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Effect of chronic moderate ethanol consumption on heart brain natriuretic peptide

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Abstract

There is experimental evidence indicating that chronic moderate ethanol consumption delays the age-dependent increase in blood pressure. Since the brain natriuretic peptide (BNP) is a potent hypotensive hormone, the effect of chronic ethanol treatment on the heart BNP system was investigated, using spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats. Chronic moderate ethanol consumption resulted in significantly lower circulating BNP levels for both SHR (206.9 ± 18.5 vs. 306.9 ± 28.1 pg/ml, $n = 12$, $P \leq 0.05$) and WKY rats (131.3 ± 20.7 vs. 220.6 ± 25.0 pg/ml, $n = 12$, $P \leq 0.05$). Left and right atrial BNP content and concentration in WKY rats and left atrial BNP content and concentration in SHR rats were augmented by the ethanol treatment, but not atrial BNP mRNA. In ventricular tissue, alcohol had no effect on total BNP content of either SHR or WKY rats, but it induced a significant elevation in ventricular BNP concentration (μg/mg protein) and BNP mRNA in SHR, but not WKY rats. Thus, chronic ethanol treatment resulted in specific alterations in the activity of the heart BNP system.

Keywords: Ethanol; BNP (brain natriuretic peptide); Hypertension

1. Introduction

Brain natriuretic peptide (BNP), originally discovered in porcine brain (Sudoh et al., 1988), is now considered the second member of the natriuretic peptide family, the first being atrial natriuretic peptide (ANP) (Davidson and Struthers, 1994; Nakao et al., 1993). BNP is an NH_2-elongated version of ANP with high sequence homology in its ring structure (Lang et al., 1992). However, in contrast to other natriuretic hormones, its amino-acid sequence displays important interspecies diversity (Davidson and Struthers, 1994). In the rat, the 45-amino-acid BNP molecule is believed to be as potent as ANP in its ability to lower blood pressure and to cause natriuresis, diuresis and vasorelaxation (Holmes et al., 1993; Lang et al., 1992). Although BNP is co-localized with ANP in atrial granules (Thibault et al., 1992), the major site of BNP synthesis and secretion in the body is the ventricle (Kohno et al., 1992a; Ogawa et al., 1991). Moreover, it appears that changes in the activity of ventricular BNP correlate better with the progression of hypertension as well as other pathophysiological conditions, such as congestive heart failure, than changes in the activity of the ANP system (Naruse et al., 1994; Lang et al., 1992; Kohno et al., 1992b).

It has been reported by Howe et al. (1989) and confirmed by us (Guillaume et al., 1995) that chronic moderate ethanol consumption prevents or delays the age-dependent increase in blood pressure in both normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. The mechanisms underlying this effect are still unknown but the activation of one or several members of the natriuretic peptide family by chronic ethanol is an attractive hypothesis. Furthermore, circulating BNP levels were recently found to be elevated following a short chronic ethanol treatment (6 weeks) (Wigle et al., 1993). However, since the prevention of the age-dependent increase in blood pressure by chronic moderate ethanol consumption is a progressive effect, observed only after 22 weeks of alcohol administration (Guillaume et al., 1995), it was the objective of the present studies to investigate the changes of the plasma and heart BNP levels following chronic

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ethanol treatment for a period sufficient to demonstrate significantly lower blood pressure values in the ethanol-treated compared to the ethanol-naive rats. Circulating, atrial and ventricular BNP levels were measured by radioimmunoassay (RIA) before and after the chronic administration of ethanol. Contents of BNP mRNA were also evaluated in the atria and ventricles of SHR and WKY rats.

2. Materials and methods

A total of 72 6-week-old rats (36 male SHR and 36 male WKY rats) were purchased from Charles River Breeding Laboratories (St.-Constant, Québec, Canada). Following a 1-week acclimatization period, the animals from each strain were randomly assigned to 1 of the following 3 groups: (1) the initial age control group, (2) the water-treated control group and (3) the ethanol-treated group. In the initial age control group, 12 7-week-old SHR and WKY rats were killed to allow estimation of the effect of age on the BNP system. In the water-treated control group, 12 SHR (SHR-water) and 12 WKY (WKY-water) rats were given free access to drinking water for the 8 months of the experimental period. In the ethanol-treated group, 12 SHR (SHR-ethanol) and 12 WKY (WKY-ethanol) rats were given free access to an ethanol solution that was gradually increased to 20% (v/v) in 15 days (5% (v/v) for 5 days, 10% (v/v) for 5 days, 15% (v/v) for 5 days and 20% (v/v) subsequently) (Howe et al., 1989). The ethanol treatment lasted 8 months. All animals were maintained on a 12 h dark/12 h light cycle (lights on at 06:00 h) and given free access to pellet chow (Purina, Richmond, VA, USA) during the experimental period. The blood pressure and the heart rate were measured monthly by the tail-cuff method (Pfeffer et al., 1971). At the end of the experimental period the animals were killed, between 09:00 and 12:00 h, during the light cycle.

