Comparison of the Hemodynamic Effects of Human and Sheep Atrial Extracts in Anesthetized Rats

Afsaneh Ranjbar[1] and Mehdi Nematbakhsh,[2] MD

Abstract

Background- Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) belong to a family of hormones that have structural similarities and some biological actions in common, such as natriuresis and vasodilatation. Previous studies have revealed the presence of ANP and BNP in the sheep atrial extract and ANP in the human atrium. The aim of the present study was to compare the hemodynamic effects of the two atrial extracts on blood pressure and hematocrit in anesthetized rats.

Methods- Human and sheep atrial extracts were prepared using saline and acid milieu method. The femoral arteries and jugular veins were cannulated for recording blood pressure and drug administration, respectively. To measure the hematocrit, we collected blood samples from the rats' eyes at the beginning and once again after 45 minutes of extract administration.

Result- The rats' mean arterial blood pressure (MAP) was reduced by the sheep atrial extract ($p<0.05$), whereas the human atrial extract did not have any significant effect on MAP. The hypotensive effect of the sheep atrial extract was higher than that of the human extract ($p<0.05$). Both extracts increased the rats' hematocrit significantly ($P<0.05$).

Discussion- The obtained results suggest that the difference between the hemodynamic effects of the two extracts could be due to the differences between the ANP and BNP clearance, cardiac output fall and the negative feedback inhibition ***(Iranian Heart Journal 2005; 6 (1,2): 78-82).***

Key words: ANP ■ BNP■ human ■ sheep ■ atrial extract ■ mean blood pressure ■ hematocrit

It has been known for a long time that the atria play an important role in the regulation of volume balance. Natriuretic peptides have been found to exist in mammalian atrial extracts. The natriuretic peptides play important roles in cardiovascular homeostasis. Three isoforms, i.e. atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) constitute the natriuretic peptide family.[1] In humans, ANP and BNP are mainly produced in cardiac atria and ventricles, respectively.

CNP is most strongly expressed in the brain but also is produced in vascular endothelial cells.[2,3,4] The combined actions of natriuretic peptides on vasculature, kidney, and adrenal glands serve both acutely and chronically to reduce systemic blood pressure as well as intravascular volume. The reduction in blood pressure is the consequence of reduced peripheral vascular resistance (mediated in part by direct relaxation of vascular smooth muscle), diminished cardiac output and decreased intravascular volume.

In the kidneys, natriuretic peptide acts on specific receptors in renal microvasculature and tubule epithelium to induce hyperfiltration, the inhibition of sodium transport and the suppression of renin release, all of which are effects responsible for natriuresis and

From the 1) Department of Physiology, Jahrom School of Medical Sciences, Ostad Motahhari Street, Jahrom Iran, and 2) Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: Ranjbar@jums.ac.ir

diuresis as well as diminished arterial blood pressure.[5,6,7] Other endocrine functions of natriuretic peptides are the inhibition of aldosterone biosynthesis and suppression of vasopressin secretion from the pituitary gland. ANP can also inhibit the water reabsorbing effect of vasopressin in collecting ducts.[7,8] There are three distinct receptors for natriuretic peptides, termed natriuretic peptide receptors A, B and C (NPRA, NPRB, NPRC, respectively). NPRA is responsible for mediating most of the biological effects of ANP and BNP, while the effects of CNP are mediated by NPRB. NPRC is a clearance receptor; it interacts with all the three natriuretic peptides in the order ANP>CNP>BNP.[8,910] Previous studies have revealed the presence of ANP and BNP in the sheep atrial extract, whereas ANP is seen in the human atrium.[11] The aim of the present study was to compare the hemodynamic effects of these two atrial extracts on blood pressure and hematocrit in anesthetized rats.

Methods

Extraction of the natriuretic activity is done with saline or in acid milieu.

Human atrial extract: 40g of the human atrial tissue (resected within 10hr post-mortem), placed in a solution containing 1M acetic acid that contained 20 mM HCl (7 times tissue volume), was boiled for 5 minutes (to denature tissue protease). After being cooled, these solutions were homogenized and centrifuged at 12000g. For pH regulation, the supernatant of the extracts was dialyzed at 4° C.[12]

Sheep atrial extract: 40g of the sheep atrial tissue was homogenized at 4°C in a buffered solution (phosphate saline) containing a protease inhibitor substance. The homogenized tissue was centrifuged at 2500g for 10 minutes, and the supernatant was boiled for 10 minutes before being centrifuged again at 1000g.[13]

In the second step, these extracts were injected to male rats (weight range 250g - 300g). The animals were housed in the animal room of Isfahan University, with lights on 12h per day, and kept at 22 ± 2^{0}C. Food and water were continuously available.

