Norepinephrine-Induced Potentiation of Arginine Vasopressin Reactivity in Arterioles of the Spontaneously Hypertensive Rat

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SUMMARY We have studied microvascular reactivity to vasopressin alone and in combination with norepinephrine in young (6- to 8-week-old) spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) controls. Closed-circuit TV microscopy was used to quantify the in vivo diameter responses of small arterioles (17 to 26 μ) to vasopressin (1.25 × 10⁻⁷ to 3.75 × 10⁻⁷ M) injected intraarterially alone and with simultaneous topical suffusion of a subthreshold concentration of norepinephrine in the cremaster muscle microcirculation. Percent decrease in luminal diameter was integrated over a 30-second period to obtain log concentration response curves. The vasoconstrictor response to vasopressin was concentration-dependent in both groups (p < 0.001). A significant increase in reactivity to vasopressin alone was exhibited by the SHR arterioles compared to the WKY vessels (p < 0.02). Maximum constriction was 55% higher in the SHR (p < 0.04). The SHR also demonstrated a greater sensitivity to vasopressin (p < 0.02).

Vasopressin-induced vasoconstriction was potentiated by norepinephrine in the SHR, demonstrated by the significant shift of the curve up and to the left of the SHR response curve to vasopressin alone (p < 0.01). The maximum response was 38% greater (p < 0.02). Sensitivity was significantly enhanced (p < 0.01). Additionally, the presence of norepinephrine stimulated a three-fold greater incidence of complete closure. In contrast to SHR results, topical suffusion of norepinephrine did not significantly alter the reactivity of the WKY arterioles to vasopressin-induced constriction. Our results support a role for vasopressin as a potential vasoconstrictor in the developing stage of SHR hypertension which may be modulated by norepinephrine and thus contribute to the elevated total peripheral resistance observed. (Hypertension 5:321-327, 1983)

KEY WORDS • vasopressin • microcirculation • hypertension • vascular reactivity • SHR • norepinephrine

ARGININE vasopressin is a potent endogenous vasoconstrictor. In the rat pressor assay, its potency is as great as, if not greater than angiotensin II. Altura observed that physiological concentrations of vasopressin (10⁻¹²-10⁻¹¹ M) can produce vascular smooth muscle constriction in the rat mesenteric microcirculation. Both in vivo mesenteric arterioles and in vitro aortic strips are one to three orders of magnitude more sensitive to vasopressin than they are to angiotensin II.

Vasopressin has been implicated as a factor in several forms of hypertension, including human essential, renal, DOCA-salt, Dahl salt-sensitive, and spontaneous hypertension. Increased pituitary content, plasma levels, and urinary excretion of vasopressin have been reported in the spontaneously hypertensive rat (SHR). Although the increased plasma levels reported in the SHR are not adequate to significantly elevate blood pressure in the normotensive organism, pressor responsiveness to vasopressin is enhanced in the adult SHR. Mohring et al. have also observed a significant blood pressure reduction with intravenous injection of vasopressin antiserum in the adult stroke-prone SHR. These studies suggest that an increased vascular reactivity to vasopressin could be one of the contributing factors responsible for the elevated total peripheral resistance characteristic of SHR hypertension.

Peripheral control of blood pressure occurs at the arteriolar level of the microcirculation. Reactivity to vasoactive agents has been commonly evaluated by several techniques: whole body reactivity (pressor responses to injected agents), isolated vascular bed preparations, and vascular strips. While these methodolo-
gies of assessing large scale reactivity may often reflect arteriolar responsiveness, they do not measure it directly. The data available on the effect of vasopressin on the small resistance vessels in hypertension is sparse. Therefore, we have investigated the in vivo diameter responses of arterioles to vasopressin in the cremaster microcirculation of the young SHR and its normotensive Wistar-Kyoto (WKY) control.

A role for the sympathetic nervous system has been suggested in the genesis of SHR hypertension. The sympathetic nervous system is also known to modulate the vascular responsiveness to vasopressin. We have developed a technique that enables us to study the interaction of vasopressin and norepinephrine, two vasoconstrictors implicated in SHR hypertension, in the same microvascular bed. Vasopressin is injected intraarterially to simulate its physiological route, while norepinephrine is topically suffused to mimic its role as a neurotransmitter.

