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Effect of natriuretic peptides on cerebral artery blood flow in healthy volunteers

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ABSTRACT

The natriuretic peptides (NPs), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), have vasoactive functions that concern humans and most animals, but their specific effects on cerebral circulation are poorly understood. We therefore examined the responsiveness of cerebral arteries to different doses of the natriuretic peptides in animals and humans. We conducted a dose-response experiment in guinea pigs (*in vitro*) and a double-blind, three-way cross-over study in healthy volunteers (*in vivo*). In the animal experiment, we administered cumulative doses of NPs to pre-contracted segments of cerebral arteries. In the main study, six healthy volunteers were randomly allocated to receive two intravenous doses of ANP, BNP or CNP, respectively, over 20 min on three separate study days. We recorded blood flow velocity in the middle cerebral artery (V_{MCA}) by transcranial Doppler. In addition, we measured temporal and radial artery diameters, headache response and plasma concentrations of the NPs. In guinea pigs, ANP and BNP but not CNP showed significant dose-dependent relaxation of cerebral arteries. In healthy humans, NP infusion had no effect on mean V_{MCA}, and we found no difference in hemodynamic responses between the NPs. Furthermore, natriuretic peptides did not affect temporal and radial artery diameters or induce headache. In conclusion, natriuretic peptides in physiological and pharmacological doses do not affect blood flow velocity in the middle cerebral artery or dilate extracerebral arteries in healthy volunteers.

1. Introduction

Natriuretic peptides, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), are structurally related hormones [1]. The biological effect of these peptides in humans and most animals is to maintain cardiovascular homeostasis through their vasoactive and diuretic properties [2–6]. ANP and BNP are predominantly produced in the heart [7,8], but all three natriuretic peptides are expressed in the brain and blood vessels [9–14].

Since their discovery, the diagnostic and therapeutic importance of natriuretic peptides has increased tremendously, especially in the field of clinical cardiology [15–18]. However, the biological effects of natriuretic peptides on cerebral circulation are still poorly understood [19]. To our knowledge, no *in vivo* or *in vitro* studies in humans of the effect of natriuretic peptides on cerebral or extracranial arteries have been conducted. Four animal *in vitro* studies have examined the vasodilatory response of natriuretic peptides on cerebral arteries. Two studies in guinea pigs and rabbits showed vasodilation [20,21] while the other two in rats showed no response [22,23]. Possible interspecies differences between humans and animals have never been investigated, but potential clinical implications of the peptides may, however, be relevant in neurovascular disorders such as stroke and migraine [19].

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We hypothesized that natriuretic peptides dilate the cerebral arteries in a dose-dependent manner. Therefore, we examined the responsiveness of cerebral arteries to natriuretic peptides with different doses in animals (*in vitro*) and in healthy volunteers (*in vivo*). In addition, we measured the diameters of the superficial temporal artery and radial artery, blood flow in face microvasculature, the headache inducing properties of the natriuretic peptides and their plasma concentrations before, during and after infusion.

2. Materials and methods

We conducted three separate experiments (Fig. 1). The first experiment was an *in vitro* animal experiment where we tested the effect of the three natriuretic peptides on isolated middle cerebral arteries (MCA) and basilar arteries (BA) from guinea pigs. The second was a pilot experiment with healthy volunteers to determine the optimal dose of the natriuretic peptides for the main experiment. In the third (and main) experiment, we examined the vasodilatory effects of all three natriuretic peptides.

All participants gave written informed consent before inclusion. The study was approved by the ethics committee of Copenhagen (H-3-2011-065) and was conducted according to the Helsinki II declaration. The study was also registered at ClinicalTrials.gov (NCT01637662).

The natriuretic peptides were purchased from CASLO ApS (Lyngby, Denmark) and prepared for infusion by central pharmacy (Marielundvej, Herlev). We performed identification test of all three peptides by monoisotopical mass spectroscopy and molecule weight determination, which showed the same results as the certificate analysis from CASLO. All stock solutions were stored at −80 °C before usage.

2.1. Design of the animal experiment

Four male guinea pigs, 350–450 g, were sacrificed in the morning by decapitation after an overdose of barbiturate. The brain was removed and the basilar artery and the middle cerebral artery were dissected free. The arteries were placed in an ice-cold buffer solution (NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, $NaHPO_4$ 1.2, $MgCl_2$ 1.2, glucose 5.5, mM, pH 7.4) aerated with 95% O_2 and 5% CO_2 and transported to the laboratory for performance of the experiment. The retrieval of animal tissue followed the ethical guidelines for animal studies in Denmark.