Trunk blood was collected in chilled 15-ml conical centrifuge tubes containing 1 mg/ml ethylenediaminetetraacetate (EDTA), 10 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO, USA; P-7626) and 5 mM pepstatin A (Sigma, P-4265). A 50-μl aliquot of whole blood was placed in 450 μl of ice-cold trichloroacetic acid (6.25% w/v, Sigma) for estimation of the blood alcohol content at the time of death using the dehydrogenase enzymatic method (Hawkins et al., 1966). The remaining blood was centrifuged and the plasma was stored frozen at −75°C until assayed for BNP content. From each group, 6 animals were killed, the ventricles, left and right atria were dissected and placed in separate tubes containing ice-cold 0.1 M HCl. The tissues were boiled for 5 min and homogenized using a Polytron (Kinematica, Luzern, Switzerland). Proteins were estimated by Bradford's method (Bradford, 1976). The atria and ventricles from the remaining 6 animals/group were dissected, immediately frozen in isopentane and stored at −75°C for estimation of BNP mRNA.

Plasma BNP was extracted from 2 ml plasma using C_{18} Sep-Pak cartridges (Millipore, Milford, MA, USA) and was measured using a second-antibody RIA (Itoh et al., 1989). Briefly, the iodination of BNP was done by the lactoperoxidase method, as described elsewhere for ANP (Gutkowska et al., 1987). The standard curve was prepared by serial dilution of synthetic rat BNP (from 12.2 to 3125 pg/ml). The standards and unknowns were incubated for 48 h at 4°C with an antibody specific for the rat BNP (Peninsula Laboratories, final dilution 1:8000). This antibody reacted 100% with rat BNP-(1–45) and cross-reacted 100% with rat BNP-(1–32), but had no cross-reactivity with rat or human ANP, porcine or human BNP, or with arginine vasopressin, angiotensin II and endothelin-1. [^{125}I]BNP (6000 cpm) was then added and incubated for 24 h at 4°C. To separate bound from free, the second-antibody (GARGG, 1:50 dilution) and the normal rabbit serum (NRS, 1:35 dilution) were added and left to incubate for 4 h at room temperature. Finally, 6.25% poly(ethylene glycol) was added prior to a 20-min ($3000 \times g$) centrifugation, the supernatants were aspirated and the pellets counted in a gammacounter (Canberra Packard). The sensitivity of this assay is 24 pg/tube. The intra- and interassay coefficients of variation were 5.8 and 9.5%, respectively. Results were expressed as pg/ml plasma.

Atrial and ventricular homogenates were centrifuged, the supernatants were appropriately diluted and the BNP content was measured directly using the same RIA procedure described for estimation of plasma BNP levels. Results were expressed as μg/tissue (total content) and μg/mg protein (concentration).

The RNA extraction from the atrial and ventricular tissues was done by the acid guanidinium thiocyanate phenol chloroform method (Chomczynski and Sacchi, 1987). Total RNA (10 μg) was separated by electrophoresis (1.5% agarose gel containing 0.22 M formaldehyde) and blot transferred to a nylon membrane. Blots were subsequently hybridized with random primed α-^{32}P-labelled cDNA probes corresponding to BNP and α-tubulin mRNA sequences. Random priming kit (Gibco, BRL, Bethesda, MD, USA) and [α-^{32}P]dCTP (3000 Ci/mmol) (Amersham, Arlington Heights, IL, USA) were used for labelling the probes. As BNP probe, a 347-bp fragment covering sequences in the 5′-untranslated region of the rat BNP cDNA and in the 3′-sequence of the second exon of the BNP gene was used (Dagnino et al., 1992). This fragment was generated by reverse transcription of rat ventricular RNA. As α-tubulin probe, a 550-bp fragment of α-tubulin cDNA was generated by reverse transcription of rat atrial RNA and amplified by the polymerase chain reaction (PCR) (Dagnino et al., 1992). Membranes were hybridized in buffer containing 120 mM Tris, 8 mM EDTA, 0.6 M NaCl, 0.01% sodium pyrophosphate, 0.02% sodium dodecyl sulfate (SDS), 0.03% heparine (Sigma;

Table 1
Body weight and daily liquid consumption in WKY and SHR rats during the 8 months of treatment