Methods

General Methods

The Okamoto-Aoki strain of SHR and its normotensive WKY control were bred in our own colony or purchased from the Charles River Laboratories (Wilmington, Massachusetts). Male SHR rats, 6 to 8 weeks old and in the developing phase of hypertension, were used in this study. The animals were allowed food and water ad libitum and maintained on a 12 hours light/12 hours dark cycle.

The rats were anesthetized with a warm solution of 2.5% chloralose-10% urethane (6 ml/kg, i.p.). Anesthetic supplements of 10% of the initial dose were administered as needed based on whisker and jaw movement and the corneal reflex. A patent airway was maintained with a tracheotomy tube. During the surgical and experimental procedures, a rectal temperature of 37°-38°C was maintained by a thermostatically controlled heating pad and infrared lamp. The carotid artery was cannulated for measurement of systemic mean arterial blood pressure (MAP) and heart rate using an Ailtech MS10C (Englewood, Colorado) transducer connected to an Offner R Dynograph (Schiller Park, Illinois) recorder. The left femoral artery, ipsilateral to the cremaster studied, was cannulated in a retrograde manner for vasoactive agent administration.

Cremaster Muscle Preparation

The microcirculatory preparation used was a covered adaptation of the Baez methodology for rat cremaster. We developed our technique to study the interaction of two vasoactive agents in the same microvascular bed. The enveloping cremaster muscle was unwrapped from the testis and stretched drumhead fashion over an 18 mm glass coverslip mounted on an adjustable plexiglass pedestal. Tension was applied evenly all around until there was no waviness in the muscle, thereby providing reproducible tone and reactivity. A brass ring (17 mm internal diameter, 3 mm depth) with an epoxied 18 mm coverslip was placed over the cremaster. The temperature of the ring apparatus was thermostatically controlled at 34.5°-35°C. A warm 4% agar solution prepared with physiological saline provided a seal between the tissue and ring and also covered the exposed portion of the cremaster proximal to the animal. A notch in the ring prevented the occlusion of the main arteriole and venule as well as the nervous supply to the tissue. The chamber (0.7 ml volume) was filled with warmed Normosol-R (Abbott Laboratory, Atlanta, Georgia), supplemented with CaCl₂ (2.3 mM). Topical administration of norepinephrine was effected through inject and outlet ports (18 gauge metal tubing soldered into the proximal and distal sides of the ring). The entire surgical board was mounted on the stage of a compound microscope. The preparation was allowed to equilibrate for an hour before agent administration.

Small arterioles (17-26 μ) of the cremaster muscle were viewed at ×80-150 through Zeiss optics. Transillumination was via a fiber optic and a long-working distance condenser. The microscope image was projected by a Sony AVC-3210 video camera (Tokyo, Japan) onto a Sony monitor screen. Vessel diameter changes were analyzed at a final magnification of approximately ×1700. These arteriolar responses were videotaped by a Sony AV3600 recorder or recorded online using image shearing techniques.

Vasoactive Agents

Arginine vasopressin (Grade VI, Sigma, St. Louis, Missouri) was dissolved in pH 7.4 adjusted Normosol-R (Abbott Laboratory) supplemented with CaCl₂ (2.3 mM) and heparin (10 U/ml). Norepinephrine bitartrate (Sigma) was dissolved in pH 7.4 adjusted Normosol containing CaCl₂ and ascorbic acid (10 mg/liter).

Intraarterial Vasopressin and Topical Norepinephrine Administration

Arginine vasopressin (1.25, 3.75, 12.5, 37.5 × 10⁻⁶ M) was injected via the femoral artery. A 50 μl bolus was visualized as a clear flush passing through the arteriole. Diameter responses were recorded during: 1) a control period (no agent administration) — 1-minute observation; and 2) vasopressin (50 μl injected within 2 seconds) — 2-minute period (minimum). Diameter responses to a vehicle control were also determined prior to the first hormone concentration administered. Diameters had to return to within 90% of control before the next dose was administered. Concentration response curves were obtained for each of the arterioles.

