EFFECTS OF BRAIN NATRIURETIC PEPTIDE-45, A CIRCULATING FORM OF RAT BNP, IN SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract

Recently rat brain natriuretic peptide-45 (rat BNP-45) was isolated from rat heart, and was shown to be a circulating form of rat BNP. We investigated the effects of rat BNP-45 in anesthetized spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY), and compared them with those of rat α-atrial natriuretic peptide (α-ANP). BNP-45 was a potent natriuretic and hypotensive agent in both strains. The effects were comparable with those of α-ANP, and were far greater than those of porcine BNP-26 reported previously. In SHR blood pressure decreased more than in WKY following injection of the highest dose (2.0 nmol/kg) of BNP-45 or α-ANP. However, WKY was more susceptible than SHR to BNP-45 for diuresis, natriuresis and urinary cGMP excretion. Moreover, a high dose of BNP-45 led to prolonged lowering of blood pressure and urinary cGMP excretion compared to α-ANP, and those features were prominent in WKY. On the other hand, BNP-45 disappeared more slowly than α-ANP, when the two peptides (2.0 μg) were injected intravenously in WKY. Thus, rat BNP-45 and α-ANP had comparable hypotensive and natriuretic potency. However, the action and plasma half-life of rat BNP-45 were more prolonged.

brain natriuretic peptide, atrial natriuretic peptide, blood pressure, natriuresis, metabolic clearance rate, rat

1. Introduction

Sudoh et al. (1988) discovered "brain natriuretic peptide (BNP)" in a porcine brain, and greater concentrations of BNP were found in porcine heart (Aburaya et al., 1989b). They established the existence of two natriuretic peptide families, atrial natriuretic peptide (ANP) and BNP, in the central nervous system and peripheral organs (Aburaya et al., 1989b; Minamino et al., 1988; Sudoh et al., 1988; Ueda et al., 1988). It has already been reported that BNP is stored in the heart and secreted into the cardiac perfusate in pigs (Saito et al., 1989) and rats (Ogawa et al., 1990). Recently, a 45 amino-acid peptide belonging to the BNP family was identified in rat heart and designated rat BNP-45 (Aburaya et al., 1989a; Kambayashi et al., 1989). Rat BNP-45 has a distinctly different sequence from porcine BNP (Aburaya et al., 1989a), and its distribution is also different; a high concentration of rat BNP-45 has been shown only in the heart (Aburaya et al., 1989c). These findings strongly suggest that rat BNP-45 may function as a circulating hormone.

Increased plasma BNP has been reported in various human diseases such as heart failure, chronic renal failure and hypertension (Mukoyama et al., 1990a; 1990b). This fact suggests the possibility that not only ANP but also BNP regulates water-electrolyte balance and blood pressure. As we have already reported, porcine BNP and ANP have similar biological activities in normal (Sudoh et al., 1988) and hypertensive rats (Kita et al., 1989a; 1989b). However, the effects of rat BNP-45 in hypertensive rats are not yet known. Therefore, to study the properties of rat BNP-

45 we investigated the effects of rat BNP-45 in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY), and compared them with the effects of rat α-ANP.

2. Materials and Methods

2.1. Animals

Twenty to 25 week-old male SHR and WKY were purchased from Charles River Inc. (Atsugi, Japan). The rats were housed in a temperature- and humidity- controlled environment and maintained on standard rat chow (Nihon CREA CE-2, Tokyo, Japan; 145 μmol Na/g) and tap water ad libitum for at least one week prior to the experiment.

2.2. Study protocol

The rats were anesthetized by an intraperitoneal (i.p.) injection of pentobarbital sodium (50 mg/kg). Anesthesia was supplemented at 20-25 mg/kg per hour (Faraci, 1989). A polyethylene catheter (PE-250) was inserted into the trachea to aid breathing. Mean blood pressure (MBP) and heart rate (HR) were monitored continuously by a right carotid artery catheter (PE-50) connected to the Statham pressure transducer (model P231D, Gould, Saddle Brook, NJ). A PE-10 catheter was inserted into the right jugular vein for administration of both maintenance solution and peptides. The bladder was exposed through a suprapubic

incision and catheterized for collection of urine.

