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Neurohypophyseal Hormones and Analogues: Rat Pressor Potency versus Contractile Potency on Rat Arterioles and Arteries[1] (38245)

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Ever since du Vigneaud's pioneering work on the isolation and synthesis of vasopressin and oxytocin, an increasing interest has been developing in the chemistry, biology and structure-activity relationships of the neurohypophyseal hormones (NHPH) and their synthetic analogues (1, 2). The contractile potency and structure-activity relationships of these peptides on peripheral vascular smooth muscle has, for the most part, been developed on the basis of the rat-pressor assay (3). But since many of these NHPH and synthetic analogues show a predilection for the muscular venules and small venous vessels in the microcirculation (4–11), the iv rat blood pressure assays (12), used routinely to assess pressor (contractile) activity of a particular NHPH or analogue, may not accurately reflect its ability or inability to effect contraction; contraction of only muscular venules would not increase blood pressure. Furthermore, iv rat-pressor assays do not enable one to determine whether maximum doses of NHPH peptides induce a 20% decrease, 50% decrease or 100% decrease in diameter of an arterial or arteriolar vessel; the latter information could be quite important since different vasoactive agents (including peptides) induce unequal maximal contractile responses on macro- as well as micro-vascular smooth muscle (11, 13–16). It is also questionable as to whether rat-pressor assays allow one to accurately assess the relative hormone-receptor affinity of a particular NHPH for its receptor on a blood vessel (17–19). In view of these considerations, interpretation of the functional importance of amino acid residues in the various nine positions of the NHPH molecules (Fig. 1) to contractile activity, on peripheral vascular smooth muscle, on the basis of the essentially iv crude rat-pressor assays may be rather limited (17–19).

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, only two direct, quantitative structure-activity studies, using only a few such NHPH, are, as yet, available for either the vascular smooth muscle cells of rat arteries or arterioles (11, 20). Accumulation of such quantitative information is critical in view of recent *in vitro* and *in vivo* studies on canine, rat and human blood vessels which, collectively, tend to question the relationship between rat-pressor potency and direct contractile potency on peripheral mammalian blood vessels (8, 11, 16, 17, 20). The present experiments employing 12 NHPH peptides, and using isolated rat aorta as well as direct, quantitative *in vivo* microscopy on rat mesenteric arterioles, were therefore undertaken to determine: (i) whether rat-pressor potency is an accurate reflection of contractile events on rat peripheral blood vessels, and (ii) the functional

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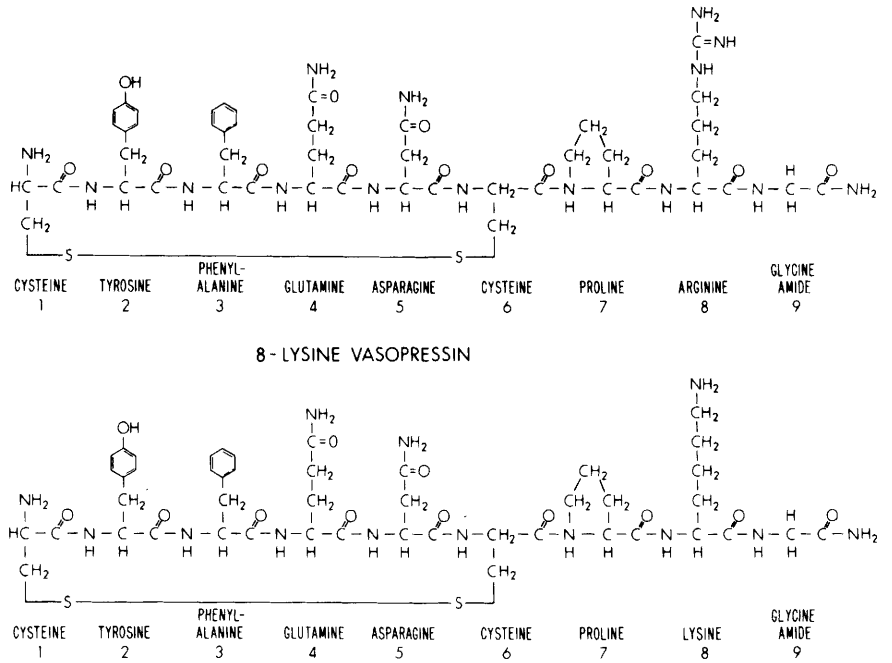

FIG. 1. Chemical structures of naturally occurring mammalian vasopressin hormones. [8-arginine]—vasopressin is found in posterior pituitary glands of most mammals, including rats and man. [8-lysine]—vasopressin is the naturally occurring hormone in the pig, peccary and hippopotamus.

importance of the amino acid residues in positions 1–3 and/or 8 of the NHPH to contractile activity on rat arteries and arterioles.