After a 10-minute reequilibration period, topical administration of a subthreshold dose of norepinephrine (mean of 3 × 10⁻⁸M for both groups) based on the individual animal was effected by continuous pump suction (Compact Infusion Pump, Harvard Apparatus, Inc., South Natick, Massachusetts) through the inject port of the brass ring. Diameter responses were recorded during: 1) a control period (no agent adminis-
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tration) — 2-minute observation; 2) a Normosol suffu-
sion (1.14 ml/min — 2-minute period; and 3) a norepi-
nephrine suffusion at a rate of 1.14 ml/min for 2
minutes followed by a rate of 0.108 ml/min. If after a
10-minute interval the vessel diameter did not change
by more than 10% from control, a second set of intraar-
terial vasopressin concentrations was injected with the
simultaneous norepinephrine suffusion. A second con-
centration response curve was thus obtained.

Diameter Measurement

Diameter measurements were made using an elec-
tronic image shearing technique.22 The vessel was ori-
ented vertically on the monitor by rotation of the cam-
era. The video image was sheared or horizontally
shifted by an amount proportional to an adjustable
voltage. The horizontal shift, proportional to the inter-
nal diameter, was read out on a digital meter and ana-
log changes were recorded on a strip chart recorder
(Offner R Dynograph). Calibration of the recorder was
achieved by videotaping a stage micrometer at the ap-
propriate magnification.

Changes in diameter were determined over the 30
second interval in which maximum vasoconstriction
occurred. Areas were integrated by planimetry. Re-
sponses were expressed as percent of control diameter.

Statistical Analysis

Percent vasoconstriction and percent responders vs
concentration for SHR and WKY arterioles were com-
pared using a two-way analysis of variance with re-
peated measures. Additionally, a Neuman-Keuls test
was used to determine which individual means were
different. Other statistical analyses were performed
using two-tailed Student's t tests.

Results

Seventeen male SHR and 20 WKY controls were
used in this study. Table 1 compares the hemodynamic
characteristics of the WKY and SHR rats at 6 to 8 weeks
of age, the developmental stage of SHR hypertension.
MAP was 59% higher in the SHR (p < 0.001). In
addition, the heart rate of the SHR was elevated by
18% (p < 0.001). The mean resting diameter of the
small resistance arterioles studied was 22 \( \mu \). Control
diameter for each dose did not vary by more than 10% over the course of the experiment. Body weights of the
SHR and WKY were not significantly different.

Figure 1 shows a representative tracing of the change
in diameter of an SHR arteriole in response to
the intraarterial administration of $12.5 \times 10^{-8}$ M vasopressin alone (A) and during simultaneous topical suffusion of norepinephrine (B). Topical administration of norepinephrine alone did not produce vasoconstriction. The shading indicates the areas integrated for determination of the 30-second maximum vasoconstriction. Responses are expressed as percentages of the control diameter prior to hormone administration. The 23 μ arteriole constricted 21% in response to vasopressin alone. Suffusion of a subthreshold dose of norepinephrine resulted in potentiation — 75% constriction in response to the same concentration of vasopressin.

The log concentration response patterns of the SHR and WKY arterioles to vasopressin alone and during simultaneous suffusion of a subthreshold concentration of norepinephrine are illustrated in figure 2. The diameter response (percent vasoconstriction) was concentration-dependent in both the SHR and WKY ($p < 0.001$). An increased reactivity to vasopressin alone was exhibited by the SHR arterioles as evidenced by the significant shift of the curve up and to the left of the WKY curve ($p < 0.02$). The SHR arterioles constricted one-to-two-fold greater than the WKY vessels ($256\%$ of the WKY response to $3.75 \times 10^{-8}$ M; $206\%$ to $12.5 \times 10^{-8}$ M $p < 0.01$; $148\%$ to $37.5 \times 10^{-8}$ M, $p < 0.01$). The maximum percent constriction attained was significantly greater in the SHR — $63.2\% + 7\%$ vs $40.9\% + 5.9\%$ ($p < 0.04$).

