The Impact of Aging on Vasa Nervorum, Nerve Blood Flow and Vasopressin Responsiveness

Mikihiro Kihara, Mitsuaki Shioyama, Kazuto Okuda, Mitsuo Takahashi

ABSTRACT: ***Objective:*** Aging impacts microvessels in a number of tissue beds. Vasopressin acts as a vasoconstrictor in most blood vessels but may also cause vasodilation. We evaluated the role of aging and vasopressin in the regulation of nerve blood flow (NBF) in rat peripheral nerve. ***Methods:*** We undertook a dose-response study to examine the impact of aging on resting NBF and its vasoreactivity to vasopressin. Nerve blood flow was measured using microelectrode hydrogen polarography. Arginine-vasopressin was administered both intra-arterially and topically. ***Results:*** In young adult rats (two months old) topical epineurial application of arginine-vasopressin produced a concentration-dependent reduction of NBF (ED_{50}= 3.8 X 10^{-5} mol/L). Intra-arterial arginine-vasopressin also reduced NBF. Nerve blood flow was lower in aged rats (12 months old) and less responsive to topically applied vasopressin. The aging group had significantly higher concentrations of vasopressin in plasma than did the younger group. ***Conclusions:*** The results suggest that vasopressin constricts vessels in peripheral nerve and that there is an age related decline in the vasoconstrictive response to vasopressin. There may be a reduction in receptor sensitivity in vascular smooth muscle cells in peripheral nerve with increasing age.

RÉSUMÉ: L'impact du vieillissement sur les vasa nervorum, le flot sanguin nerveux et la réponse à la vasopressine. ***Objectif:*** Le vieillissement affecte les microvaisseaux d'un certain nombre de lits tissulaires. La vasopressine agit comme vasoconstricteur dans la plupart des vaisseaux sanguins, mais elle peut également causer une vasodilatation. Nous avons évalué le rôle du vieillissement et de la vasopressine dans la régulation du flot sanguin nerveux (FSN) au niveau de nerfs périphériques de rats. ***Méthodes:*** Nous avons effectué une étude dose-réponse pour examiner l'impact du vieillissement sur le FSN au repos et sa vasoréactivité à la vasopressine. Le FSN a été mesuré par polarographie au moyen de microélectrodes à hydrogène. De l'arginine-vasopressine a été administrée par voie intra-artérielle et topique. ***Résultats:*** Chez de jeunes rats adultes (âgés de deux mois), l'application épineurale topique d'arginine-vasopressine a provoqué une réduction du FSN concentration-dépendante (ED_{50} = 3.8 X10^{-5} mol/L). L'administration intra-artérielle d'arginine-vasopressine a également réduit le FSN. Le flot sanguin nerveux était moindre chez les rats âgés (12 mois) et moins sensible à la vasopressine topique. Le groupe plus âgé avait des concentrations significativement plus élevées de vasopressine plasmatique que le groupe plus jeune. ***Conclusions:*** Ces résultats suggèrent que la vasopressine contracte les vaisseaux des nerfs périphériques et qu'il y a un déclin de la réponse vasoconstrictive à la vasopressine lié à l'âge. Il pourrait exister une diminution de la sensibilité du récepteur des cellules musculaires lisses des vaisseaux des nerfs périphériques avec l'âge.

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Systemic blood pressure and a balance between nerve vessel vasoconstrictors and vasodilators regulate regional nerve blood flow (NBF).[1] Vasoconstriction is known to be mediated by epineurial α-adrenergic,[2,3] endothelin 1[4,5] and angiotensin II receptors.[6] Although vasopressin acts as a vasoconstrictor in most vascular beds,[7,8] some recent studies have also reported a vasodilator effect in the basilar,[9,10] coronary, pulmonary,[11] and forearm arteries.[12]

The aging process causes morphological and functional changes in vascular smooth muscle and endothelial cells.[13] The increased peripheral resistance and decreased NBF observed in older animals may reflect decreased nerve vascular caliber and progressively less energy substrate.[14] Reductions in NBF during aging are not understood but may be associated with altered vasoreactivity.[15] Vasopressin fibers are widespread in CNS[16] and

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may be susceptible to degenerative changes with aging.[17] Clinical studies have suggested that there is a relationship between vasopressin and memory, and vasopressin has been advocated as a potential treatment in senile dementia.[18] These previous reports indicate that vasopressin plays a key role in the aging process and in the regulation of circulation in the peripheral nervous system. The present study was designed to determine vascular reactivity to vasopressin in peripheral nerve and to test the hypothesis that aging rats have reduced vasoconstrictive responses induced by vasopressin.