Isotonic saline (20 µl/min) was infused throughout the experiment. After equilibration for at least 60 min, urine was collected every 10 min during a 20-min control period. Rat BNP-45 or rat α-ANP (0.1, 0.2, 0.5, 1.0 and 2.0 nmol/kg) dissolved in saline containing 1 % bacitracin (Sigma, St. Louis, MO) was injected intravenously, and urine was collected continuously for three to six 10-min periods following each dose. There was a 30- to 60-min rest interval between each injection, to allow urine volume to return to a steady baseline value. Urine volume was determined by weight. Urinary sodium and potassium were measured by flame photometry (model 205D, Hitachi, Tokyo, Japan). The concentration of cyclic guanosine 3', 5'-monophosphate (cGMP) in urine was measured by radioimmunoassay using a cGMP assay kit (Amersham, Tokyo, Japan).

2.3. Pharmacodynamics of the peptides

Another group of WKY (n = 10) was anesthetized by i. p. injection of pentobarbital sodium (50 mg/kg) and the trachea, jugular vein and carotid artery were catheterized. A bolus i.v. injection of rat BNP-45 or rat α-ANP (2.0 µg, dissolved in 150 µl saline) was given, after which 300 µl blood samples were drawn at 0.5, 1, 1.5, 2, 3, 5 and 10 min via the carotid artery catheter. The blood taken was replaced by an equal volume of saline. The remaining blood was finally taken at 30 min after the injection. Blood was collected with aprotinin (500 KIU/ml) and EDTA-2Na (1 mg/ml) and centrifuged at 3,000 x g for 10 min at 4°C.

Plasma was stored at -20°C until assayed. Plasma was loaded on a Sep-Pak C18 cartridge, and ANP and BNP were extracted as described previously (Kato et al., 1988). The concentrations of the peptides were measured using specific radioimmunoassays for rat BNP-45 (Aburaya et al., 1989c) and rat α-ANP (Kato et al., 1987) as described previously.

2.4. Peptides

Rat BNP-45 was donated by Shionogi Research Laboratories (Osaka, Japan); the peptide was synthesized by the solid phase method, and its homogeneity was confirmed by reverse phase high-performance liquid chromatography and amino acid analysis. Rat α-ANP [rat ANF (99-126)] was purchased from Peptide Institute Inc. (Osaka, Japan).

2.5. Statistical Analysis

All data were expressed as means ± S.E.M. Dose-dependent relationships were expressed by maximal changes as compared to the mean of two pre-injection measurements. The time course study was evaluated by one way analysis of variance and significant differences were subsequently determined by Dunnett's t-test. Unpaired t-tests were used for comparisons between SHR and WKY and between ANP and BNP. Values of $P < 0.05$ were considered statistically significant.

3. Results

Table 1 shows basal values of MBP, HR, urinary volume (UV), urinary sodium and potassium excretion (UNaV, UKV) and urinary cGMP excretion (UcGMPV). There were no significant intrastrain differences in these parameters. MBP and HR were significantly higher in SHR than in WKY in both BNP-45 and α-ANP groups.

Fig. 1 shows dose-response relationships of hypotensive effect induced by rat BNP-45, rat α-ANP and porcine BNP-26. At the highest dose, all three peptides lowered MBP more in SHR than in WKY. Porcine BNP-26 had less potency than the other peptides in both strains. Fig. 2 shows natriuretic effects of the three peptides in SHR and WKY. Rat BNP-45 and α-ANP caused dose-dependent natriuresis in both strains. The natriuretic effect of porcine BNP-26 was weak. On the other hand, at 0.5 nmol/kg of BNP-45, WKY excreted more water and sodium than SHR (UV; 152.8 ± 19.9 vs. 70.3 ± 9.9 μl/min per kg, $P < 0.01$, UNaV; 25.86 ± 3.81 vs. 11.33 ± 1.68 μEq/min per kg, $P < 0.05$, for WKY and SHR, respectively). Urinary potassium was also greater in WKY than in SHR at 0.5 nmol/kg (8.91 ± 0.37 vs. 5.91 ± 0.84 μEq/min per kg, $P < 0.01$, for WKY and SHR, respectively). There was no such difference in the α-ANP data.

The time courses of MBP in SHR and WKY are illustrated in Fig. 3. Intravenous administration of at least 0.5 nmol/kg rat BNP-45 was followed by a rapid dose-dependent decrease in MBP in both strains. Injection of 1.0 or 2.0 nmol/kg rat BNP-45 resulted in prolonged reduction in MBP in both groups. The effects of the highest dose of BNP

lasted over 60 min in WKY. On the other hand, at 2.0 nmol/kg the maximal α-ANP-induced decrease in MBP was equal to the decrease caused by the same dose of BNP. The hypotensive effect of α-ANP lasted 50 min. Heart rate was unchanged at all doses in both groups (data not shown).