	Body weight (g)								
	0 month	1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months
WKY-water	288 ± 8	327 ± 9	362 ± 12	384 ± 13	387 ± 15	407 ± 16	435 ± 16	447 ± 16	462 ± 13
WKY-ethanol	290 ± 9	304 ± 16	330 ± 18	357 ± 17	369 ± 18	380 ± 16	403 ± 18	416 ± 17	434 ± 18
SHR-water	286 ± 11	327 ± 11	370 ± 12	386 ± 17	410 ± 15	423 ± 16	439 ± 19	452 ± 19	451 ± 15
SHR-ethanol	290 ± 9	313 ± 10	360 ± 9	386 ± 8	410 ± 10	421 ± 12	430 ± 10	439 ± 8	460 ± 6
	Daily liquid consumption (ml/day)								
	0 month	1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months
WKY-water	41 ± 4	49 ± 7	42 ± 4	64 ± 6	49 ± 7	50 ± 3	50 ± 4	58 ± 2	60 ± 5
WKY-ethanol	53 ± 5	53 ± 4	41 ± 7	56 ± 3	56 ± 5	52 ± 4	57 ± 7	67 ± 7	71 ± 6
SHR-water	44 ± 4	55 ± 3	46 ± 5	67 ± 6	62 ± 5	56 ± 3	51 ± 3	63 ± 6	61 ± 5
SHR-ethanol	51 ± 4	61 ± 7	44 ± 7	64 ± 5	59 ± 4	55 ± 6	46 ± 4	76 ± 5	71 ± 7

Values shown are mean ± S.E.M. ($n = 10$).
Body weights are recorded on the last day of each month.
Daily fluid intake of each animal represents the mean fluid intake for 7 consecutive days at the end of each month.

H7005), pH 7.4 and supplemented with 10% dextran sulphate and 10^6 dpm/ml of radioactive-labelled probe. Washing was done in 2 × sodium chloride-sodium citrate buffer (SSC)/1% SDS (10 min at room temperature), 0.1 × SSC/0.01% SDS (15–30 min at 65°C) and 0.1 × SSC (10 min at room temperature). The intensity of the hybridization signals were subsequently quantitated by densitometry using the PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA). In preliminary studies, we used both α-tubulin and GAPDH probes to control for the hybridization signal. Our results indicated that there was no significant effect of ethanol on either α-tubulin or GAPDH mRNAs. Thus, to correct for differences in the amount of total mRNA applied on the electrophoresis gel, α-tubulin was used as internal control and the results are expressed as the ratio of BNP/α-tubulin mRNAs.

RT-PCR was conducted according to previously described procedures (Dagnino et al., 1992). Normalized input of cDNA with 60 ng of liver gDNA, 0.2 mM dNTPs, 10 μCi ^{32}P (final concentration) and 40 pmol of forward and reverse primers corresponding to sequences in the 1 and 2 exon of the BNP gene, were added for amplification with 2.5 U of *Thermus aquaticus* (*Taq*) DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT, USA). The following primers were used for the PCR amplification reaction: BNP forward, 5′-CCATCGCAGCTGCCTGGCCCATCACTTCTG-3′, BNP reverse, 5′-GACTGCGCCGATCCGGTC-3′. PCR amplification was carried out with 25 cycles of 1 min at 94°C, 1 min at 61°C and 3 min at 72°C. Amplification of the cDNA and the genomic DNA templates yielded a 347- and a 546-bp fragment, respectively. After separation on 1.5% agarose gels, BNP cDNA (lower band) and genomic cDNA (upper band) were measured on PhosphorImager (Molecular Dynamics) and BNP cDNA amplification products were normalized to the amount of genomic fragments.

Data are presented as mean ± S.E.M. The significance of difference among the various groups was calculated by

Table 2
Systolic blood pressure and heart rate in WKY and SHR rats during the 8 months of treament

	Blood pressure (mmHg)								
	0 month	1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months
WKY-water	109 ± 4	101 ± 3	99 ± 4	112 ± 3	110 ± 4	118 ± 4	124 ± 4	132 ± 3	130 ± 2
WKY-ethanol	118 ± 4	118 ± 5	107 ± 3	112 ± 5	105 ± 3	117 ± 4	111 ± 4	111 ± 3 [a]	115 ± 2 [b]
SHR-water	123 ± 5	116 ± 3	140 ± 5	146 ± 5	169 ± 5	184 ± 5	186 ± 5	191 ± 2	188 ± 4
SHR-ethanol	131 ± 4	125 ± 5	132 ± 5	142 ± 6	158 ± 4	170 ± 5	167 ± 4 [b]	174 ± 1 [b]	160 ± 4 [c]
	Heart rate (beats/min)								
	0 month	1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months
WKY-water	384 ± 12	380 ± 17	370 ± 8	396 ± 11	346 ± 9	348 ± 13	368 ± 14	373 ± 3	397 ± 5
WKY-ethanol	386 ± 11	395 ± 9	372 ± 9	382 ± 12	322 ± 10	328 ± 9	344 ± 5	346 ± 5 [b]	380 ± 9
SHR-water	440 ± 8	432 ± 11	446 ± 16	428 ± 12	424 ± 5	448 ± 13	418 ± 6	434 ± 9	475 ± 5
SHR-ethanol	430 ± 11	422 ± 7	440 ± 5	415 ± 15	407 ± 11	438 ± 6	382 ± 8	404 ± 12	446 ± 8 [b]

