an image-splitting television microscope recording system (10). For the latter male rats (Wistar strain, 135 ± 20 g) anesthetized with im pentobarbital

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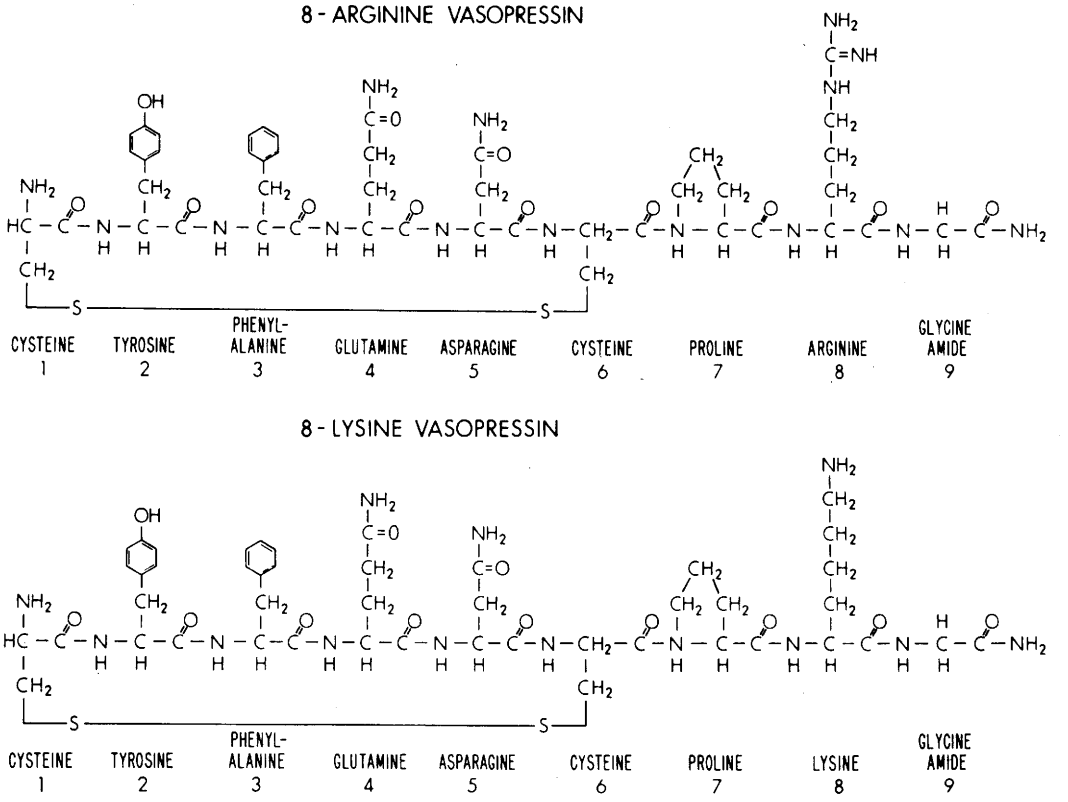

FIG. 1. Chemical structures of naturally occurring mammalian vasopressin hormones. [Arg^8]-vasopressin is found in posterior pituitary glands of most mammals, including rats and man. [Lys^8]-vasopressin is the naturally occurring hormone in the pig, peccary and hippopotomus.

(Nembutal, 3 mg/100 g body weight) were utilized. Only male rats were employed in these *in vitro* and *in vivo* studies, since estrogenic hormones are known to affect not only the blood pressure response to the systemic administration of vasopressin hormones (11) but to the local administration of these peptides as well (12). The rat mesentery was prepared and kept under physiologic conditions according to procedures described previously (13). Measurements for changes in arteriolar lumen size were made before (control) and after topical application of graded doses (8 to 12 in number) of the pure, synthetic vasopressin peptides (9). *In vivo* microscopic observations for discrete drug effects were made at magnifications up to 4000 $\times$ using the image-splitting television microscope recording system (10). The Ringer gelatin irrigation (13) of the mesentery was temporarily interrupted during topical drug applications. The effects of the vasopressin hormones and analogues on lumen diameter were recorded for at least 2–3 min after topical application either to the point where the contractile response stabilized or until complete lumen occlusion. Complete lumen occlusion is defined here as a touching of both internal walls of an arteriolar vessel and was simultaneously observed visually on the TV monitor and recorded on the polygraph. Leitz-Ultrapak water immersion objectives, 32$\times$ and 55$\times$, were used in conjunction with 10$\times$ Bausch and Lomb oculars on a Bausch and Lomb Dyna-Zoom microscope equipped with a trinocular head. The use of the image-splitting device allows one to make measurements with an accuracy 10 times that of the resolving power of the light microscope (10). Such a system has very recently been effectively used to make rapid *in vivo* micrometric measurements from which complete log

dose–response curves have been constructed for drug, as well as peptide, effects on various kinds of muscular microvessels, including arterioles (12, 14–16).