Fig. 4 shows the time course of UcGMPV in SHR and WKY. Dose-dependent increases of UcGMPV were observed in both strains, and the effects lasted over 60 min with 1.0 and 2.0 nmol/kg of BNP-45. Injection of α-ANP was also followed by a marked increase in UcGMPV, which returned relatively quickly to the basal value. The effects of BNP-45 were more prolonged in WKY than in SHR. The natriuresis elicited by 2.0 nmol/kg of rat BNP-45 also lasted for 50 min in WKY, while the effect of an equimolar dose of α-ANP lasted only 20 min.

Fig. 5 illustrates the disappearance of the peptides after bolus injection. The immunoreactive BNP-45 and α-ANP disappeared very quickly from the circulation during the initial phase of clearance, with $T_{\frac{1}{2}}$ equal to 45 sec and 55 sec for BNP-45 and α-ANP, respectively. The remaining BNP-45 disappeared more slowly than α-ANP during the second phase ($T_{\frac{1}{2}}$; 6.95 min vs. 4.03 min for BNP-45 and α-ANP, respectively). Therefore, there was a relatively high concentration of rat BNP-45 in the circulation 30 min after injection (BNP-45; 308.0 ± 24.7 pg/ml vs. α-ANP; 158.2 ± 12.6 pg/ml, $P < 0.01$).

4. Discussion

The major findings of the present study are 1) rat BNP-45 and α-ANP showed similar pharmacological spectra in both WKY and SHR, and their effects were far greater than those of porcine BNP-26 in the same animals(Kita et al., 1989a); 2) renal actions of rat BNP-45 were lower in SHR than in WKY; 3) rat BNP-45 resulted in more prolonged natriuresis, urinary cGMP excretion and lowering of MBP than rat α-ANP, and these effects were seen more clearly in WKY than in SHR; and 4) the plasma clearance of rat BNP-45 was slower than that of rat α-ANP in WKY.

There have been many studies of the importance of the ANP system in modulating blood pressure and water-electrolyte metabolism in the central nervous system and peripheral organs. The tissue concentration of BNP in porcine whole brain was about ten times higher than that of ANP (Ueda et al., 1988). However, the concentration of BNP in the cardiac atria was much higher than that in the brain (Aburaya et al., 1989b). Moreover, we note that a high concentration of rat BNP has been found only in the heart (Aburaya et al., 1989c). Rat, porcine and human atria contain high and low molecular weight forms of BNP (Aburaya et al., 1989a; Minamino et al., 1988; Tateyama et al., 1990), namely γ-BNP and the processed form of BNP. The low molecular weight form of BNP has also been found in the blood stream (Aburaya et al., 1989b; Saito et al., 1989; Togashi et al., 1989). While the concentration of BNP was lower than that of ANP in normal porcine (Aburaya et al., 1989b; Saito et al., 1989), human (Tateyama et al., 1990) and rat hearts (Aburaya et al., 1989c; Ogawa et al., 1990), a remarkably high concentration was

observed in diseases such as congestive heart failure (Mukoyama et al., 1990b). These findings indicate that both ANP and BNP have potent functions as circulating hormones. Therefore it is interesting to clarify the properties of BNP and investigate its effect in models of hypertension.

In contrast to the similarities among mammalian ANPs (Matsuo and Nakazato, 1987), porcine, rat and human BNP show considerable differences in amino-acid sequence (Kojima et al., 1989). In rat, circulating form of ANP has 28 residues, but BNP is composed of 45 amino acids and has a long N-terminal extension. We expected that the N-terminal extension would be important in the action of rat BNP. However, BNP-32, the C-terminal 32-amino acid analogue of BNP-45, and BNP-45 had almost equal actions in our assay system (data not shown). On the other hand, porcine BNP-26, which has significantly different sequence from rat BNP, had only one half to one quarter of the bioactivity of BNP-45 or α-ANP. When administered in humans, porcine BNP-26 was less active than human ANP (McGregor et al., 1990). These data suggest species-specific actions based on heterogeneity in the amino-acid sequence of BNP.