Values shown are mean ± S.E.M. ($n = 10$).
Levels of significance refer to the difference between treatments (water vs. ethanol): [c] $P \leq 0.001$, [a] $P \leq 0.01$, [b] $P \leq 0.05$.
Blood pressure and heart rate of each animal represent the mean of 3 consecutive tail-cuff measurements at the end of each month.

Table 3
Protein levels in the cardiac compartments of WKY and SHR rats

	Protein levels (mg/total tissue)		
	Right atria	Left atria	Ventricles
WKY-initial	1.28 ± 0.17	1.00 ± 0.19	4.40 ± 0.40
WKY-water	7.08 ± 0.18 [a]	6.19 ± 0.58 [a]	8.54 ± 1.12 [b]
WKY-ethanol	7.12 ± 0.42	5.24 ± 0.17	8.19 ± 1.26
SHR-initial	1.50 ± 0.13	0.86 ± 0.07	4.49 ± 0.28
SHR-water	6.51 ± 0.19 [a]	6.36 ± 0.17 [a]	14.13 ± 1.82 [c,d]
SHR-ethanol	6.66 ± 0.27	6.10 ± 0.34	7.88 ± 1.40 [e]

Values shown are mean ± S.E.M. ($n = 6$).
Effect of age (initial vs. water): [a] $P \leq 0.001$, [c] $P \leq 0.01$, [b] $P \leq 0.05$.
Effect of strain (WKY vs. SHR): [d] $P \leq 0.05$.
Effect of treatment (water vs. ethanol): [e] $P \leq 0.05$.

a two-way analysis of variance (ANOVA). This analysis was followed by the Neuman-Keuls multiple comparison test. A P value of ≤ 0.05 was considered significant.

3. Results

3.1. Effect of age and ethanol on body weight, blood pressure, heart rate and total protein content in atrial and ventricular tissues

SHR and WKY rats received a 20% (v/v) solution of ethanol for 8 months (32 weeks). Controls of both strains had free access to normal water. Table 1 shows the changes in body weight and daily fluid intake at various steps during the experimental period. No significant differ-

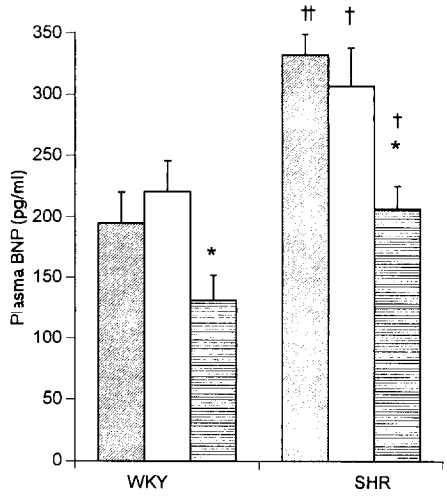

Fig. 1. Circulating BNP levels in WKY rats and SHR at 7 weeks of age (vertical lines bars) and after 8 months of water (plain bars) or 20% v/v ethanol consumption (horizontal lines bars). Values are presented as means ± S.E.M. ($n = 12$). Significant difference between ethanol- and water-treated animals of the same strain: * $P \leq 0.05$. Significant difference between SHR and WKY rats of the same age and treatment: † $P \leq 0.05$, †† $P \leq 0.01$.