Methods. Thoracic aortas were obtained from male rats (Wistar strain, 200–375 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 (14). All vascular strips were equilibrated for 2 hr in muscle chambers containing Krebs–Ringer bicarbonate solution, the composition of which has been given previously (17). Magnesium (1.2 m*M*) was utilized since this concentration is essential for optimum NHPH action on these isolated vessels [(21), 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 (17), were obtained for 12 different synthetic NHPH analogues.[3] The results for these experiments are expressed in: (i) percentage of maximal [8-arginine] vasopressin contractile responses since the latter is the native rat pituitary hormone; and (ii) the concentration required to elicit 50% of the maximal contractile response (i.e., ED_{50}). The latter is a measure of hormone-receptor affinity, while the former is a measure of the intrinsic activity or effective-

[3] The pure, synthetic vasopressin hormones and analogues used were [8-arginine]-vasopressin (approx assay by rat-pressor method = 400 IU/mg), [8-lysine]-vasopressin (270 IU/mg), [8-ornithine]-vasopressin (360 IU/mg), [2-phenylalanine, 8-ornithine]-vasopressin (153 IU/mg), [2-phenylalanine, 8-lysine]-vasopressin (55 IU/mg), [3-isoleucine, 8-arginine]-vasopressin (252 IU/mg), (3-isoleucine, 8-ornithine]-vasopressin (104 IU/mg), [8-N$^{\epsilon}$-formyl-lysine]-vasopressin (32 IU/mg), [2-phenylalanine, 3-isoleucine, 8-ornithine]-vasopressin (120 IU/mg), 1-deamino-[8-arginine]-vasopressin (370 IU/mg), 1-deamino-[2-phenylalanine, 8-arginine]-vasopressin (30 IU/mg), and [3-isoleucine, 8-leucine]-vasopressin (5 IU/mg).

ness of the drug-receptor complex (22).

In vivo quantitative microscopic observations were carried out on rat mesenteric arterioles (20–37 μm) by means of an image-splitting television microscope recording system (23). For the latter male rats (Wistar strain, 135 ± 20 g) anesthetized with im pentobarbital (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 NHPH (24) but to the local administration of these peptides as well (15). The rat mesentery was prepared and kept under physiologic conditions according to procedures described previously (25). Measurements for changes in arteriolar lumen size were made before (control) and after topical application of graded doses (8–12 in number) of the pure, synthetic NHPH peptides.[3] *In vivo* microscopic observations for discrete drug effects were made at magnifications up to 4000 times using the image-splitting television microscope recording system (23). The protocol for studying the NHPH and analogues was identical to that described previously (20).

Results and Discussion. If rat-pressor assays are to be considered as reliable estimates of the ability or inability of NHPH and synthetic analogues to stimulate strongly, weakly, etc., the effector smooth muscle cells of peripheral blood vessels, and thus be a reflection of structure-activity relationships (SAR's) at the hormone-receptors (3), then one might expect to see a parallelism between the descending order of relative rat-pressor potencies and contractile potencies of a series of analogues on rat blood vessels. If, however, the SAR's for many of these peptides vary with the particular type of rat blood vessel (e.g. artery vs arteriole vs metarteriole, etc.) (8–11, 20), then one should not see a parallelism between rat-pressor potencies and contractile potencies. The relative ED_{50}'s shown in Table I do, indeed, suggest that the relative contractile potencies of a series of 12 NHPH and analogues are widely divergent on 2 different types of rat vessels, viz., aorta and mesenteric arteriole. These results also indicate that the relative descending order of contractile potency (i.e., relative ED_{50}'s) for this series of peptides is different for the 2 rat blood vessels. For example, (i) [8-ornithine]-vasopressin is 2.5 times as potent as [8-arginine]-vasopressin on the aorta but one-ninth as potent as the latter on the arteriole, (ii) 1-deamino-[8-arginine]-vasopressin is only the seventh most potent analogue on the aorta but on the arteriole it is the fourth most potent analogue, (iii) although [3-isoleucine, 8-ornithine]-vasopressin is a fairly potent analogue on the aorta, it is a very weak molecule on the arteriole (Table I), etc.

