Influence of Dietary Salt Intake on the Response of Isolated Perfused Mesenteric Veins of the Dog to Vasoactive Agents

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Background: Previous work has established that a high dietary salt intake results in enhanced arterial vasoconstrictor responses to stimulation with agonists. This investigation was designed to investigate the effects of dietary salt on the responses of isolated capacitance vessels (third order mesenteric veins).

Methods: Dogs were fed diets containing low, intermediate, and high levels of dietary salt (0.4, 3.0, and 6.0 mmol kg/day). The animals were killed, and lengths of mesenteric vein were mounted in a perfusion myograph with changes in lumenal diameter measured using a video tracking device. Responses to cumulative doses of norepinephrine (NE) and acetylcholine (Ach) were then determined.

Results: The vasoconstrictor responses to NE were greater in the veins from dogs on a high salt diet. Acetylcholine also caused venoconstriction that also was greater in the high salt group of animals. Responses to Ach were unaffected by Nω-nitro-L-arginine methyl ester but were abolished by atropine.

Conclusions: These results indicate that mesenteric veins from dogs fed a high salt diet constrict more powerfully in response to agonists, which could contribute to the hypertensive effects of high intakes of dietary salt. Am J Hypertens 2003;16:6–10 © 2003 American Journal of Hypertension, Ltd.

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their food for 4 weeks before the experiment. The diets were made from suet, lean meat, and sucrose and were similar to those described by Gupta et al.\textsuperscript{15} with sodium chloride added as calculated to bring the daily sodium intake to 0.4 mmol kg/day (low salt, LS), 3.0 mmol kg/day (intermediate salt, IS), or 6.0 mmol kg/day (high salt, HS). The sodium content of the IS diet was equivalent to normal kennel food. The quantity of sodium in the diets was determined from samples of known weight, which were desiccated and ashed at 500°C in an oven. The ash was dissolved in hydrochloric acid, diluted, and analyzed using a flame photometer.

Before and at the end of the period of dietary control in some dogs, we estimated systolic BP by the tail cuff method as described by Bunag and Teravainen,\textsuperscript{16} using appropriate cuff sizes for the shaved dog tail (IITC Inc., Woodland Hills, CA).

At the end of the period of dietary control, all dogs were anesthetized using α-chloralose (100 mg/kg body weight) and the baroreflex was studied in initial experiments by examining the reflex response to carotid occlusion. This procedure was carried out in all experiments. After completion of these studies and while animals were still under chloralose anesthesia, a section of mesentery was harvested and quickly placed in physiologic salt solution (PSS) at 4°C. The composition of the PSS was (in mmol/L): NaCl, 119; NaHCO\textsubscript{3}, 25; KCl, 4.2; CaCl\textsubscript{2}, 1.6; MgSO\textsubscript{4}, 7; H\textsubscript{2}O\textsubscript{2}, 1.17; KH\textsubscript{2}PO\textsubscript{4}, 1.18; Na\textsubscript{2}EDTA, 0.026; and glucose, 5.5. The mid-ileal segment of the mesentery, the animal was then killed by exsanguination by tying off the gut in this region, cutting the superior mesenteric artery between two ligatures, and exsanguinating while under deep anesthesia. The vein was identified from the corresponding artery. Third order branches were then carefully dissected out from the accompanying artery and vessels, and straight venous segments approximately 1 cm in length were removed. The vessel was then trimmed to provide a vein about 7 mm in length. This was mounted between two glass cannulas in a pressure myograph\textsuperscript{17} (Living Systems Instrumentation, Burlington, VT). The vessel was perfused and superfused with PSS at 37°C and pH of 7.4 from a 100-mL reservoir that was gased with 5% CO\textsubscript{2}:95% O\textsubscript{2}. The perfusion pressure within the vein was set at 10 mm Hg, controlled by a servo pump. The lumenal diameter was continuously determined using an inverted microscope (Nikon, model TMS-F, Surrey, United Kingdom) and video tracking equipment. The output of the lumenal diameter measurement was recorded on paper using a heated stylus recorder (Devices Instruments, Herts, UK).

After pressurizing the vessel, a stabilization time of 60 to 90 min was allowed. Cumulative dose-responses to NE and Ach were then determined with concentrations of $10^{-8}$ to $10^{-5}$ mol/L of the agent. For the response to Ach, an initial small constriction of about 10% was obtained with $10^{-7}$ mol/L NE before adding Ach. In some experiments, we determined the responses to Ach after addition of $10^{-4}$ mol/L of N\textsuperscript{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, or atropine ($10^{-5}$ mol/L). Atropine was administered into the perfusate and left for 20 min before experiments were carried out. In some experiments, the responses to $10^{-5}$ mol/L sodium nitroprusside (SNP) were also determined in preconstricted veins in the three groups of dogs.