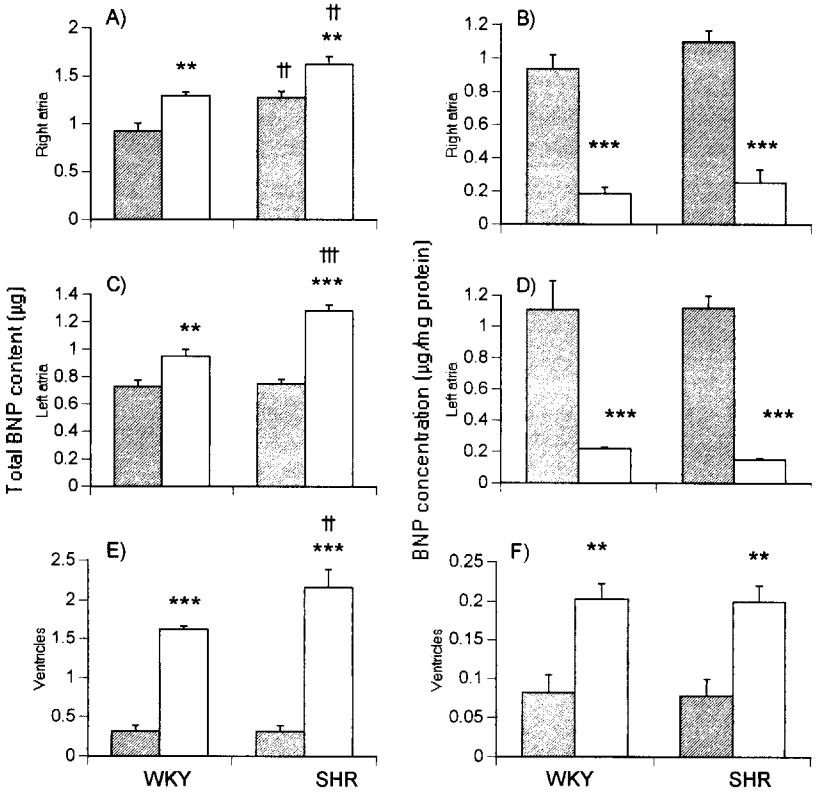

Fig. 2. Effect of age on right atrial (A-B), left atrial (C-D) and ventricular (E-F) heart BNP content and concentration. Vertical lines bars represent 7-week-old animals and plain bars represent 38-week-old rats. Values are presented as means ± S.E.M. ($n = 6$). Significant difference between the 7-week- and 38-week-old (water) animals of the same strain: ** $P \leq 0.01$, *** $P \leq 0.001$. Significant difference between the SHR and WKY rats of the same age: †† $P \leq 0.01$, ††† $P \leq 0.001$.

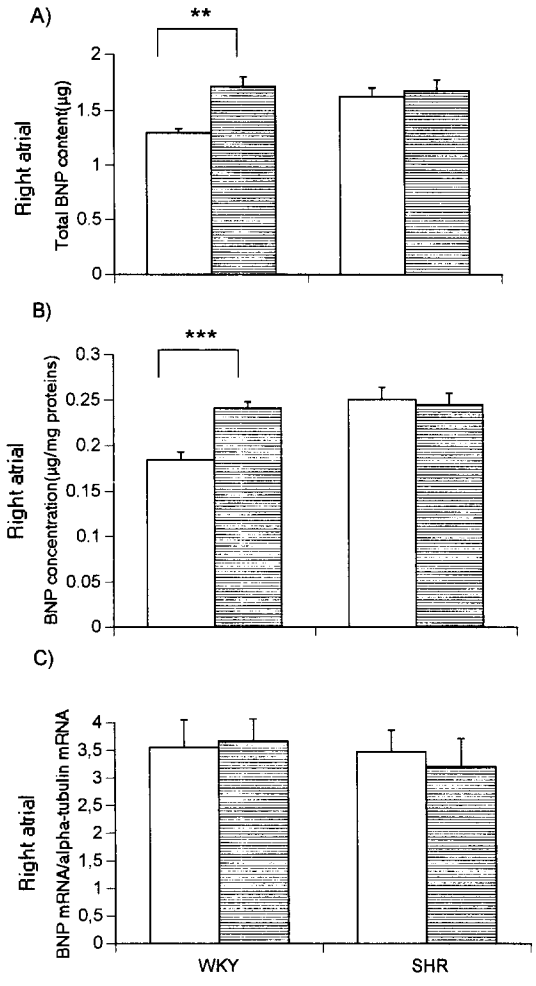

Fig. 3. Effect of water (plain bars) or chronic ethanol treatment (horizontal lines bars) on the right atrial BNP system (total content (A), concentration (B) and mRNA (C)) in WKY rats and SHR. Values are presented as means ± S.E.M. ($n = 6$). ** $P \leq 0.01$, *** $P \leq 0.001$, ethanol vs. water.