The hypotensive effect of rat BNP or ANP was greater in SHR than in WKY, and this observation agrees with a previous report (Sasaki et al., 1985). However, in the middle of the dose range, BNP-45 caused less urinary electrolyte and cGMP excretion in SHR than in WKY, while responses to α-ANP did not differ between strains. By contrast, at the highest dose of BNP-45, SHR excreted at least as much urine and urinary electrolytes as WKY. Blood pressure reached critically low values in

WKY at the highest dose of BNP-45. It has been reported that cGMP production was less in SHR than in WKY when ANP was administered in vivo (Marsh et al., 1985) and in vitro (Sauro et al., 1988). However, there have been reports that SHR, compared to WKY, showed increased (Gellai et al., 1986; Kondo et al., 1985) or decreased (Marsh et al., 1985) renal electrolyte excretion in response to exogenous ANP administration. From the present data we cannot explain why renal responses to medium doses of BNP-45 diminished in SHR but not in WKY. However, these observations raise the possibility that the SHR kidney responds abnormally to BNP. The differential reactivity of SHR and WKY to BNP-45 may suggest the potential importance of this peptide in hypertension.

The peak action of rat α-ANP was equal to or greater than that of rat BNP-45. In contrast, the activity of ANP disappeared quickly, and that of BNP-45 was more prolonged, especially in WKY. This phenomenon is probably caused by the longer plasma half-life of rat BNP-45. The half-life of α-ANP was, as previously reported, 15-60 sec and 3-5 min in the first and second phase, respectively (Kondra et al., 1988; Katsube et al., 1986, Widimsky et al., 1990). The first phase of BNP-45 clearance was similar to that of ANP, but the second phase was longer (Fig. 5). BNP and ANP are reported to share clearance and biological receptors (Hirata et al., 1988; Maeda et al., 1990; Oehlenschlager et al., 1989). Nakao and Imura (1990) reported that human BNP showed less affinity to the clearance receptor than ANP did. So it seems likely that the half-life of rat BNP-45 was longer due to lower affinity to the clearance receptor. Recently, the role of enkephalinase (or endopeptidase) in the degradation of ANP has attracted much attention

(Gros et al., 1990). Vogt-Schaden et al. (1989) reported that endopeptidase cleaved porcine BNP as well as ANP. A different affinity to the endopeptidase may prolong the half-life of rat BNP, so it is necessary to investigate the role of endopeptidase in the clearance of rat BNP. The decreased clearance of BNP is very interesting, since a low clearance results in a high plasma concentration despite extraordinary excretion, and finally increases bioactivity. The remarkable increase of human BNP in patients with heart failure may be explained in part by decreased clearance.

Recently, a new BNP receptor that induces greater cGMP production than ANP has been identified from the human placental cDNA library (Chang et al., 1989). The prolonged action of rat BNP-45 in WKY might result from greater cGMP production associated with this receptor. However, the distribution or fluctuation of this receptor is not yet known. Many ANP and BNP receptor-mediated actions still require examination.

In conclusion, rat BNP-45 had comparable potency, and prolonged action and half-life, as compared to rat α-ANP. This new peptide should permit us to elucidate the pathophysiological importance of the natriuretic peptide families in Hypertension.

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Figure legends

Fig. 1. Dose-response relationships of maximal reduction in mean blood pressure (MBP) following injection of rat BNP-45, rat α-ANP and porcine BNP-26 in SHR and WKY. All data are means ± S.E.M.

Fig. 2. Dose-response relationships of maximal increase in urinary sodium excretion (UNaV) following injection of rat BNP-45, rat α-ANP and porcine BNP-26 in SHR and WKY. All data are means ± S.E.M.

Fig. 3. Time course of mean blood pressure (MBP) following administration of rat BNP-45 and rat α-ANP in SHR and WKY. All data are means ± S.E.M. Filled symbols indicate that values are significantly different from the pretreatment baselines ($P < 0.05$, Dunnett's _t_-test).

Fig. 4. Time course of urinary cGMP excretion (UcGMPV) following administration of rat BNP-45 and rat α-ANP in SHR and WKY. All data are means ± S.E.M. Filled symbols indicate that values are significantly different from the pretreatment baselines ($P < 0.05$, Dunnett's _t_-test).

Fig. 5. Kinetics of exogenously administered rat BNP-45 and rat α-ANP (2.0 μg, i. v.) in WKY.