METHODS

Animal preparation

We used 68 male Sprague Dawley rats (two months; 2M, n=56 and 12 months; 12M, n=12). These animals were housed in cages and received an unrestricted supply of water and rat chow (CE-2; CLEA, Japan, Inc.).

Nerve preparation

The details of the nerve preparation have been described previously.[2,4] In brief, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60mg/kg) that induced general anesthesia in rats for two to three hours. Rectal temperature was monitored and maintained between 36.5-37.5ºC using a thermister probe connected to a digital thermometer attached to a control feedback unit (BWT-100, Research Center Co Ltd. Japan) and an infra-red lamp. A tracheotomy tube was inserted and a polyethylene catheter (PE-50) was placed into the left common carotid artery for monitoring of mean arterial blood pressure (MAP) and for measurement of blood gases and pH. Artificial ventilation with a mixture of nitrogen and oxygen was provided by a rodent respirator (Model 683, Harvard Apparatus, Mills, MA, USA). The animals were paralyzed with tubocurarine (15-20 U/kg, intra-arterial or intra-peritoneal) which maintains muscle relaxation for three to four hours. The MAP was measured with a pressure transducer (ASI202, NEC, Japan) and recorded on a polygraph chart recorder (Model R-202 Rikadenki, Japan). Depth of anesthesia was assessed by continuous monitoring of MAP, which showed no spontaneous fluctuations and no change in response to manipulation of tissue, under sufficiently deep anesthesia. When such fluctuations appeared, intra-arterial bolus injections of 8-12 mg/kg pentobarbital were given through the arterial line. When necessary, additional intra-arterial doses of 3-5 U/kg tubocurarine were given slowly to abolish muscle twitches. Arterial blood was sampled every 60 minutes to ensure that pH and gas values remained within the physiological range (PaO_2;120±10mmHg, $PaCO_2$;40±5mmHg, pH;7.40±0.05). A polyethylene tube (3 mm diameter) filled with 2M-KCl and 3% agar was sutured into the subcutaneous tissue of the abdominal wall. The other end of this tube was placed in a 2M-KCl solution into which a silver chloride electrode connected to the reference terminal of a current-sensitive amplifier (Chemical Microsensor Model 1201, Diamond Electro Tech, Ann Arbor, MI, USA) was also placed. A length of left sciatic nerve, not exceeding 2 cm, was exposed and a pool was formed with the surrounding muscle and skin. The pool was filled with physiological saline maintained at 33.5±0.1ºC using a servo-controlled infrared lamp.

Measurements of nerve blood flow

Nerve blood flow was measured by microelectrode H_2-

Table: Effects of topical and intra-arterial administration of vasopressin on NBF, VR and MAP

Vasopressin Treatment (Log M)	N	NBF (ml/100g/min) Before	NBF (ml/100g/min) After	VR (mmHg/ml/100g/min) Before	VR (mmHg/ml/100g/min) After	MAP (mmHg) Before	MAP (mmHg) After
-2	8	15.8±1.4	6.0±1.4	6.5±1.0	24.3±6.3	102±2.0	102±l.4
-3	8	16.0±1.4	5.5±0.8	6.4±0.7	22.7±4.1	102±1.8	101±1.2
-6	8	15.0±1.5	8.5±1.0	6.5±1.0	13.3±2.1	102±1.4	103±1.0
-9	8	15.4±0.9	12.4±1.4	6.6±0.9	8.8±1.1	101±1.0	101±1.4
-12	8	15.6±1.8	13.7±1.4	6.9±0.8	8.1±0.7	108±5.9	108±3.9
-20	8	15.8±1.6	15.4±2.3	6.7±0.8	9.0±0.7	106±2.1	104±2.6

Intra-arterial administration of vasopressin

Vasopressin Treatment (Log M)	N	NBF (ml/100g/min) Before	NBF (ml/100g/min) After	VR (mmHg/ml/100g/min) Before	VR (mmHg/ml/100g/min) After	MAP (mmHg) Before	MAP (mmHg) After
-4	8	16.8±0.8	9.0±1.0	6.2±0.9	14.3±5.8	104±2.3	103±1.8

Values are means±SE; N = number of rats; NBF = nerve blood flow; VR = vascular resistance; MAP = mean arterial pressure
Results are in 2M rats.