The data presented in Table II suggest that most of the NHPH and analogues exhibit relative rat-pressor potencies which do not parallel the relative contractile potencies obtained on rat aortas or mesenteric arterioles. For example, although four neurohypophyseal peptides—namely, [8-lysine]-, [2-phenylalanine, 8-lysine]-, [N^{ϵ}-for-lys]-[8], and 1-deamino-[2-phenylalanine, 8-arginine]- vasopressin-exhibit similar potencies relative to [8-arginine]-vasopressin in both the rat-pressor assay and in contraction of rat aorta (Table II), all of the other peptides show differences between these 2 assay systems which are statistically significant ($P < 0.01$, t test). Furthermore, all of the NHPH analogues, when tested on arterioles (the major sites of peripheral resistance), exhibit potencies relative to [8-arginine]-vasopressin which are statistically different from those obtained in the rat-pressor assays (Table II). These findings, when taken together with previous findings (5, 7–11, 20), thus raise a question as to the validity of crude iv rat-pressor assays to reflect (or assess) contractile activity (or SAR's) at the peripheral effector vascular smooth muscle cells, at least in the rat. The *in vitro* and *in vivo* data in this communication clearly show that previously observed discrepancies between contractile actions on isolated canine blood vessels and rat-pressor assays (17) cannot be due to: (i) species (i.e., dog vs rat), (ii) *in vitro* vs *in vivo* studies (i.e., isolated arteries vs blood pressure), or (iii) the fact that not enough

TABLE I. Relative Hormone-Receptor Affinities and Intrinsic Activities of Neurohypophyseal Hormones and Synthetic Analogues on Rat Aortic and Mesenteric Arteriolar Smooth Muscle.

Peptide (-vasopressin)	Affinity ($\times 10^{-9}$ M) [a]		Intrinsic activity (i.a.) [b]	
	Aorta	Arteriole	Aorta	Arteriole
[Arg^8]-	3.5 ± 0.2	0.7 ± 0.05	1.00 ± 0.03	1.00 ± 0.06
[Orn^8]-	1.6 ± 0.2[c]	6.3 ± 0.4[c]	0.99 ± 0.02	1.18 ± 0.00[c]
[Lys^8]-	2.9 ± 0.3	9.0 ± 0.5[c]	0.89 ± 0.04[c]	0.99 ± 0.04
[Phe^2, Orn^8]-	5.0 ± 0.4[c]	73.0 ± 5.2[c]	0.84 ± 0.05[c]	0.96 ± 0.05
[Phe^2, Ileu^3, Orn^8]-	5.6 ± 0.4[c]	33.0 ± 2.0[c]	0.79 ± 0.03[c]	1.18 ± 0.07[c]
[Ileu^3, Orn^8]-	6.2 ± 0.4[c]	95.0 ± 7.4[c]	0.96 ± 0.02	0.53 ± 0.10[c]
1-Deamino-[Arg^8]-	13.0 ± 0.5[c]	28.0 ± 0.8[c]	0.91 ± 0.05	0.79 ± 0.07[c]
[Phe^2, Lys^8]-	18.0 ± 0.7[c]	130.0 ± 25.0[c]	0.75 ± 0.07[c]	0.97 ± 0.08
[Ileu^3, Arg^8]-	25.0 ± 0.8[c]	700.0 ± 45.0[c]	1.03 ± 0.03	0.97 ± 0.02
1-Deamino-[Phe^2, Arg^8]-	28.0 ± 0.9[c]	160.0 ± 27.0[c]	0.55 ± 0.04[c]	0.61 ± 0.10[c]
[N$^\epsilon$-For-Lys^8]-	54.0 ± 2.3[c]	280.0 ± 32.0[c]	0.98 ± 0.05	0.94 ± 0.08
[Ileu^3, Arg^8]-	2,200.0 ± 70.0[c]	15,000.0 ± 342.0[c]	0.87 ± 0.04[c]	0.54 ± 0.07[c]

[a] Mean concentration of agonist (±SEM) required to produce 50% of the maximal contractile response to vasopressin peptide [topical vol (0.1 ml) for arterioles converted to mole/liter]. N = 6–30 different vessels for each peptide. No more than one peptide was tested on any one vessel type.

[b] Where maximal contractile response to [Arg^8]-vasopressin is taken as 100% (i.e., i.a. = 1.00).

[c] Significantly different from [Arg^8]-vasopressin ($P < 0.02$) (Student's t test).