Table 1. Basal values of mean arterial pressure, heart rate, urinary output, urinary sodium and potassium excretion and urinary cGMP excretion in WKY and SHR.

	rat BNP-45		rat α-ANP	
	WKY (n=8)	**SHR** (n=7)	**WKY** (n=8)	**SHR** (n=7)
Mean arterial pressure (mmHg)	128.9 ± 1.3	188.6 ± 1.0**	129.0 ± 1.9	188.3 ± 1.3**
Heart rate (beats/min)	296.0 ± 10.8	356.6 ± 5.3**	292.9 ± 10.5	341.3 ± 8.9**
Urinary output (µl/min per kg)	12.0 ± 1.0	12.4 ± 0.8	12.4 ± 0.9	12.4 ± 1.0
Urinary sodium excretion (µEq/min per kg)	0.43 ± 0.13	0.33 ± 0.11	0.45 ± 0.10	0.51 ± 0.14
Urinary potassium excretion (µEq/min per kg)	2.91 ± 0.40	3.06 ± 0.26	2.59 ± 0.48	3.05 ± 0.42
Urinary cGMP excretion (pmol/min)	27.0 ± 3.1	27.4 ± 1.4	28.0 ± 2.6	28.5 ± 1.3

Values are means ± S.E.M.; n, no. of rats. ** $P < 0.01$ when compared with values in WKY given BNP-45 or α-ANP.

Fig. 1

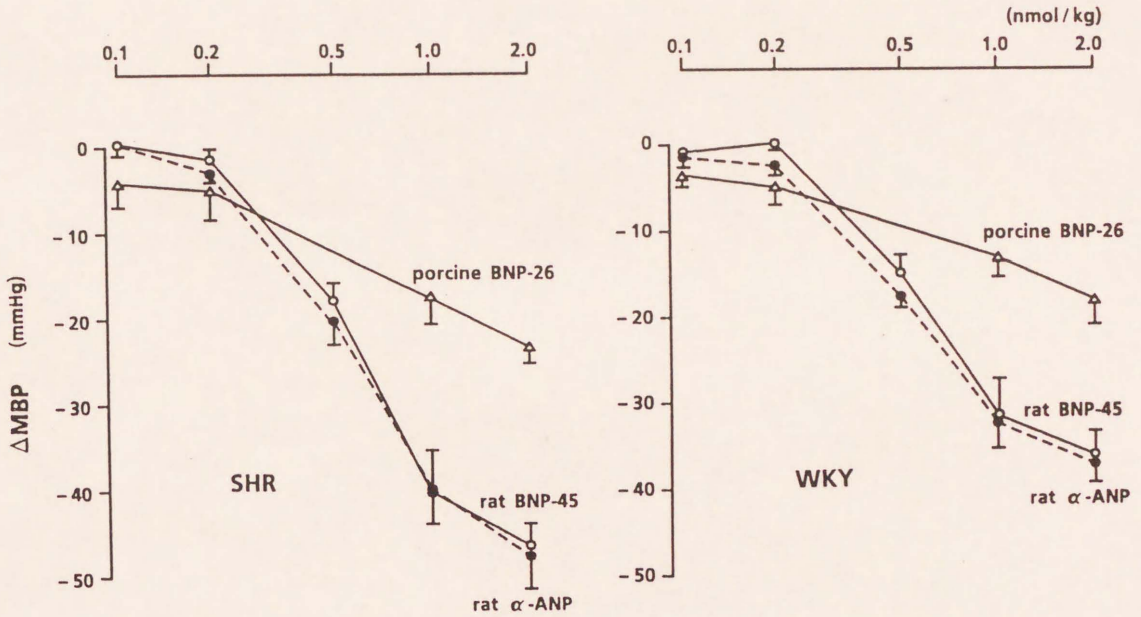

(nmol / kg)
0.1
0.2
0.5
1.0
2.0
ΔMBP (mmHg)
0
-10
-20
-30
-40
-50
SHR
WKY
porcine BNP-26
rat BNP-45
rat α-ANP