Results are given as means ± SEM. Statistical significance was assessed using either the Student $t$ test or one-or two-way analysis of variance with the Tukey-Kramer post-test.

**Results**

Systolic BP were successfully measured using the tail cuff method in five HS, four IS, and five LS. Values at the beginning and end of the period of dietary control were, respectively, as follows (in mm Hg): in LS, 149.0 ± 4.8 and 139.0 ± 5.1; in IS, 143.8 ± 4.7 and 146.3 ± 5.5; and in HS, 146.0 ± 4.1 and 157.0 ± 2.4 respectively. In the LS group, BP was significantly decreased ($P < .05$, Student $t$ test), whereas in the HS group, BP was significantly increased ($P < .05$, Student $t$ test).

**Response to NE**

Concentration responses to NE were determined from veins taken from eight LS, six IS, and eight HS dogs. Fig. 1 shows concentration–diameter recordings taken from
one HS and one LS dog, and illustrates the greater constriction obtained from the HS animal. Average cumulative concentration response curves are shown in Fig. 2. This shows the dose dependency of the responses. It clearly shows the greater responsiveness to NE in vessels from the dogs fed higher quantities of salt. The responses in the HS dogs were significantly greater than in the LS dogs \((P < .01\), analysis of variance). The maximal reductions in diameter at the highest concentration of NE (\(10^{-5}\) mol/L) in the vessels from LS, IS, and HS dogs were 22.9% \(\pm\) 6.1%, 35.7% \(\pm\) 6.6%, and 50.8% \(\pm\) 4.4% respectively. The maximum response in the HS dogs was significantly greater than that in the LS dogs \((P < .01\), Student \(t\) test).

**Responses to Ach**

In four of 22 vessels, at concentrations of Ach between \(10^{-8}\) and \(3 \times 10^{-7}\) mol/L, there was a small (<5%) dilation. This was observed in two of eight vessels from LS dogs and in two of six vessels from IS dogs; however, none of the HS vessels showed dilator responses at any dose of Ach. Higher concentrations of Ach \((>3 \times 10^{-6}\) mol/L) invariably resulted in constriction of all veins tested from all animals. The vasoconstrictor responses tended to be greater in vessels from the HS and IS dogs than in those from LS dogs (Fig. 3), although this did not reach statistical significance. The maximum reduction in diameter in the LS, IS, and HS groups were 24.0% \(\pm\) 6.9%, 33.8% \(\pm\) 6.9%, and 35.0% \(\pm\) 4.0%.

The vasoconstrictor responses to Ach were not enhanced after administration of L-NAME. The maximal responses before and after L-NAME in veins from four LS dogs were 22.8% \(\pm\) 5.6% and 15.0% \(\pm\) 5.0%, and in four HS 32.3% \(\pm\) 1.4% and 24.0% \(\pm\) 6.9%. The response to Ach was completely abolished by atropine \((10^{-5}\) mol/L) in veins from two dogs in each group tested (Fig. 4).

**Responses to Sodium Nitroprusside**

Veins from four LS, three IS, and four HS dogs were preconstricted with \(10^{-7}\) mol/L to \(10^{-4}\) mol/L NE, sufficient to preconstrict the veins by approximately 40%. The actual changes in diameter were 41.3% \(\pm\) 6.1%, 40.6% \(\pm\) 7.4%, and 43.5% \(\pm\) 6.3% in LS, IS, and HS, respectively. Sodium nitroprusside was then added at a concentration of \(10^{-5}\) mol/L. This resulted in relaxation, in relation to the initial preconstriction diameter, of 24.5% \(\pm\) 13.5%, 73.7% \(\pm\) 10.5%, and 88.3% \(\pm\) 13.6% in LS, IS, and HS vessels, respectively (Fig. 5). The responses in veins from HS dogs were significantly greater than those from the LS dogs \((P < .05\) by analysis of variance) but not IS dogs.

**Discussion**

Previous studies have established that high levels of dietary salt enhance the responses of arterial smooth muscle to vasoconstrictor agents\(^{4,18}\) but much less is known concerning the responses of veins. We have used the pressure...
The myograph technique, which was developed for the study of responses of perfused small arteries. This technique has not been extensively used for the study of veins, but it would seem to be eminently suited for this purpose. The principal advantages are that it is possible to study veins that are much smaller (600 to 1000 μm) than those that can be studied using other techniques such as vessel rings. Also, these small veins are believed to be of particular physiologic importance in capacitance control. The physiologic condition of the vessel was ensured not only by surrounding it by a physiologic solution, but also by perfusing its lumen with the same solution and at a pressure (10 mm Hg), which is similar to that recorded from similar vessels in vivo. One difference from the normal in vivo conditions was that the veins were superfused and perfused with oxygenated PSS rather than venous blood. However, in vivo, the blood supply to the venous structures is actually arterial through the vasa venorum, and it has previously been shown that the responses of isolated perfused veins are not different when perfused with arterial or venous blood.

