SYNTHETIC ATRIAL NATRIURETIC FACTOR DOES NOT DILATE RESISTANCE-SIZED ARTERIES

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SUMMARY The effects of synthetic atrial natriuretic factor and atriopeptin III on induced tone in resistance-sized arteries from the rat were examined in vitro. Cylindrical segments of small mesenteric or cerebral arteries were mounted on a microcannula and pressurized to a transmural pressure of 75 mm Hg. After equilibration, the level of tone in cerebral arteries was on the order of −35% change in diameter; addition of atrial natriuretic factor or atriopeptin III in cumulative doses from $10^{-10}$ to $10^{-7}$ M did not produce any transient or sustained changes in diameter. Similarly, atrial natriuretic factor or atriopeptin III did not alter the contractile responses of cerebral vessels to serotonin or prostaglandin F$_2$_. Mesenteric arteries, which do not possess an intrinsic myogenic tone, were precontracted with potassium (30 mM), norepinephrine (10$^{-6}$ M), or prostaglandin F$_2$ (1.1 $\times$ 10$^{-5}$ M) and exposed to the synthetic natriuretic peptides, also without effect. Transmural electrical stimulation (0.3-msec pulses; 180 mA; 4/second) relaxed cerebral and contracted mesenteric arteries; preincubation in $10^{-7}$ M atrial natriuretic factor or atriopeptin III did not alter subsequent responses. These observations suggest that the hypotensive action of atrial natriuretic factor cannot be attributed to direct vasodilation of splanchnic or cerebral resistance-sized arteries. (Hypertension 8: 606-610, 1986)

KEY WORDS • atrial peptides • mesenteric arteries • cerebral arteries • pressurized arteries

A rapid and prolonged decrease in blood pressure has been observed in vivo during infusion of synthetic atrial natriuretic factor (ANF), and Garcia et al. suggested that this effect was due to peripheral vasodilation. Synthetic ANF is a potent relaxing agent in precontracted large conduit arteries; however, to lower blood pressure significantly, ANF would have to dilate smaller, resistance-sized arteries as well. Synthetic analogues of ANF are now available that differ in their natriuretic potency and vasorelaxant ability. Our purpose was to evaluate the effects of two types of synthetic ANF on resistance-sized mesenteric and cerebral arteries from the rat. Although comparable in size, the two arterial preparations used have different functional and behavioral characteristics. Hence, potential interactions of ANF with a variety of activation mechanisms could be explored. In terms of their vasoactive properties, the two peptides used in this study are among the more potent analogues presently available.

Materials and Methods
Small Artery Preparations

To obtain small arteries, 20- to 30-week-old Wistar-Kyoto rats (WKY) or stroke-prone spontaneously hypertensive rats (SHRSP), from a colony maintained at the University of Vermont, were lightly anesthetized with ether and killed by decapitation. For cerebral vessel preparation, the entire brain was removed from the cranial cavity and placed in a dissecting dish filled with oxygenated physiological salt solution (PSS) at room temperature. A branch of the posterior cerebral artery was carefully dissected free from surrounding connective tissue and transferred to the experimental chamber of an arteriograph (Living Systems Instrumentation, Burlington, VT, USA), which was also filled with oxygenated physiological salt solution (PSS) at room temperature. A branch of the posterior cerebral artery was carefully dissected free from surrounding connective tissue and transferred to the experimental chamber of an arteriograph (Living Systems Instrumentation, Burlington, VT, USA), which was also filled with oxygenated PSS at 25°C. The chamber contains a glass microcannula (120 μm outer diameter) mounted on a bulkhead to facilitate positioning and has inlet and outlet ports for circulating oxygenated, prewarmed PSS. For mesenteric vessel preparation, the abdominal cavity was opened and a section of the mesenteric arcade 5 to 10 cm distal to the pylorus was removed and pinned in a dissecting dish. An arterial segment arising from a tertiary branch point was excised and cannulated. The cylindrical segments of mesenteric and cerebral vessels used for these studies were 1 to 2...
mm long and of comparable diameters in a relaxed state.