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polarography (tip size 2-5μm). The details of the procedure have been described previously.[2,4] In brief, the hydrogen sensitive microelectrode, which was chosen for linear response to varying concentrations of hydrogen in saline, was inserted into the endoneurium. The free end of the electrode was connected to the current-sensitive amplifier and was polarized positively with +0.25V. The tissue was then saturated with hydrogen until reaching a plateau (indicating hydrogen saturation in endoneurium), then the hydrogen supply was shut off and a hydrogen clearance curve was recorded on a polygraph chart recorder for more than 30 minutes, until the current reached baseline. The signal was fed into a computer (PC-XT) and was stored (Lotus 123, Lotus Development Corp., MA, USA). The obtained hydrogen clearance curves were fitted to a mono- or biexponential washout curve using the nonlinear regression analysis program based on the Marquardt algorithm. Nerve vascular resistance (VR) was calculated as MAP/NBF.

Effects of epineurially applied vasopressin

The left sciatic nerve was exposed, and a pool was formed with surrounding muscle and skin and filled with Mammalian Ringer solution. Once the resting NBF was measured, we infused known concentrations of vasopressin (arginine-8-vasopressin acetate; Sigma Chemical, St Louis MO) to this pool surrounding the nerve and repeated the NBF measurement.

Effects of intra-arterial administration

Vasopressin was administered by intra-arterial infused injection via the femoral catheter, according to previously reported methods.[3] The end of the catheter was placed within 2 mm of the aortic bifurcation and 10ml (1 X 10^{-4} M) of vasopressin was infused for 60 minutes. During the first 30 minutes, a plateau in hydrogen current was achieved, and then a clearance curve was recorded over the last 30 minutes.

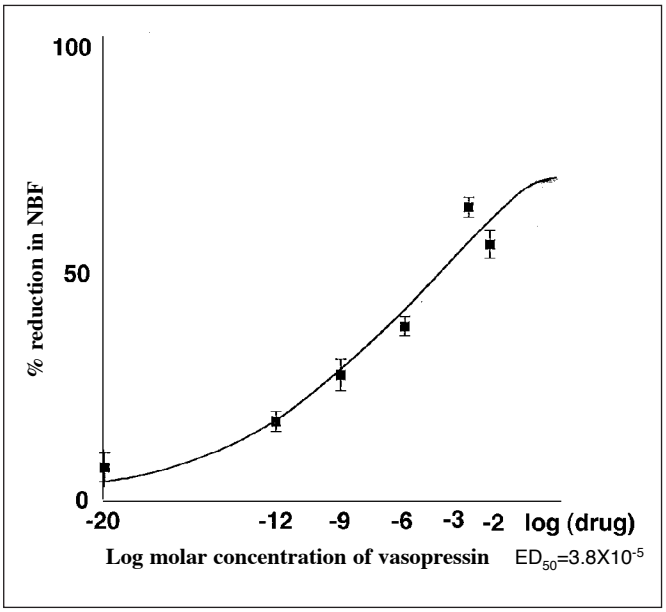

Figure 1: *Dose-response study relating nerve blood flow, expressed as percent reduction against log-molar concentration of epineurial topically applied vasopressin (mean ±SE). Results are in 2M rats.*

Determination of plasma vasopressin

A radioimmunoassay (RIA) method was used for the determination of vasopressin. Blood samples for the determination of plasma vasopressin were collected into tubes containing EDTA. Plasma was separated immediately by centrifugation at 4ºC and 3000 rpm, and stored at -80ºC for extraction and assay. Plasma vasopressin was measured in duplicate using RIA kits (Mitsubishi Petrochemical Co. Ltd. Tokyo, Japan) after extraction using SepPak C_{18} (Water Associates, Milford,MA). The sensitivity of this RIA was 0.06 pg/tube, and the recovery of plasma vasopressin ranged from 71.1%~85.5%. The interassay coefficients of variation were 7.6% and 7.2% for concentration of 1.4 and 2.4 pmol/L, respectively, and the interassay coefficients of variation were 12.5% and 4.5% for concentrations of 0.2 and 2.7 pmol/L, respectively. The results were corrected for recovery, which was determined by adding known amounts of plasma vasopressin to duplicate samples. All samples from an individual subject were analyzed at the same time.