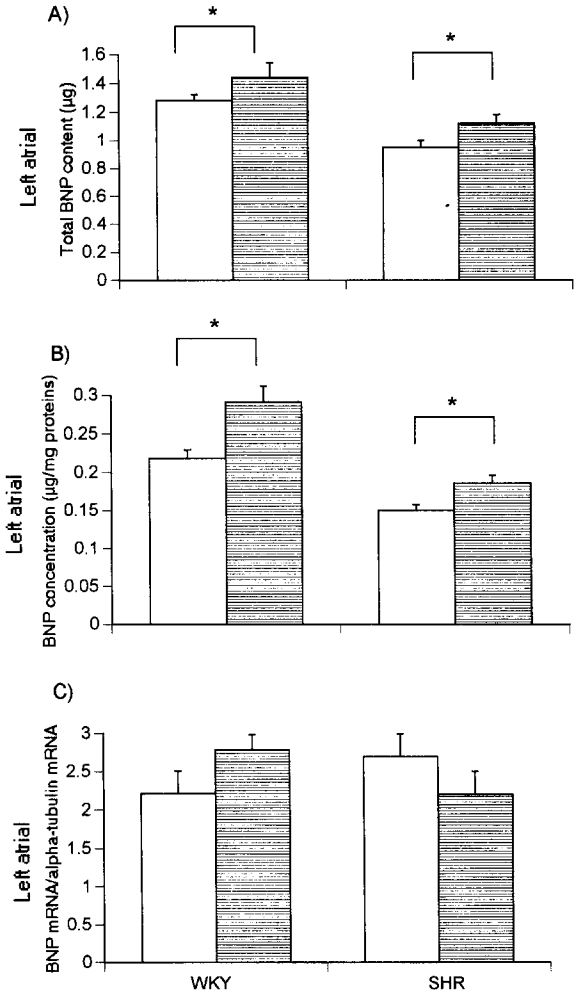

Fig. 4. Effect of water (plain bars) or chronic ethanol treatment (horizontal lines bars) on the left atrial BNP system (total content (A), concentration (B) and mRNA (C)) in WKY rats and SHR. Values are presented as means ± S.E.M. ($n = 6$). * $P \leq 0.05$, ethanol vs. water.

ences were observed between the body weights of the water- vs. the ethanol-treated animals, or between the daily fluid intake of the water- vs. the ethanol-treated rats. For each strain of rats, a two-way ANOVA with the treatment as the first independent variable and time as the second independent variable was performed for the blood pressure and heart rate (Table 2). For the blood pressure, significant effect of treatment ($F(1,162) = 17.92$, $P \leq 0.001$), time ($F(8,162) = 34.50$, $P \leq 0.001$) and a significant interaction of treatment with time ($F(8,162) = 4.95$, $P \leq 0.001$) are demonstrated for SHR rats. Similarly, significant effects of treatment ($F(1,162) = 4.01$, $P \leq 0.05$), time ($F(8,162) = 4.77$, $P \leq 0.001$) and treatment by time interaction ($F(8,162) = 3.53$, $P \leq 0.01$) are present for WKY animals. Indeed, chronic moderate ethanol consumption resulted in significantly lower BP values from the 6th (in SHR) and 7th (in WKY) month of treatment onward, as compared to age-matched water-treated controls (Table 2). For the heart rate, the two-way ANOVA indicates a significant effect of treatment ($F(1,162) = 19.81$, $P \leq 0.001$) and time ($F(8,162) = 8.64$, $P \leq 0.001$), but no significant interaction between treatment and time ($F(8,162) = 1.85$, $P = 0.08$) for SHR rats. A significant effect of treatment ($F(1,162) = 4.50$, $P \leq 0.05$) and time ($F(8,162) = 19.67$,

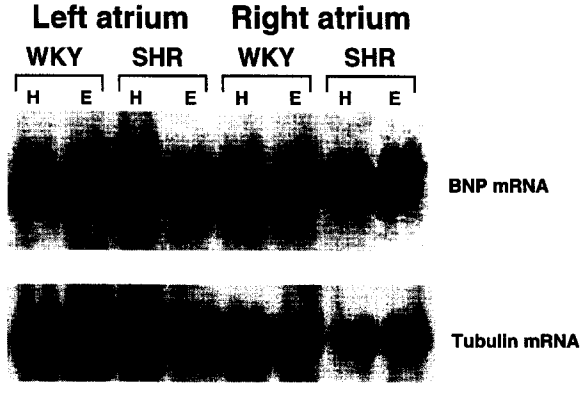

Fig. 5. Autoradiogram of a representative Northern blot of atrial BNP mRNA in WKY and SHR rats after 8 months of water (H) or 20% (v/v) ethanol treatment (E). Tubulin mRNA is used as internal control. The BNP/tubulin ratios are presented in Fig. 3C and 4C.

$P \leq 0.001$), but no significant interaction of treatment by time ($F(8,162) = 1.04$, $P = 0.41$) is also observed in WKY animals. Chronic moderate ethanol consumption significantly lowered heart rate values during the 7th month of treatment in WKY rats and during the 8th month of treatment in SHR animals (Table 2).