Fig. 2

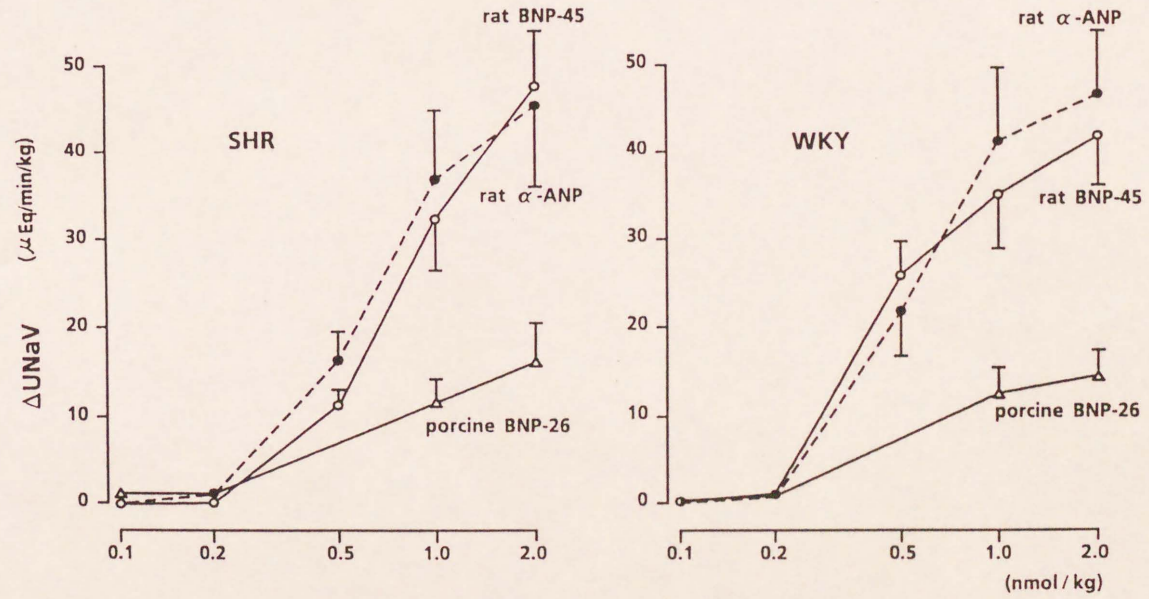

ΔUNaV (μEq/min/kg)
50
40
30
20
10
0
SHR
WKY
rat BNP-45
rat α-ANP
porcine BNP-26
0.1
0.2
0.5
1.0
2.0
(nmol / kg)

Fig. 3

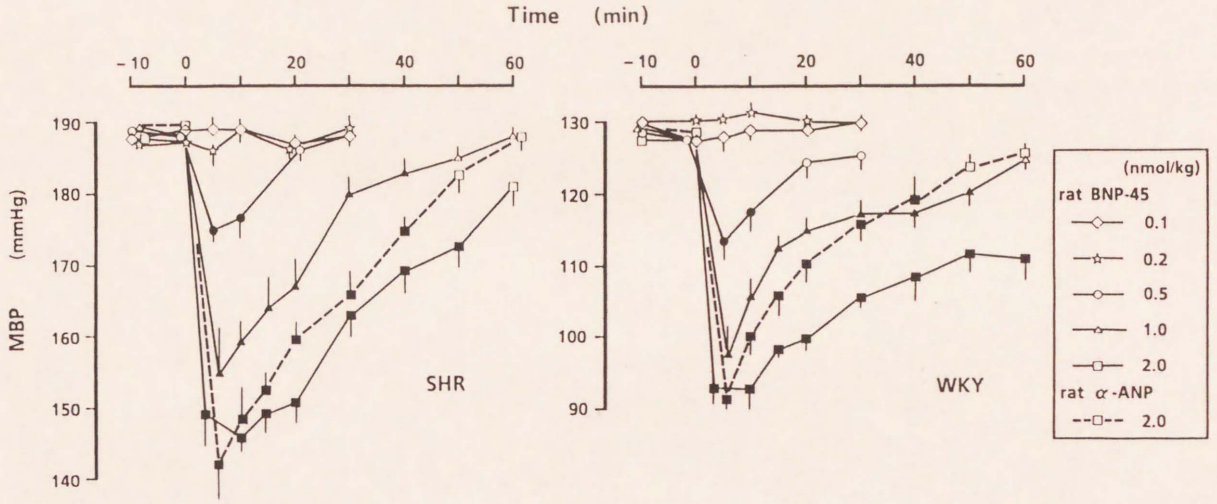

Time (min)
-10 0 20 40 60
MBP (mmHg)
190 180 170 160 150 140
130 120 110 100 90
SHR
WKY
(nmol/kg)
rat BNP-45
0.1
0.2
0.5
1.0
2.0
rat α-ANP
2.0