To determine vessel tension, each segment was mounted on two metal wires 40 μm in diameter in a myograph (Model 610 M, Danish Myo Technology, Denmark). The buffer solution was continuously aerated with 5% CO_2 in O_2 to maintain a stable pH of 7.4. The artery segments were allowed to equilibrate for approximately 30 min. The vessels were stretched to the internal circumference the vessel would have if relaxed and exposed to a passive transmural pressure of 100 mmHg (13.3 kPa). This was in order to achieve maximal active force development [24]. Following a second 30 min equilibration period, the vessels were constricted twice with 60 mM KCl in a modified buffer solution in which NaCl was substituted for KCl on an equimolar basis. The contraction amounted to 4.65 ± 0.15 mN/mm (n = 16) in basilar and 1.98 ± 0.16 mN/mm (n = 16) in middle cerebral arteries. In order to study the relaxant effect of ANP, BNP and CNP the arteries were pre-contracted with 3×10^{-6} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In our preparations, it resulted in a stable tension of 3.63 ± 0.40 mN (n = 16) in basilar and 1.87 ± 0.17 mN (n = 16) in middle cerebral arteries to which the agonist was added in cumulative concentrations. The tension lasted for at least 20–30 min without a significant fall in tone.

Stock solutions of ANP, BNP and CNP (10–4 M) and $PGF_{2\alpha}$ (Sigma, USA) were prepared by dissolving the peptides in distilled water and further diluted in buffer immediately prior to the experiment.

2.2. Design of the pilot experiment

In a double-blind, three-way cross-over design, four healthy volunteers were randomly allocated to receive three high pharmacological doses (Fig. 1) of ANP, BNP or CNP over 20 min on 3 study days, separated by at least a week. The natriuretic peptides were administered intravenously and technicians, who were blinded in respect to the natriuretic peptides and their doses, performed all measurements. The purpose was to determine the optimal dose of the natriuretic peptides for the main study. We wanted to find a dose that would cause detectable changes in mean blood flow velocity of the middle cerebral artery (V_{MCA}) by transcranial Doppler (TCD) without intolerable adverse effects

Inclusion criteria for the pilot experiment were: healthy individuals 18–35 years old and weighting 50–90 kg. Fertile women were included only if using either oral contraceptives or intrauterine devices. Exclusion criteria were: a history of migraine, first degree relatives with migraine, episodic tension-type headache more than once a week, history or clinical findings suggesting neurological or cardiovascular disorders, asthma or bronchospasm and daily intake of any kind of medicine except oral contraceptives.

The subjects arrived at the clinic at 8:00 a.m. on each study day fasting and received intravenous natriuretic peptides in step-wise increasing doses. Subjects had to be free from headache for at least the last 48 h. Each infusion lasted 20 min separated by a 100-min washout period. V_{MCA}, diameter of superficial temporal artery and radial artery, blood flow in face microvasculature, end-tidal partial pressure of CO_2 ($P_{et}CO_2$), vital signs, ECG, headache intensity and adverse events were recorded at baseline and then every 10 min until 60 min after start of each infusion.

2.3. Design of the main experiment

In a double-blind, three-way cross-over design, six healthy volunteers were randomly allocated to receive two doses (a high physiological and a pharmacological dose) of ANP, BNP or CNP over 20 min on 3 study days, separated by at least a week (Fig. 1). The doses used in the main experiment were based on the results from the pilot experiment. The natriuretic peptides were administered intravenously and skilled technicians, who were blinded in respect to the natriuretic peptides and their doses, performed all measurements.

Inclusion criteria for the main experiment were the same as in the pilot experiment. Full medical history and examination was performed prior inclusion.

Each study day consisted of two administration rounds separated by a washout period. The participants arrived at the clinic at 8.30 a.m. fasting for the last 8 h and were offered a glass of water (200 ml) to avoid dehydration. Coffee, tea, cocoa or other methylxanthine-containing foods or beverages was not allowed for at least 8 h before start of the study. The participants had to be free from headache for at least 48 h. They were informed that the natriuretic peptides might induce headache in some individuals, but the timing or the type of headache was not discussed. None of the participants had previously participated in similar studies.

All procedures were performed in a quiet room and the room temperature was kept between 23 and 26 °C. The participants were placed in the supine position and venous catheters (Venflon®) were inserted into both antecubital veins for infusion and blood sampling. The subjects then rested for 30 min before baseline recordings. Infusion was given for 20 min using a time and volume controlled infusion pump (Braun Perfusor, Melsungen, Germany).