Results and Discussion. If the degree of basicity of the amino acid residue in position 8 is critical for optimizing contractile activity on blood vessels, then [8-arginine]-vasopressin (with arginine exhibiting a pK_3 of 13.2) (17) should not only elicit the greatest maximal contractile (or constrictor) response but, in addition, should show the greatest affinity (or lowest EC_{50}, ED_{50}) for the vasopressin receptor on all rat blood vessels. However, a glance at Figs. 2 and 3 (as well as Table I) reveals that although [8-arginine]-vasopressin does show the greatest affinity for the vasopressin receptor (indicated by greatest parallel shift of log dose–response curve to left) on rat mesenteric arterioles (*e.g.*, Fig. 3), it: (i) exhibits two and one-half times less affinity for the vasopressin receptor than does [8-ornithine]-vasopressin on isolated rat aorta (Fig. 2 and Table I), and (ii) elicits a significantly smaller maximal contraction on rat mesenteric arterioles (Fig. 2) than does [8-ornithine]-vasopressin. This is especially interesting in view of the fact that ornithine in [8-ornithine]-vasopressin is much less basic (*e.g.*, $pK_3 = 10.76$) (17) than arginine. Although the basicity of lysine ($pK_3 = 10.28$) (17), in [8-lysine]-vasopressin, is not appreciably different from ornithine, its dose–response curves (on both rat arterioles and aorta) are shifted almost twofold to the right of [8-ornithine]-vasopressin (Figs. 2 and 3). In addition, the maximum responses elicited by [8-lysine]-vasopressin are approximately 80% of [8-ornithine]-vasopressin on both types of blood vessels. It is important, however, to note that although the side chain length in position 8 of [8-lysine]-vasopressin is approximately equivalent to that of [8-arginine]-vasopressin it is longer than that of [8-ornithine]-vasopressin (Table I). These present findings could be used to suggest that while basicity in position 8 might be very important in affinity of the hormone for its receptor on mammalian vascular smooth muscle, an optimum side chain length in position 8 may be critical in determining maximum biologic activ-

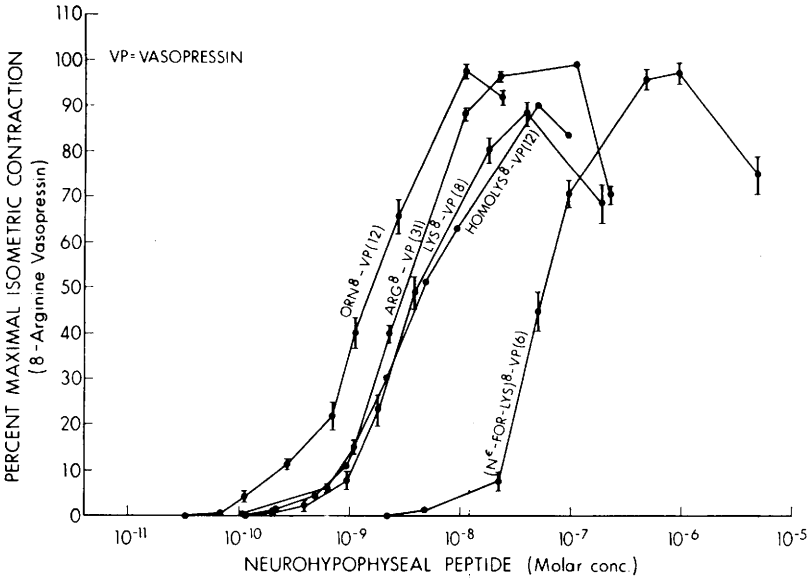

FIG. 2. Cumulative dose–response curves of vasopressin hormones and analogues on *in vitro* rat aortic strips mounted isometrically in Krebs–Ringer bicarbonate solution containing 1.2 m*M* magnesium. Maximal contractile response to [8-arginine]-vasopressin is taken as 100%. All other contractile responses are expressed as a percentage of this value. Values are mean responses ± 1 SEM. Numbers in parentheses are numbers of aortas studied from different male rats.