TABLE II. Relative Order of Potency of Neurohypophyseal Hormones and Synthetic Analogues on Rat Blood Pressure, Aorta and Mesenteric Arteriole.

Peptide (-vasopressin)	Potency (%[Arg^8]-vasopressin)			Potency ratio	
	Blood pressure (BP) [a]	Aorta (A) [b]	Arteriole (AL) [b]	(BP/A)	(BP/AL)
[Arg^8]-	100	100	100	1.0	1.0
1-Deamino-[Arg^8]-	93	27[c]	2.5[d]	3.3	37.2
[Orn^8]-	90	238[c]	11.1[d]	0.4	8.1
[Lys^8]-	68	83	7.8[d]	0.8	8.7
[Ileu^3, Arg^8]-	63	14[c]	0.1[d]	4.5	630.0
[Phe^2, Orn^8]-	38	70[c]	1.0[d]	0.5	38.0
[Phe^2, Ileu^3, Orn^8]-	30	63[c]	2.1[d]	0.5	14.3
[Ileu^3, Orn^8]-	26	56[c]	0.7[d]	0.5	37.2
[Phe^2, Lys^8]-	14	19	0.5[d]	0.7	28.0
[N$^\epsilon$-For-Lys^8]-	8	6.5	0.3[d]	1.2	26.6
1-Deamino-[Phe^2, Arg^8]-	7.5	12.5	0.4[d]	0.6	18.7
[Ileu^3, Leu^8]-	1.3	0.16[c]	0.002[d]	8.1	650.0

[a] Based on rat-pressor assay potencies (see METHODS).

[b] ED_{50} (a measure of hormone-receptor affinity) values from Table I were utilized to get approximate relative potencies.

[c] Significantly different from rat-pressor (BP) potencies ($P < 0.01$).

[d] Significantly different from rat-pressor (BP) potencies and aortic strip potency ($P < 0.01$).

NHPH and analogues were examined (i.e., 7 vs 12 peptides).

It becomes apparent from the present study that the marked differences observed between the SAR's for contraction of blood vessels (Table II) versus effects on blood pressure are in all probability most likely due to at least 5 major factors: (i) Since many of these NHPH and analogues show a predilection for the muscular venules and other capacitance vessels (4–11), the rat blood pressure assays (12), used routinely to assess pressor (contractile) activity of a particular NHPH or analogue, may not accurately reflect its ability or inability to effect contraction; contraction of solely muscular venules would not increase blood pressure. (ii) Complex autonomic reflex actions probably complicate pressor assays (18). (iii) Heterogeneity of vasopressin receptors which subserve contraction may exist with respect to the SAR's of the NHPH on blood vessels (11, 14–18, 20, 26). The widely divergent relative hormone-receptor affinities (i.e., ED_{50}'s) and intrinsic activities of 12 structurally different NHPH and analogues on rat aorta and mesenteric arterioles seen in this study (Table I) could not only be used as support for the latter contention but could lend further support to our previous suggestion that the vasopressin receptor may not be identical on all blood vessels (11, 16, 17, 26). (iv) Extracellular, ionized magnesium concentration [Mg^{2+}] may also be a factor. It has recently been demonstrated that [Mg^{2+}] is not only necessary for NHPH-induced contractions of mammalian blood vessels (26–28), but that different blood vessels within a single species may show different [Mg^{2+}] dependencies for the same NHPH (26). In addition, preliminary findings in our laboratory indicate that different NHPH and analogues can have different dependencies on [Mg^{2+}] on the same rat blood vessel (21), and that this may be brought about through an effect on calcium permeability and translocation (28, 29). (v) Cardiac actions *in vivo* may also interfere in the determination of NHPH SAR's by the rat-pressor assay method (18). The following findings, in this study, also serve to demonstrate the difficulties one has in extrapolating rat-pressor assay SAR's to direct effects on peripheral rat blood vessels.