The proximal end of the arterial segment was grasped with extra-fine-point No. 5 microforceps, slipped onto the microcannula, and secured using a single strand obtained by teasing apart a 1-cm segment of surgical suture. Any residual blood in the arterial lumen was gently flushed, and the distal end of the artery was occluded with another strand of surgical suture and held in position with a Lexan pinch clamp. Transmural pressure, monitored with a Micro Switch 140PC pressure sensor (Freeport, IL, USA), was increased up to 75 mm Hg with a motorized syringe, and axial length was adjusted to remove any buckle from the artery. Pressure was then lowered to 30 mm Hg, and the arteriograph was connected to a 500-ml reservoir of PSS, which was vigorously bubbled with 95% O₂, 5% CO₂ through a gassing stone. The PSS was circulated at a flow rate of 20 to 50 ml/min, and a heat exchanger connected to a heating pump (Haake, Berlin, West Germany) prewarmed the PSS to 37°C. Temperature was monitored continuously with a thermistor probe (Yellow Springs Instrument, Yellow Springs, OH, USA) immersed directly into the bath. By adjusting the reservoir gassing rate or PSS circulating rate, or both, a pH of 7.40 ± 0.02 was maintained throughout each experiment. Samples of the PSS were periodically withdrawn from the bath and injected into a blood gas analyzer (Radiometer, Copenhagen, Denmark) for measurement of CO₂ tension, which was between 29 and 33 mm Hg under these conditions.

The arteriograph, containing a mounted, pressurized artery, was placed onto the stage of a microscope (Carl Zeiss, Oberkochen, West Germany) fitted with a television camera (RCA, Lancaster, PA, USA) attached to the viewing tube, and the vessel was allowed to equilibrate for 1 hour. Transmural pressure was then increased to 75 mm Hg, and the diameter was allowed to stabilize before the addition of activating agents.

**Experimental Protocols**

Two specific protocols, summarized in Figure 1, were used for pharmacological interventions. In each protocol, vessels were precontracted with a particular agonist until a stable and reproducible contraction was obtained. After washout, vessels were preincubated with 10⁻⁷ M of ANF or atriopeptin III (AP III) for 5 to 10 minutes and then challenged again with the same agonist or electrical stimulus (Protocol A). Alternatively, a contraction would be induced and a cumulative ANF or AP III dose-response curve (10⁻¹⁰ to 10⁻⁷ M) obtained in the continued presence of the agonist (Protocol B).

**Diameter Measurement**

Lumen diameter was measured continuously using an electronic system (Living Systems Instrumentation, Burlington, VT, USA) similar to that initially described by Wiederhielm⁴ and used by others for in vivo measurements of blood vessel diameter. Briefly, changes in optical density corresponding to vessel walls were used to generate triggered pulses that initiated sampling of a voltage ramp. These voltages were then converted into linear wall and lumen dimensions available both as a direct digital readout in microns and as analog signals for recording. Both the system and the myogenic properties of rat cerebral vessels are described in detail elsewhere.⁵

**Solutions**

The PSS contained the following concentrations (in mM): NaCl, 119; NaHCO₃, 24; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.17; CaCl₂, 1.6; glucose, 5.5; sodium ethylenediaminetetraacetic acid, 0.026. High potassium (125 mM) activating solution consisted of PSS containing an equimolar substitution of KCl for NaCl and a CaCl₂ concentration of 5 mM. Relaxing solution consisted of the PSS with CaCl₂ replaced by 1 mM ethylene glycol bis(β-aminoethyl ether), N,N,N',N'...
N'-tetraacetic acid (EGTA). The 30 mM K-PSS contained 5 mM CaCl₂, 30 mM KCl, and 94 mM NaCl; concentrations of the remaining constituents were identical to those of PSS.

Stock solutions of norepinephrine, serotonin, and prostaglandin F₂α (PGF₂α; Sigma Chemical, St. Louis, MO, USA) were made fresh on the day of each experiment and added to the reservoir to achieve the desired final concentration. Stock solutions of AP III (Peninsula Laboratories, Belmont, CA, USA) and synthetic ANF (Merck Institute for Therapeutic Research, West Point, PA, USA) were dissolved in distilled water, frozen, and stored at -20°C. Doses corresponding to the approximate 50% effective concentration were determined in preliminary experiments. Norepinephrine and PGF₂α were used to precontract mesenteric arteries, while PGF₂α and serotonin were used on cerebral arteries. Mesenteric arteries were contracted with 30 mM K⁺-activating solution at the beginning and with 125 mM K-PSS at the end of each experiment as an index of viability; cerebral arteries were contracted (125 mM K-PSS) at the end of an experiment only, since the development of myogenic tone during equilibration provided an indication of viability. The agents used to contract cerebral and mesenteric arteries are summarized in Figure 2.