Fig. 4

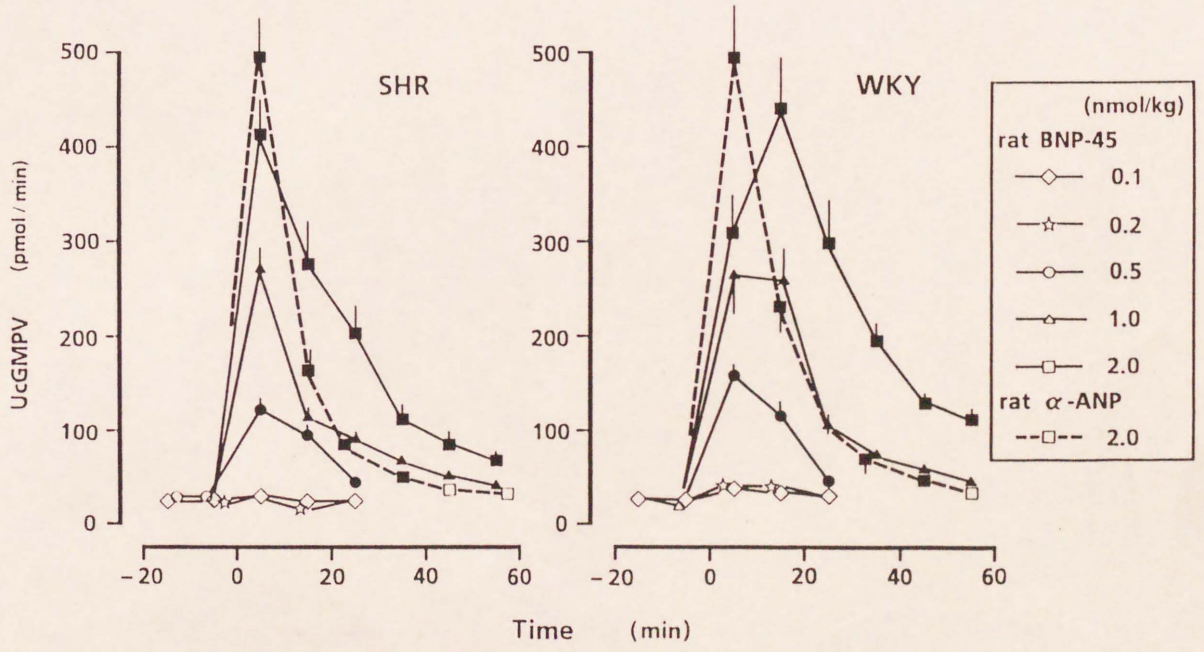
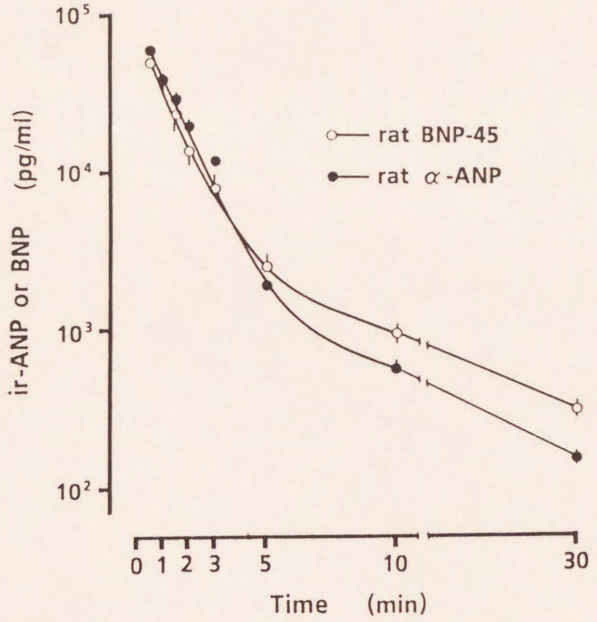

UcGMPV (pmol / min)
500 400 300 200 100 0
SHR
WKY
-20 0 20 40 60
Time (min)
(nmol/kg)
rat BNP-45
0.1
0.2
0.5
1.0
2.0
rat α-ANP
2.0

Fig. 5


10^5
10^4
10^3
10^2
ir-ANP or BNP (pg/ml)
rat BNP-45
rat α-ANP
0 1 2 3 5 10 30
Time (min)