Statistical analysis

Statistical analysis was performed on a Macintosh computer system using the Stat View (ABACUS Concept, Berkeley, CA, USA) statistical program. The data were analyzed using chi-squared, nonparametric comparison, analysis of variance when comparing differences between more than the three groups (effects of epineurial application). Data within two groups were

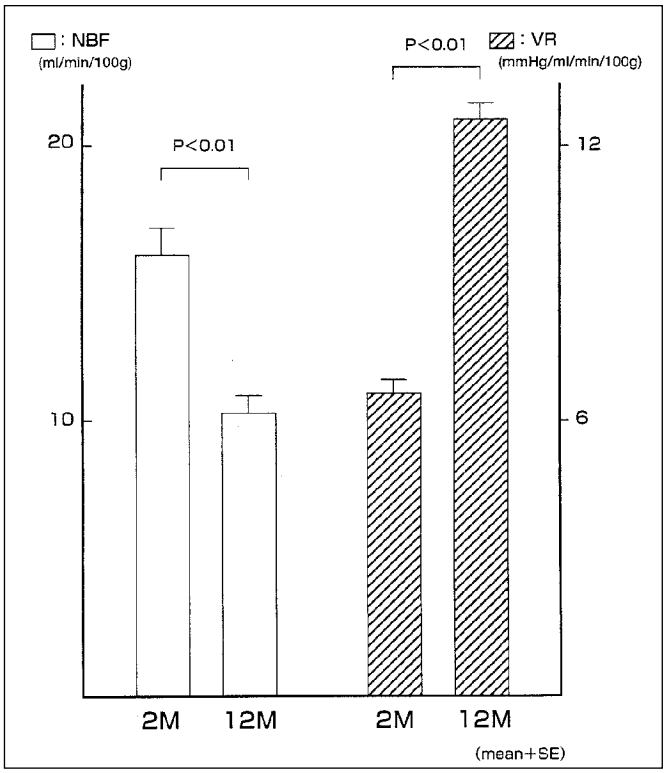

Figure 2: *Nerve blood flow (NBF) and vascular resistance (VR) measured in 2 month-old (2M) and 12 month-old (12M) rats without vasopressin application. There is significant reduction in blood flow in aging rats on NBF and VR.*

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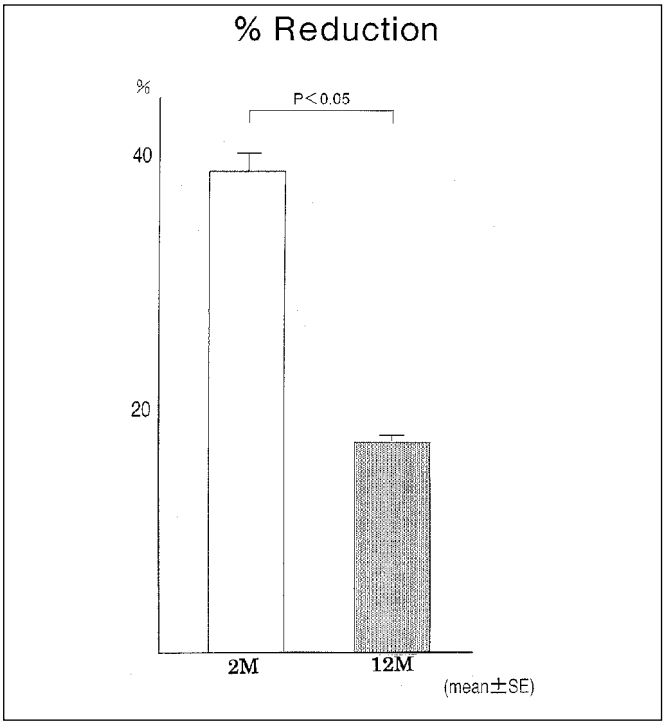

Figure 3: *Effect of application of vasopressin on sciatic nerve in two-month-old (2M) and 12 month-old (12M) rats given 10^{-5} topically. There was a significant difference in the percent change in NBF between 2M and 12M.*

analyzed using the unpaired group t test (Welch method; NBF of aging, effect of intra-arterial administration, determination of plasma vasopressin). Values are expressed as mean±SEM and significance was accepted when $p<0.05$.

Results

Topically epineurial applied vasopressin (10^{-20}, 10^{-12}, 10^{-9}, 10^{-6}, 10^{-3} and 10^{-2} mol/L) on the sciatic nerve of 2M rats decreased NBF in a dose-dependent fashion (Table, Figure 1). The ED_{50} value was $3.8X10^{-5}$ mol/L. Similarly, the intra-arterial administration of vasopressin resulted in significant reduction of NBF in 2M rats (Table).

The 12M rats had a reduction in resting NBF and rise in VR when compared with 2M rats (Figure 2). The topical application of vasopressin (10^{-5}M) caused a reduction of NBF and an increase of VR in both 2M and 12M rats. There was, however, a significant difference in the percent change in NBF in response to vasopressin between 2M (-38.9±7.2%) and 12M (-17.0±2.6) rats (Figure 3).