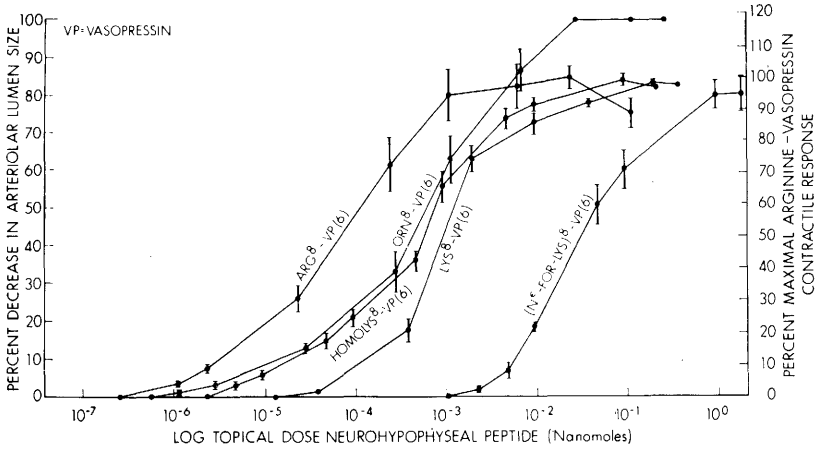

FIG. 3. Graded contractile responses of rat mesenteric arterioles to vasopressin hormones and analogues. Each point represents the mean value obtained from measurements on vessels of different male rats (indicated by numbers in parentheses). One type of vasopressin peptide was tested on each rat mesentery. The bars represent one SEM. The mean control lumen sizes for the arterioles (μm) were: Arg^8-vasopressin (28.9 $\pm$ 1.0), Orn^8-vasopressin (30.8 $\pm$ 1.8), Lys^8-vasopressin (33.5 $\pm$ 2.2), Homolys^8-vasopressin (28.7 $\pm$ 1.6), and (N^ϵ-for-lys)^8-vasopressin (33.4 $\pm$ 2.4).

ity (*i.e.*, degree or magnitude of contraction).

If the latter hypothesis is correct, then one might not expect vasopressin analogues having no or very little basicity in position 8 together with side chain lengths equivalent to that of lysine or longer than ornithine (*e.g.*, [N^ϵ-for-lys]^8-vasopressin) to exhibit any significant change in maximal contractile response (see Figs. 2 and 3). Such molecules would be expected to exhibit marked losses in affinity for the vasopressin receptor on blood vessels (indicated by parallel shifts of log-dose–response curves to right). Figures 2 and 3 do, indeed, show that the dose–response curves for [N^ϵ-for-lys]^8-vasopressin are shifted markedly to the right of the parent hormone, [8-lysine]-vasopressin, suggestive of losses in hormone–receptor affinity. But the ability of this hormone analogue (devoid of basicity in position 8) to induce a near maximal contraction is not impaired. The finding of reduced hormone–receptor affinity could explain the marked reduction in rat pressor activity seen with [N^ϵ-for-lys]^8-vasopressin when it is compared to [8-lysine]-vasopressin (18). Interestingly, lengthening the side chain of [8-lysine]-vasopressin by a single carbon atom (*e.g.*, [8-homolysine]-vasopressin) (4) does not result in a change of either hormone–receptor affinity or maximal contractile response when compared to the parent hormone (Figs. 2 and 3). (Since [8-homolysine]-vasopressin has the same basicity as [8-lysine]-vasopressin its affinity for the hormonal receptor should not change if the above hypothesis is correct.)

In this context, it is of interest to note that a combination of reduction in side chain length and loss of basicity in position 8 (*e.g.*, [3-isoleucine, 8-leucine]-vasopressin) not only results in 1000- to 21,000-fold shifts of the dose–response curves to the right of [8-arginine]-vasopressin (indicative of marked losses in hormone–receptor affinity) on these rat blood vessels (19) as well as on canine blood vessels (5) but, in addition, marked losses in ability to induce maximal contractile responses (5, 19). Although the latter findings, with [3-isoleucine, 8-leucine]-vasopressin, could also be reflections of steric changes, alterations in hydrophobicity, etc. (5), preliminary findings with [8-α,γ-diaminobutyric acid]-vasopressin (an analogue with a side chain one carbon atom *shorter*

TABLE I. The Effect of the Structure of the Side Chain of Amino Acids Substituted in the 8-Position of Vasopressin on Hormonal Affinity and Maximal Contractile Response in Rat Mesenteric Arterioles and Aorta.