The total protein content in the atria and ventricles of SHR and WKY rats is shown on Table 3. In most cases, changes in total protein content represent changes in the total mass of the tissue and are usually proportional to changes in tissue weight (Szabo et al., 1979; Deshaies et al., 1975). With age, both SHR and WKY rats presented similar increases in the total protein content in the atria, suggesting similar increase in the atrial weight. However, even though at 7 weeks of age the total protein content in the ventricles of SHR and WKY rats is similar, at 38 weeks of age the ventricles of the SHR rats presented significantly higher total protein content compared to age-matched WKY rats, indicating that aging induced a larger increase in the ventricular tissue weight of the SHR animals. Chronic moderate ethanol consumption had no significant effect on the total protein content in the atria and ventricles of the WKY rats and in the atria of SHR animals. However, ethanol prevented the age-induced increase in ventricular total protein content in SHR rats, suggesting the prevention of the ventricular hypertrophy usually observed with age in this strain.

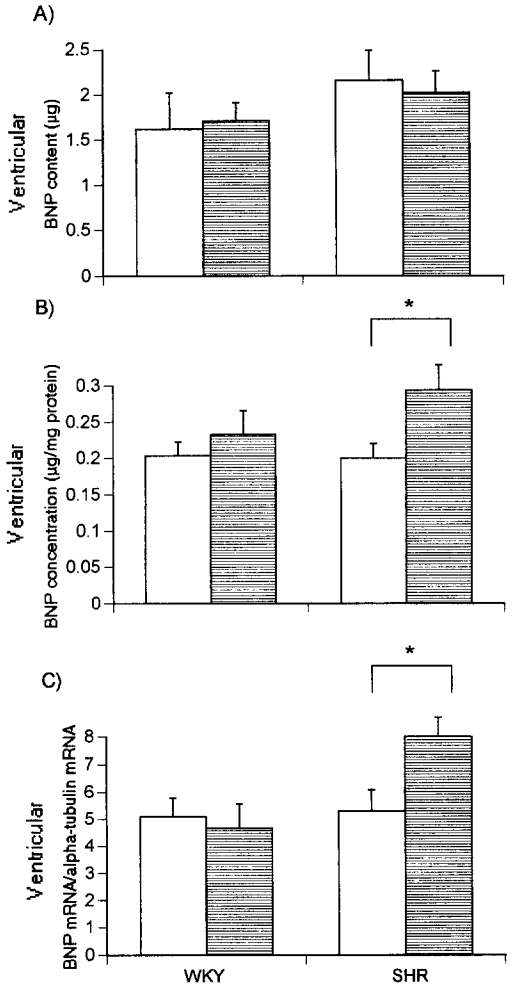

Fig. 6. Effect of water (plain bars) or chronic ethanol treatment (horizontal lines bars) on the ventricular BNP system (total content (A), concentration (B) and mRNA (C)) in WKY rats and SHR. Values are presented as means ± S.E.M. ($n = 6$). * $P \leq 0.05$, ethanol vs. water.

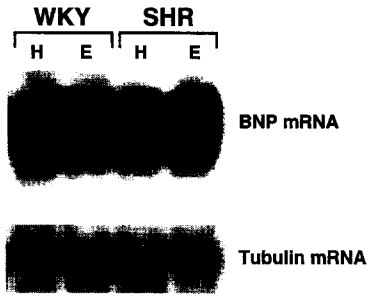

Fig. 7. Autoradiogram of a representative Northern blot of ventricular BNP mRNA in WKY and SHR rats after 8 months of water (H) or 20% (v/v) ethanol treatment (E). Tubulin mRNA is used as internal control. The BNP/tubulin ratios are presented in Fig. 6C.

3.2. Effect of age on BNP

Young (7-week-old) and adult (38-week-old) SHR rats have significantly higher plasma BNP values than WKY animals of the same age (332.3 ± 16.9 vs. 194.9 ± 25.5 pg/ml, $n = 12$, $P \leq 0.01$, for 7-week-old animals and 306.9 ± 28.1 vs. 220.6 ± 25.0 pg/ml, $n = 12$, $P \leq 0.05$, for 38-week-old rats) (Fig. 1). Total BNP content (µg) is significantly increased with age in both the right and the left atria of SHR and WKY rats (Fig. 2A,C), while the concentration (µg/mg protein) is significantly decreased (Fig. 2B,D). As observed for the atria, total ventricular BNP content is increased with age in both strains of animals (Fig. 2E). However, contrary to the atria, ventricular BNP concentration is also augmented in the adult compared to young animals (Fig. 2F). Comparison of the heart BNP content between the SHR and WKY rats of the same age indicated: (a) 7-week-old SHR rats presented a higher total BNP content in the right atria, but not in the left atria and the ventricles, compared to 7-week-old WKY rats. No significant difference is observed in the concentration of BNP in the right and left atria or in the ventricles between the 7-week-old SHR and WKY rats. (b) At 38 weeks of age, the total BNP content is significantly higher in the right and left atria and in the ventricles of SHR compared to WKY rats. However, this significant difference in the total BNP content is not associated with a significant difference in the BNP concentration (µg/mg protein) in either the right and left atria or the ventricles of the 38-week-old SHR vs. WKY rats.