When a subthreshold concentration of norepinephrine was constantly suffused over the cremaster, the vasopressin-induced constriction of the SHR arterioles was significantly enhanced compared to that in the SHR before suffusion ($p < 0.01$, fig. 2). The SHR constriction to vasopressin and norepinephrine was 877% of the response to $1.25 \times 10^{-8}$ M alone, 305% to $3.75 \times 10^{-8}$ M ($p < 0.01$), 134% to $12.5 \times 10^{-8}$ M ($p < 0.05$), and 137% to $37.5 \times 10^{-8}$ M ($p < 0.01$). The maximum percent constriction attained was 38% greater in the SHR during norepinephrine suffusion — $89.9\% + 5.6\%$ vs $63.2\% + 7.0\%$ ($p < 0.02$). A greater frequency of complete closure was also exhibited by the SHR during norepinephrine suffusion; 54% of SHR arterioles reached 100% constriction when a subconstrictor concentration of norepinephrine was simultaneously suffused, whereas only 18% of SHR vessels attained closure in response to vasopressin alone.

In contrast to the SHR results, topical suffusion of norepinephrine did not significantly alter the reactivity of the WKY arterioles to vasopressin-induced constriction (fig. 2). Maximum constriction was similar, being $40.9\% + 5.9\%$ in response to vasopressin alone and $33.6\% + 5.8\%$ during simultaneous norepinephrine suffusion. Only 5% of WKY vessels attained 100% constriction in the presence of vasopressin; none reached complete closure when norepinephrine was suffused concomitantly.

Sensitivity to vasopressin was also evaluated by determining the percentage of arterioles responding with a greater than 5% constriction over a 5-second period at each concentration (table 2). Overall, a significantly greater percentage of SHR arterioles constricted in response to vasopressin alone than did WKY arterioles ($p < 0.02$). Calculated EC$_{50}$ values, the concentrations at which 50% of the arterioles responded, for the SHR and WKY were $4.3 \times 10^{-8}$ M and $8.7 \times 10^{-8}$ M vasopressin, respectively. In the SHR, the percentage of responders was dramatically increased when norepinephrine was simultaneously suffused ($p < 0.01$). Since 54% of SHR arterioles responded to the first

![Figure 2. Vasoconstrictor response of SHR (---) and WKY (----) arterioles to the intraarterial administration of vasopressin alone (open symbols) and during simultaneous suffusion of a subthreshold concentration of norepinephrine (closed symbols). SHR n = 13-17; WKY n = 15-21. SHR-vasopressin vs WKY-vasopressin, p < 0.02; SHR-vasopressin vs SHR-vasopressin + norepinephrine, p < 0.01.](image-url)

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</table>

C1 = $1.25 \times 10^{-8}$ M; C2 = $3.75 \times 10^{-8}$ M; C3 = $12.5 \times 10^{-8}$ M; C4 = $37.5 \times 10^{-8}$ M.
concentration of vasopressin in the presence of norepinephrine, EC$_{50}$ values were calculated — SHR 3.6 $\times$ 10^{-8} M vs WKY 28.2 $\times$ 10^{-8} M vasopressin. The percentage of WKY responders to vasopressin was not altered by norepinephrine suffusion.

Discussion

Several recent studies have suggested that vasopressin may play a role in SHR hypertension. Crofton et al. observed increased pituitary content, plasma levels, and urinary excretion of vasopressin in the young SHR compared to WKY controls. Morris et al. reported decreased hypothalamic content, increased pituitary concentration and elevated plasma levels of vasopressin in 10-week-old SHR rats, suggesting hypothalamic hypersecretion. Although the magnitude of the plasma elevations that have been reported are small (13% to 55% above control), Hoffman et al. demonstrated an increased pressor responsiveness to intravenously injected vasopressin in the adult SHR. Mohring et al. found that plasma vasopressin concentrations were twofold higher in the benign and four- to fivefold higher in the adult malignant stroke-prone SHR than in normotensive controls. The height of the blood pressure correlated significantly with the log of the plasma vasopressin level. Injection of vasopressin antiserum reduced the same response with intraarterial administration. An overall decrease in diameter of 20% can elevate resistance by more than twofold and significantly increase blood pressure. Therefore, we contend that it may be possible for physiologically attainable vasopressin levels to produce constriction in the SHR, especially during times of stress.