Amino acid in 8-position	Structure of side chain	Arteriole		Aorta	
		Affinity[a] ($\times 10^{-9}$ moles/liter)	% Maximal[b] response	Affinity[a] ($\times 10^{-9}$ moles/liter)	% Maximal[b] response
Ornithine	$-CH_2-CH_2-CH_2-NH_2$	6.3 ± 0.4	100 ± 0.0	1.6 ± 0.2	99 ± 4.0
Arginine	$-CH_2-CH_2-CH_2-NH-C(=NH)-NH_2$	0.7 ± 0.05[c]	85 ± 6.0[e]	3.5 ± 0.2[f]	100 ± 3.0
Lysine	$-CH_2-CH_2-CH_2-CH_2-NH_2$	9.0 ± 0.5[c]	85 ± 2.0[e]	2.9 ± 0.3[f]	80 ± 5.0[g]
Homolysine	$-CH_2-CH_2-CH_2-CH_2-CH_2-NH_2$	5.6 ± 0.9[d]	84 ± 2.0[e]	3.0 ± 0.4[f]	81 ± 4.0[h]
Formyl-Lysine	$-CH_2-CH_2-CH_2-CH_2-NH-CH=O$	280.0 ± 32.0[c]	80 ± 8.0[e]	54.0 ± 2.3[c]	98 ± 5.0

[a] Mean concentration of agonist (± SEM) required to produce 50% of the maximal contractile response to vasopressin peptide [topical vol (0.1 ml) converted to moles/liter].

[b] % maximal (mean values ± SEM) attainable contractile response.

[c] Significantly different from all other analogues ($p < .02$) (Student's t test).

[d] Significantly different from all other analogues except [Orn^8]-vasopressin ($p < .02$).

[e] Significantly different from [Orn^8]-vasopressin ($p < .02$).

[f] Significantly different from [Orn^8]- and [Formlys^8]-vasopressin ($p < .02$).

[g] Significantly different from all other analogues except [Homolys^8]-vasopressin ($p < .02$).

[h] Significantly different from all other analogues except [Lys^8]-vasopressin ($p < .02$).

than ornithine, 30% shorter than lysine, and basicity *approximately equivalent* to lysine) (20) on rat mesenteric arterioles and aorta indicate that this vasopressin analogue also exhibits losses in maximal contractile activity even though its hormone–receptor affinity approximates that of [8-lysine]-vasopressin) (unpublished data). Such *in vitro* and *in vivo* findings on rat arterial and arteriolar smooth muscle, thus, lend further support to the concept that an optimum interaction between length of side chain and basicity in position 8 may, indeed, be necessary for maximizing vasopressin-induced contractile responses in mammalian somatic vascular muscle (5).

Although the present preliminary observations tend to suggest that a degree of basicity in position 8 of vasopressin may be extremely important in maintaining hormone–receptor affinity, while an optimum length of side chain may be critical in determining maximal biologic (contractile) activity on mammalian somatic blood vessels, further work with more synthetic analogues (especially analogues with isosteric or isofunctional substitutions) (21) will be required to buttress this hypothesis. These findings indicating widely divergent degrees of relative hormone–receptor affinities and maximal contractile responses for a variety of vasopressin hormone analogues on rat aorta versus rat mesenteric arterioles (Table I) could be used to explain some or all of the structure–activity relationship discrepancies observed between previous *in vitro* (5) (and present) observations and crude rat pressor assay. For example, since the blood pressure responses obtained with rat pressor assays are reflections of the overall average effects of the vasopressin molecules on many different small arteries and arterioles in many vascular beds, it is distinctly probable that blood vessels from different regions exhibit slightly different structure–activity relationships for a given series of vasopressin peptides. The present data when taken together with previous observations (5, 12, 15, 19, 22–26) emphasize the importance of direct studies on peripheral blood vessels rather than *indirect* pressure assays if one desires to gain insight into the relationship between chemical structure and contractile activity of the vasopressin hormones at the effector vascular smooth muscle cells.

It was recently demonstrated, by the image-splitting TV microscope recording system, that rat mesenteric metarteriolar vessels respond (*i.e.*, constrict) to arginine-vasopressin in physiologic concentrations (15). The present study demonstrates that not only do arterioles also respond to physiologic doses (Fig. 3) of vasopressin[3] (27) but are, on the average, 10 times more sensitive to this naturally occurring posterior pituitary hormone than are the metarterioles (15). In view of these quantitative data, one must consider the possibility that vasopressin plays an important role in control of arteriolar and metarteriolar pressure in the microcirculation.