The animals were randomly divided into three groups of 5 animals each. The control group received an injection of 1ml saline, whereas the experimental group 1 received the injection of 1ml of the human atrial extract and the experimental group 2 the injection of the same amount of the sheep atrial extract.

The rats were anesthetized by intraperitoneal (i.p.) injection of pentobarbital (60mg/kg) and prepared for bioassay. The femoral arteries and jugular veins were cannulated for recording blood pressure and drug administration, respectively. Systolic and diastolic pressures were measured by physiograph. Mean arterial blood pressure (MAP) was calculated as:

MAP=diastolic pressure + 1/3 (systolic pressure - diastolic pressure)

To measure the hematocrit, we collected blood samples from the rats' eyes at the beginning and once again after 45 minutes of extract administration.

Statistical analysis

Results are expressed as mean±SEM. Paired t-test was used to compare hematocrit at the beginning and after 45 minutes of extract administration.

MAP data were assessed by one–way ANOVA and differences were examined by the Duncan range test.

Differences between the groups were considered significant at $P<0.05$.

Results

Table I presents changes in MAP before and after the injection of the two extracts. Fig.1 shows that the injection of the sheep atrial extract decreases MAP at 10, 15, 20, 25 and 30 minutes of injection (P<0.05). However, after 30 min. the reduction of MAP was not significant. Further analyses showed that human atrial extract did not have any significant effect on MAP. ANOVA indicated significant differences among the animals which had been administered the sheep and human atrial extracts. The hypotensive effect of the sheep atrial extract was higher than that of the human extract (P<0.05). Both extracts increased the rats' hematocrit significantly.

Table I. Changes in the mean arterial blood pressure (MAP) in the control group and the experimental groups 1 and 2 (MAP, mmHg, n=5).

Group	Control Group	Exp. Group (1)	Exp..Group (2)
Time (min)	Mean ± SEM	Mean ± SEM	Mean ± SEM
Before Injection	117.68± 0.65	120.76±11.37	119.46±4.57
5 After Injection	118.47±0.48	116.40±8.22	114.39±5.55
10After Injection	119.73±o.65	110.40±7.35	∗ 80.67±12.24
15 After Injection	115.80±o.48	100.8 0±12.62	∗52±13.06
20 After Injection	115.80±o.48	104.53±10.33	∗ 43.73±1
25 After Injection	115.80±o.48	117.73±9.57	∗ 34.13±6.20
30 After Injection	115.80±o.48	97.60±19.42	∗ 50.39±10.45
35 After Injection	114.86±0.08	97.33±19.28	70.93±20.57
40 After Injection	115.86±0.32	93.06±21.37	74.13±19.26
45 After Injection	115.86±0.32	92.26±23.25	75.47± 17.63
50 After Injection	115.86±0.32	101.46±17.86	94.13±2.93

∗ P<0.05

Values are presented as mean ± SEM for the control group (injection of 1ml saline), experimental group 1(the injection of 1 ml human atrial extract) and the experimental group 2 (injection of 1ml sheep atrial extract).

Table II. Changes in hematocrit values in the control group and the experimental groups 1 and 2 (n=5).

Group	Control Group	Experimental Group (1)	Experimental Group (2)
Before injection	46.0± 2.75	46.8± 1.01	48.33±2.05
After injection	46.20± 3.1	∗ 49.90± 1.10	∗51.66± 1.31

∗ P<0.05

Values are presented as mean ± SEM for the control group (the injection of 1 ml saline), experimental group No. 1 (the injection of 1 ml human atrial extract) and the experimental group No.2 (the injection of 1 ml sheep atrial extract).