The *in vitro* and *in vivo* findings with oxytoxin (i.e., [3-isoleucine, 8-leucine]-vasopressin) presented in this study, support a recent tenet that basicity in position 8 of the NHPH may not be critical for intrinsic (contractile) activity on mammalian blood vessels (8, 11, 16, 17, 20); prior to these studies basicity (on basis of rat-pressor assay) was thought to be critical for contraction of blood vessels (30). Oxytocin, a NHPH lacking a free basic amino group in position 8, although showing marked losses in hormone-receptor affinity (i.e., high ED_{50}'s), is capable of eliciting fairly potent contractile responses on both rat aorta and mesenteric arterioles (Table I). It is, thus, probably this drastic decrease in hormone-receptor affinity which accounts for the low specific activity of oxytocin in the rat-pressor assay (i.e., 5 IU/mg) rather than the intrinsic (contractile) activities which are quite high (Table I). It should be noted that on human umbilical arteries and veins oxytocin cannot only elicit potent contractile responses but exhibits greater i.a.'s and affinities for the NHPH receptor on these vessels than does [8-arginine]-vasopressin (16). The effect of modifying position 8 with regard to side chain length (e.g., [8-ornithine]-,[8-lysine]-, and [8-homolysine]-vasopressin) on contractile activity in rat aorta and mesenteric arteriole has been discussed previously (20).

The findings in this report demonstrate that modification of the vasopressin molecule in other than the 8-position can also affect its ability to induce contraction on rat aorta and mesenteric arterioles. For example, loss of the OH group of the phenolic amino acid tyrosine in position 2 (as in [2-phenylalanine, 8-ornithine]-vasopressin and [2-phenylalanine, 8-lysine]-vasopressin) or replacement of the aromatic amino acid phenylalanine in position 3 by an aliphatic amino acid (as in [3-isoleucine, 8-arginine]-vasopressin and [3-isoleucine, 8-ornithine]-vasopressin) not only reduces the affinity of the altered vasopressin molecules to their receptors (see increased ED_{50}'s in

Table I) but also results in losses in intrinsic activity (Table I). These data thus lend firm support to previous suggestions that the phenolic and aromatic groups in positions 2 and 3, respectively, are involved in both the affinity and the intrinsic activity of the NHPH on vascular muscle (17).

The absence of only the functional amino group in position 1 also results in reductions in both hormone-receptor affinity and intrinsic activity in rat aorta and mesenteric arterioles (Table I). This is, thus, quite different from that which has been reported for this functional group in oxytocin activity on uterine smooth muscle (31) and suggests that the amino group may be very important for the binding of the hormone to the vasopressin receptor in mammalian blood vessels. This finding, solely on the basis of the rat-pressor assay, would not be anticipated since 1-deamino-[8-arginine]-vasopressin (370 IU/mg) is approximately equivalent to [8-arginine]-vasopressin (400 IU/mg) (3). Absence of both the functional amino group and the phenolic hydroxyl (e.g., 1-deamino-[2-phenylalanine, 8 arginine]-vasopressin) results in a molecule which exhibits further losses in both affinity and intrinsic activity of the altered hormone on rat blood vessels (Table I). These latter findings could aid in explaining the rather marked losses previously noted in rat-pressor potency (e.g., 30 IU/mg) when compared to 1-deamino-[8-arginine]-vasopressin (3). Overall, the present observations, when coupled with previous observations on canine and rat blood vessels (17, 20), indicate that the amino, phenolic and aromatic groups in positions 1, 2 and 3, respectively, are all necessary to interact with a basic amino acid residue in position 8 to promote optimum contractile activity on mammalian arteries and arterioles.

At the very least, the present direct study on rat blood vessels when viewed in light of previous and recent findings on mammalian blood vessels (4–11, 16–18, 20, 21, 26, 27) makes it difficult, if not impossible, to predict structure-action relationships for NHPH (and analogues) on peripheral mammalian vascular smooth muscle solely on the basis of rat-pressor assays.

Summary. The present quantitative results demonstrate that the terminal amino group, phenolic hydroxyl, aromatic ring and basicity in positions, 1–3 and 8, respectively, of the neurohypophyseal hormones are important for optimizing hormone-receptor affinity and intrinsic (contractile) activity on both rat aortas and mesenteric arterioles; basicity in position 8 is, however, definitely not an absolute requirement for vasopressin-induced contractions of blood vessels. The use of 12 synthetic neurohypophyseal hormones and analogues, and direct *in vitro* and *in vivo* studies on rat aortas and mesenteric arterioles respectively, indicates many discrepancies between hormone potency obtained through rat-pressor assays versus those obtained through direct studies on peripheral vascular smooth muscle. The present findings thus question the use of rat-pressor assays in assessing structure-activity relationships of neurohypophyseal hormones on rat arterial or arteriolar smooth muscle. In addition, the data supports the notion that a heterogeneity of the vasopressin receptor, which subserves contraction in mammalian vascular muscles, may exist in different types of blood vessels.

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