Summary. The present quantitative results demonstrate that although an optimum interaction between length of side chain and a certain degree of basicity of the amino acid residue in position 8 is necessary for maximizing vasopressin-induced contractions of both rat aortas and mesenteric arterioles, basicity in position 8 is definitely *not* an absolute requirement for vasopressin-induced contractions of rat blood vessels. The length of the amino acid residue in position 8, in contrast to what is currently believed, may be quite important in determining the strength of vasopressin-induced contractions on both macro- and microscopic rat blood vessels. In view of the extremely low doses of arginine-vasopressin required to elicit arteriolar constriction, one must entertain the possibility that vasopressin plays an important role in control of arteriolar blood pressure.

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[3] Since 0.1 ml volumes of Ringer gelatin were superfused over the entire rat mesocecum, the actual threshold doses must be appreciably less.

1. Katsoyannis, P. G., and Du Vigneaud, V., Arch. Biochem. Biophys **78**, 555 (1958).

2. Boissonnas, R. A., *in* "Polypeptides which Affect Smooth Muscles and Blood Vessels" (M. Schachter, ed.), p. 1. Pergamon, London (1960).

3. Huguenin, R. L., Helv. Chim. Acta **47**, 1934 (1964).

4. Bodanzsky, M., and Lindeberg, G., J. Med. Chem. **14**, 1197 (1971).

5. Altura, B. M., Amer. J. Physiol. **219**, 222 (1970).

6. Altura, B. M., and Altura, B. T., Eur. J. Pharmacol. **12**, 44 (1970).

7. Altura, B. M., and Altura, B. T., Amer. J. Physiol. **219**, 1698 (1970).

8. Altura, B. M., and Burton, R. W., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **31**, 542 (1972).

9. The pure, synthetic vasopressin hormones and analogues used were [8-arginine]-vasopressin (approx assay for rat pressor method = 400 IU/mg), [8-lysine]-vasopressin (270 IU/mg), [8-ornithine]-vasopressin (360 IU/mg), [8-homolysine]-vasopressin (250 IU/mg), and [N^{ϵ}-formyl-lysine]^{8}-vasopressin (35 IU/mg).

10. Baez, S., Appl. Physiol. **211**, 299 (1966).

11. Lloyd, S., and Pickford, M., J. Physiol. (London) **155**, 161 (1961).

12. Altura, B. M., Microvasc. Res. **3**, 361 (1971).

13. Zweifach, B. W., and Metz, D. B., Ergeb. Anat. Entwicklungsgesch. **35**, 176 (1956).

14. Altura, B. M., Proc. Soc. Exp. Biol. Med. **138**, 273 (1971).

15. Altura, B. M., Proc. Soc. Exp. Biol. Med. **140**, 1270 (1972).

16. Altura, B. M., Eur. J. Pharmacol. **20**, 261 (1972).

17. Stecher, E., "Merck Index." Rahway, NJ (1968).

18. Boissonnas, R. A., Guttmann, S., Huguenin, R. L., Jaquenod, P. A., and Sandrin, E., Helv. Chim. Acta **46**, 2347 (1963).

19. Altura, B. M., *in* "Chemistry and Biology of Peptides" (J. Meienhofer, ed.). p. 441. Ann Arbor Sci. Pub., Ann Arbor, MI (1972).

20. Zaoral, M., and Sorm, F., Collect. Czech. Chem. Commun. **31**, 90 (1966).

21. Rudinger, J., *in* "Drug Design" (E. J. Ariëns, ed.), Vol. 2, p. 319. Academic Press, New York (1972).

22. Altura, B. M., Zweifach, B. W., and Hershey, S. G., Proc. Soc. Exp. Bol. Med. **119**, 258 (1965).

23. Altura, B. M., and Hershey, S. G., Angiology **18**, 428 (1967).

24. Krejčí, I., Kupková, B., Vávra, I., and Rudinger, J., Eur. J. Pharmacol. **13**, 65 (1970).

25. Altura, B. M., Malaviya, D., Reich, C. F., and Orkin, L. R., Amer. J. Physiol. **222**, 345 (1972).

26. Altura, B. M., Advan. Exp. Med. Biol. **21**, 187 (1972).

27. Ginsburg, M., *in* "Handbook of Experimental Pharmacology" (B. Berde, ed.), Vol. 23, p. 286. Springer-Verlag, Berlin (1968).

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