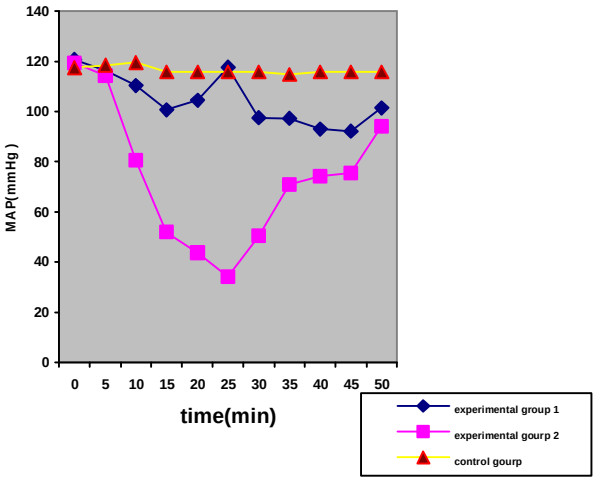

Fig. 1. Effects of the human and sheep atrial extracts on mean arterial blood pressure (MAP) during a 50-minute period for the control group (injection of 1ml saline), the experimental group 1 (injection of 1ml human atrial extract) and the experimental group 2 (injection of 1ml sheep atrial extract) (*p<0.05).

Discussion

A milestone was reached in cardiophysiology in 1981 when Deblod demonstrated that the heart functioned as an endocrine gland by injecting an extract of atrial muscle into rats, resulting in an induction of natriuresis and a drop in blood pressure.[14] Soon thereafter, ANP molecule was purified from the atrium and sequenced.[15] A few years later, BNP and CNP were also discovered from the porcine brain.[16,17] ANP and BNP are cardiac hormones and are involved in the regulation of blood pressure and fluid homeostasis.[18] In humans, ANP and BNP are mainly produced in cardiac atria and ventricles, respectively.[19] In normal sheep, although highest levels of BNP have been found in the atrial tissue (15-fold those of the ventricle), the BNP/ANP concentration ratio in the ventricle is 10-20 fold higher than the ratio calculated for the atrial tissue.[10,20] Pervious studies

have shown that the mammalian atrial extract has physiological effects on rats.[21] Peptides with natriuretic activity can be extracted from the atria of various species: rats, pigs, monkeys, rabbits, man, and sheep.[22,8] The good cross-reactivity between the different species points to similar amino acid sequences. Thus, the rat and human ANP differ only by the amino acid isoleucine instead of methionine in position 12. [22]

In the present study, the injection of sheep and human atrial extracts produced a reduction in blood pressure. The fall in blood pressure was perhaps the result of natriuretic peptide effects on the smooth muscles of blood vessels and/or the inhibition of the renin-angiotensin system and cardiac output decrease. Also, natriuretic peptides act to reduce blood pressure and intravascular volume by inhibiting aldosterone biosynthesis, both indirectly by inhibiting renin secretion from the renal juxtaglomerular apparatus and directly by a receptor-mediated action on adrenal glomerulosa cells. In addition, natriuretic peptides have been shown to inhibit the firing of vasopressin neurons in paraventricular nuclei.[7,8] On the other hand, ANP can inhibit the function of vasopressin in collecting ducts.[8] For the sheep atrial extract, the duration of the primary decline in blood pressure continues 25 minutes after injection, but for the human extract it continues only 15 minutes (Fig. 1). Our results could be elucidated through the following: the half life of BNP is higher than that of ANP, which is perhaps due to two factors: 1-the higher clearance of ANP compared to BNP; and 2-the fact that the negative feedback of ANP is higher than that of BNP. Notably, in the three species (man, cows, rats), the affinity of the clearance receptors NPRC for ANP, CNP and BNP is in descending order (ANP>CNP>BNP).[22,23,24] The hypotensive effect of the sheep atrial extract was higher than that of the human extract (Fig. 1). This is due to a less cardiac output decrease after the injection of the human extract, possibly because of the lack of BNP. In this regard, other studies have demonstrated the higher potency of BNP compared to ANP in reducing the cardiac output.[9]

In addition to the first hypotensive effect of the extracts, another decline in MAP was observed after about 45 minutes of the injection, possibly due to increased renal tubular excretion of salt and water (Fig. 1), which could suggest that the renal effects of both extracts may be the same. This result has also been shown by other investigators.[9,10] After 35 minutes of the injection, the reduction of MAP in group 2 was not statistically significant. It could be due to the possibility that the effectiveness of ANP and BNP could have decreased with time. Furthermore, the rats' hematocrit values increased after the i.p. injection of either extracts. This is probably through the renal excretion of salt and water.[21] The data generated in the present investigation, using the human and sheep atrial extracts, indicate that the presence of both ANP and BNP in the sheep atrial extract could have a synergistic or additive effect in reducing blood pressure. This remains to be confirmed by further studies.

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