There was a significant difference in the concentration of plasma vasopressin between 2M (n=18, 17.5±20.3 pg/ml) and 12M rats (n=12, 87.5±69.4)($p<0.01$).

Discussion

Five major findings were obtained from this study. The first finding was a reduction in NBF observed with increasing age associated with an increase in nerve VR. The second finding was that topically applied vasopressin reduced epineurial NBF in young adult rats in a dose-dependent fashion, with an ED_{50} of $3.8X10^{-5}$ mol/L. The third finding was that intra-arterial vasopressin also reduced NBF. The fourth finding was that there was a significant difference in the percent change of NBF reduction induced by vasopressin between younger and older rats (less responsive in older rats). The fifth finding was that the plasma concentration of vasopressin in older rats was significantly higher than in younger rats.

We had demonstrated previously, using Fisher and Sprague Dawley rats, that NBF regressed negatively with increasing age and that this decline was associated with an increase in nerve VR.[15,19] The present results support these studies.

The combination of microelectrode polarography and topical superfusion of test agents in peripheral nerve vasoreactivity has two major advantages. The first is that microvasculature is much less vasoreactive than other tissues and responds to changes in blood pressure in a largely passive fashion. Therefore NBF alterations following the systemic administration of vasoreactive agents may reflect changes in blood pressure rather than those of arteriolar tone.[1] The second is that the exogenous application of our preparation is as effective as direct application on endothelial receptors in epineurial arterioles.[20]

The effects of vasopressin on NBF are not well-known. Although it is well-known that vasopressin influences vasoconstriction that is mediated by V1 receptor on vascular smooth muscle cells,[7] vasopressin has also been reported to have a vasodilator action, mediated by the V2 receptor on the endothelial cell.[10,11,21] Moreover the V2 receptor mediates vasodilatation in forearm vessels.[12,22] Our results show that vasopressin caused vasoconstriction in peripheral nerve in spite of a potential vasodilatation action. Since vasodilatation is dependent on binding to endothelial cells and epineurial application would activate smooth muscle before endothelial cells, we were concerned that the weaker vasodilator action might be masked by more potent direct smooth muscle-mediated vasoconstriction. We therefore undertook a study using the intra-arterial injection of vasopressin because with this administration, the initial and major action is on endothelial cells. However, we did not find any evidence of vasodilatation using intra-arterial vasopressin on peripheral nerve. Our vasopressin infusion time may have been too long (60 minutes), allowing vasopressin to reach both endothelial cells and smooth muscle.

Our data show that topically applied vasopressin decreased NBF with a dose-dependent fashion in normal rats (ED_{50} value was $3.8X10^{-5}$ mol/L). There are regional differences in vasopressin action. Altura et al[7] found the effective concentration of vasopressin to be between 10^{-6} to 10^{-3} mol/L in rat terminal arterioles and from 10^{-10} to 10^{-8} mol/L in rat aorta. Our ED_{50} data suggest that the vasoconstriction seen with vasopressin in nerve vessels resembles that of Altura et al's terminal arterioles.

We have also observed that many vasoconstrictive agents regulate endoneurial NBF through epineurial arterioles after local epineurial application. Based on our previous dose-response studies, the vasoconstrictive effect of vasopressin has almost the same potency as noradrenaline[2] and angiotensin II.[6] However the ED_{50} for endothelin was about three orders of magnitude lower at 10^{-8} mol/L.[4] These potent vasoconstrictor actions are balanced by vasodilatation, mediated by nitric oxide,[4]

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the prostaglandins,[23] calcitonin gene-related peptide and substance P.[24]

During aging, there is a decline of vasoconstrictive responses to noradrenaline, endothelin-I and angiotensin II in peripheral nerve.[15] Handa and Dukles[25] also showed that the vascular adrenergic responses of rat hindlimb decreased in old rats. Our data and Handa's observation supports the finding that there is also a decline of vasoconstrictive response to vasopressin in peripheral nerve with aging. Our results show that aging is accompanied by an increase in plasma vasopressin concentrations. Most large clinical studies of healthy subjects have reported that plasma vasopressin concentrations increase with age in a linear fashion.[26-28] In contrast, Duggan et al[29] reported that there was no association between plasma vasopressin concentration and age. Recently, Hosoya et al[30] reported that the binding capacity for receptors of vasopressin in kidney was significantly increased with age. Our data suggest that aging might reduce the sensitivity of vasopressin receptors in peripheral nerve microvessels.

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