Most investigators report an enhanced reactivity to vasoconstrictors in the adult SHR as assessed by isolated hindquarter, mesenteric artery, microcirculation, and renal perfusion experiments and whole animal pressor responsiveness. This generalized increase in reactivity has been attributed to structural alterations in the vasculature such as an increased wall-to-lumen ratio, which could be a result of hypertension. However, since reactivity is not enhanced to the same degree with each agent and increased wall thickness has not been observed in all vascular beds, a functional alteration in the vascular smooth muscle is also implicated.

Evaluation of reactivity in the young SHR has not been as extensive and the results have been conflicting. Vascular reactivity to vasoconstrictors, including norepinephrine and angiotensin II, is unaltered as evaluated by isolated renal perfusion and microcirculation studies and whole body pressor responsiveness. Henrich and Eder also reported that the response of SHR mesenteric arterioles to efferent nerve stimulation of the mesenteric plexus is not different from that of young normotensive controls. Additionally, Bohlen and Lobach reported that the wall thickness of the small resistance arterioles in the cremaster microcirculation of the young SHR was not significantly different from that of young normotensive controls. Henrich and Eder also reported that compensatory vascular hypertrophy had not occurred. Therefore, the increased reactivity to vasopressin that we have observed does not appear to be part of a generalized phenomenon or due to structural alterations. A specific functional change at the level of the vascular smooth muscle is suggested.

In addition to enhanced reactivity to vasopressin, Berecek et al. have reported increased responsiveness to norepinephrine and angiotensin II in renal perfusion experiments in the young stroke-prone SHR. These results indicate that there are important differences between the SHR and its stroke-prone substrain since Collis and Vanhoutte reported no difference in reactivity to these vasoconstrictors between young SHR and WKY kidneys.

Our data also demonstrate that norepinephrine potentiates the responsiveness of SHR arterioles to vasopressin. SHR arterioles exhibited a significantly greater constriction and sensitivity, and an increased incidence of closure in the presence of norepinephrine. Elevated functional closure has been reported in the young SHR. Dusseau and Hutchins determined the
arteriolar density of the cremaster microcirculation in the young SHR (40 days). In vivo microscopy was used to estimate the number of open, functional arterioles; the total number of arterioles present was determined by latex infusion of the microvasculature. Among WKY controls, 44% of the small arterioles were observed to be open to blood flow, compared with only 24% of the SHR vessels. Our results suggest a possible contributing factor to the increased closure observed.

There is evidence that the sympathetic nervous system is involved in the development and maintenance of hypertension in the SHR. Spinal cord transection, pithing, and immunological and pharmacological sympathectomy have significantly decreased blood pressure in the SHR.17, 26 Although both norepinephrine plasma levels39, 40 and vascular reactivity30-34 in the young SHR (6 to 8 weeks old) are not increased, the modulation of vasopressin responsiveness by norepinephrine suggests a mechanism that could contribute to the elevation of total peripheral resistance characteristic of hypertension.

The exact mechanism of the vasopressin-norepinephrine potentiation at the level of the vascular smooth muscle is not known. In the isolated rabbit ear artery, Gerk et al.41 observed that the synergistic vasoconstrictor effect of octapressin (synthetic lysine vasopressin analog) and norepinephrine was specific soconstrictor effect of octapressin. In the presence of alpha-adrenergic blockers, potentiated the pressor response to vasopressin in rats. In isolated rat aortic strips, phenoxybenzamine potentiated the pressor response to vasopressin in rats. In vitro, exogenous norepinephrine must be added to produce the potentiation. Other possibilities include norepinephrine modification of the vasopressin receptor resulting in increased affinity for vasopressin, or increased intracellular calcium. Goldberg et al.43 have demonstrated that transcellular calcium movement is resulting in increased affinity for vasopressin, or in the presence of norepinephrine. This evidence suggests that there is a common pathway in vascular smooth muscle contraction activated by both norepinephrine and vasopressin. In the presence of alpha-adrenergic blockade in vivo, endogenous norepinephrine is freed from the adrenergic receptor sites to produce the potentiation. Other possibilities include norepinephrine modification of the vasopressin receptor resulting in increased affinity for vasopressin, or increased intracellular calcium. Goldberg et al.43 have demonstrated that transcellular calcium movement is involved in the pressor responses to both norepinephrine and vasopressin in rats. The mechanism of this potentiation has not been investigated at the microcircular level.

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