The main findings from this research were 1) that the small mesenteric veins constricted in response to both NE and to Ach, and 2) the constriction was greater in animals that had been fed a high salt diet for the previous month. The enhancement of arterial segment responses to NE by a high salt diet is already known, but this is the first report on the effects of dietary salt on the response of small veins. The vasoconstrictor response to Ach was qualitatively different from that seen in arteries, in which the response is a dilatation mediated through endothelial derived nitric oxide. It thus appears that, at least in mesenteric veins, endothelium-derived nitric oxide release is not of major importance, despite the fact that these veins did dilate in response to exogenous nitric oxide (from SNP).

In four of the 22 vessels, there was a very small dilatation (<5%) to low doses of Ach, although higher doses produced constriction in all vessels. That these vessels were able to respond to a vasodilator was demonstrated by administration of the nitric oxide donor, SNP, which consistently dilated all vessels. It does not appear that the failure to obtain a large and consistent vasodilation in response to Ach was due to a vasodilator effect being masked by a more powerful vasoconstriction, as blocking the potential vasodilator effect with L-NAME did not enhance the vasoconstriction. Vasoconstriction in response to Ach seems surprising. However, it is not a new observation, as it has previously been shown that Ach constricts these vessels.

The responses to SNP were particularly interesting because, despite the same initial preconstriction in the three groups of vessels, those from the HS and IS animals showed a much greater dilation than those from the LS animals (88.8%, 73.7%, and 24.5%, respectively). The explanation for this is not clear, but one possible mechanism may be that there is an alteration in the activity of soluble guanylate cyclase, the mediator of the action of SNP. However, the physiologic significance of this (if any) remains to be established.

The observation that small mesenteric veins from animals given high salt diets constrict more powerfully in response to vasoconstrictor agents compared to the veins from those given low salt is a new finding. Although an increase in basal venous tone, as determined by mean circulatory filling pressure in DOCA-salt hypertensive rats, has been reported, this effect has not been seen in other rats fed different quantities of dietary salt. The mechanism for the enhancement by a high salt diet of the constrictor response to NE and Ach is uncertain; however, one possibility is that it may be related to the mobilization of calcium ions from extracellular stores, either through receptor-operated or voltage-dependent vascular ion channels, as well as factors involved in the intracellular modulation of smooth muscle contraction by calcium ions.

The results of these experiments suggest that the enhanced contractility of mesenteric veins in response to increased levels of dietary salt could be a factor in the development of hypertension. However, any translation of these findings to the development of human hypertension must be made with caution. First, there is considerable interspecies variation in the sensitivity of BP to dietary salt, with the Dahl salt-sensitive rats being at one extreme. Second, the amount of dietary salt in these animals has to be related to the various dietary intakes in both animals and humans. We selected levels of salt intake that seemed to be compatible with those normally consumed by both dogs and humans. The intermediate salt level was equivalent to that consumed by dogs on their normal kennel food and was equivalent to a high-normal intake for humans. The low level is probably less than that normally consumed in the human diet in most western societies but is well above that consumed by certain other societies that are known rarely to develop hypertension. Also, the high level was higher than that in most western societies.
but was less than that consumed by some populations such as in northern Japan.\(^1,2\) It should be emphasized also that the period for which the diets were controlled (4 weeks) was quite short. It is conceivable that longer periods of dietary control, particularly during the developmental phase, might result in greater changes. Thus, both the duration of the dietary control and the likely differences in reaction to dietary salt between humans and dogs must be taken into consideration, particularly when making quantitative comparisons between effects in the two species. However, most aspects of cardiovascular control do seem to be qualitatively similar in the two species, so it is likely that high levels of dietary salt would also enhance venous responses in humans.

The significance of the enhanced venous response to vasoconstrictor agonists is likely to be related to the important role of these vessels in capacitance control. Veins in the abdominal circulation, unlike those in most other regions of the circulation, constrict powerfully in response to sympathetic stimulation.\(^1,12\) The main effect of capacitance vessel constriction is to enhance venous return to the heart and thereby to increase cardiac output.\(^12\) Enhanced venoconstrictor responses, therefore, are likely to contribute to the development of hypertension. It is, of course, not the only possible cause of salt-related hypertension, which is a multifactorial condition with likely contributions from arterial hypersensitivity,\(^4,21\) increased plasma and extracellular volume,\(^15,28\) and possible alterations in reflex responses.\(^29\) However, in combination with the other factors, increased venoconstriction could contribute to the development of increased BP, which is often associated with increased dietary salt intake.

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**References**