Electrical Stimulation

Transmural nerve stimulation was delivered transverse to the axial dimension of the artery with two 20-gauge platinum electrodes positioned approximately 0.5 cm on either side of and parallel to the vessel. The output of a dual-channel stimulator (Ortec, Oak Ridge, IL, USA) was amplified with a bipolar operational amplifier (Kepco, Flushing, NY, USA) to obtain the required currents. Trains of unipolar rectangular pulses (0.3 msec pulse width; 4/second) were used throughout. Since effective voltage or current in the tissue is not easily monitored during transmural stimulation, a single beam storage oscilloscope (Tektronix, Beaverton, OR, USA) was used to record electrical parameters. Bath voltage could thus be determined directly and current indirectly by measuring the voltage across a 1-ohm resistor connected in series with the stimulating circuit (when resistance = 1 ohm, voltage = current by Ohm's law). Bath resistance, calculated by dividing voltage by current, was on the order of 70 ohms.

Large Artery Preparation

After rats were decapitated, segments of the abdominal aorta were excised and mounted on two canulas and perfused with PSS at flow rate of approximately 5 ml/min and a steady pressure of 90 mm Hg. Norepinephrine (10⁻⁷ M) was added to the superfusate to precontract the vessel, and once a stable contraction was attained, ANF or AP III was then added to the superfusate. Changes in outer diameter were measured with the video-electronic system just described and recorded on a Gould strip chart recorder (Cleveland, OH, USA).

Results

The comparative dimensions and reactivity of mesenteric (n = 4) and cerebral (n = 6) arteries are summarized in Table 1. When pressurized, cerebral arteries spontaneously acquired a myogenic tone that remained stable for hours. Arteries from WKY were quiescent, while those from SHRSP frequently (80-100% of the time) showed tetrodotoxin-resistant and indomethacin-resistant vasomotion, in which 2 to 10% oscillations in diameter occurred very regularly at a rate of 15 to 20/minute. Pharmacologically, rat posterior cerebral arteries were insensitive to norepinephrine, contracted in response to serotonin and PGF₂α,

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Pharmacological agents and concentrations (M) used to precontract arteries before application of atrial natriuretic factor (ANF) or atriopeptin III (AP III) and electrical parameters during transmural electrical stimulation (TES). PGF₂α = prostaglandin F₂α; NE = norepinephrine; 5-HT = serotonin.
and dilated in response to electrical stimulation. These dilations were not blocked by preincubation in 3.3 $\times$ 10^{-7} M tetrodotoxin. Conversely, mesenteric arteries contracted vigorously to norepinephrine and PGF_{2a}, were relatively insensitive to serotonin, and contracted when stimulated by the same electrical stimulus parameters given to the cerebral arteries. The basis for the contraction was probably $\alpha$-adrenergic, since tetrodotoxin (3.3 $\times$ 10^{-7} M) or phentolamine (10^{-6} M) completely abolished the response.

Addition of ANF or AP III according to either protocol did not alter the contractile responses of mesenteric vessels, nor did it affect the tone, vasomotion, contraction, or dilation of cerebral arteries in any way. Conversely, when the rat aorta was precontracted with norepinephrine (10^{-7} M), complete relaxation was readily induced by AP III (Figure 3A) and ANF (Figure 3B).

**Discussion**

The recent isolation and synthesis of ANF factor has generated much interest in view of its therapeutic potential in the treatment of essential hypertension. The cardinal symptom of essential hypertension is an elevation in total peripheral resistance, and the purpose of the present study was to examine the direct effects of ANF on resistance-sized arteries from two vascular beds. Previously, experiments on the vasorelaxant effects of ANF have been limited to large vessels, in which a potent vasodilating effect was described. The splanchnic circulation is thought to make a major contribution to total peripheral resistance and therefore to be involved in blood pressure regulation. The cerebral bed is autoregulatory, and unlike mesenteric arteries, cerebral vessels possess an intrinsic myogenic tone. By using these two preparations, we were able to study the effects of ANF on intrinsic (tone, vasomotion) and extrinsic (pharmacological, electrical) activation mechanisms. Initially, we examined normoten-

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References


Synthetic atrial natriuretic factor does not dilate resistance-sized arteries.

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