3.3. Effect of ethanol on BNP

At the time of killing, the blood alcohol content detected was very low for both strains (29.97 ± 11.24 and

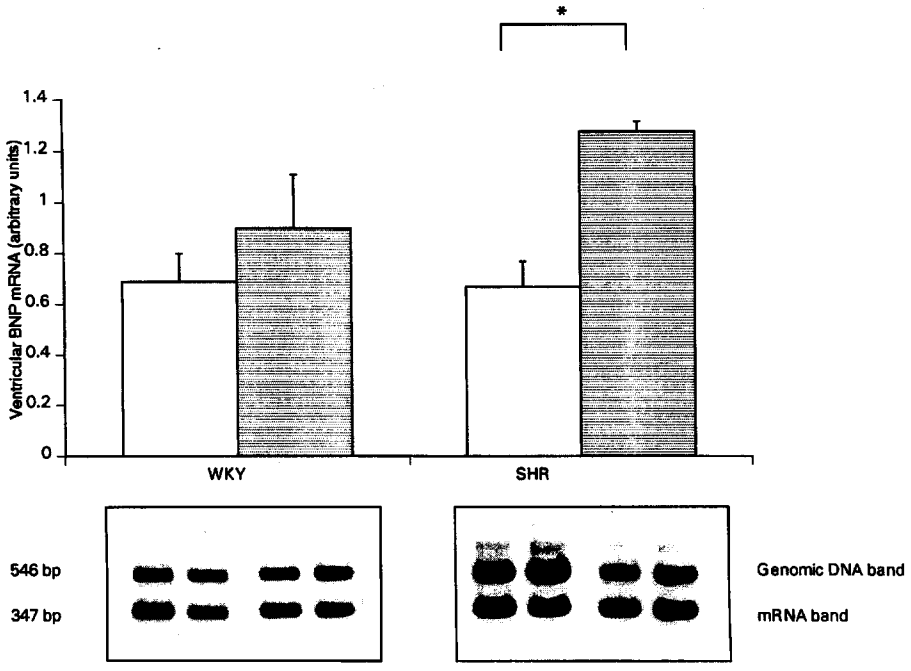

Fig. 8. Ventricular BNP mRNA by RT-PCR in adult WKY and SHR rats. Values are calculated as BNP mRNA band/genomic DNA band ($n = 3$). Significant difference between water (plain bars) and ethanol treatment (horizontal lines bars): * $P \leq 0.05$.

38.27 ± 17.82 mg/dl, $n = 6$, in WKY-ethanol and SHR-ethanol, respectively). This low blood alcohol content is due to the fact that the animals are killed during the light cycle (between 09:00 and 12:00 h) when they are not actively consuming ethanol. Fig. 1 demonstrates that the circulating BNP levels are significantly lower in the ethanol-treated rats of both strains. Plasma BNP is estimated to be 306.9 ± 28.1 and 206.9 ± 18.5 pg/ml ($n = 12$, $P \leq 0.05$) in SHR-water and SHR-ethanol, respectively, a 33% decrease, and 220.6 ± 25.0 and 131.3 ± 20.7 pg/ml ($n = 12$, $P \leq 0.05$) in WKY-water and WKY-ethanol, a 40% decrease.

The ethanol-induced changes in the total content (μg) and concentration (μg/mg protein) of BNP, as well as BNP mRNA, in the heart right and left atria and ventricles are shown in Figs. 3–7. WKY rats have increased right atrial BNP content and concentration following chronic moderate ethanol consumption (Fig. 3) whereas right atrial BNP levels in SHR are similar following the 8 months of either water or ethanol administration. In left atrial tissue, SHR and WKY rats have significantly elevated BNP levels, both in terms of total content (μg) and concentration (μg/mg protein) (Fig. 4). However, the contents of BNP mRNA in both atria are unchanged by the ethanol treatment (Fig. 5). The ethanol treatment prevented the development of ventricular hypertrophy in SHR rats, as indicated by the lower total protein content in the ventricles of ethanol- compared to water-treated SHR animals (Table 3). The chronic ethanol consumption also significantly increased both ventricular BNP concentration ($n = 6$, $P \leq 0.05$) and ventricular BNP mRNA ($n = 6$, $P \leq 0.05$) in SHR rats (Figs. 6 and 7). In contrast, total ventricular BNP content and concentration and ventricular BNP mRNA were not affected by the ethanol treatment in normotensive WKY animals (Figs. 6 and 7).

3.4. Ventricular BNP mRNA by RT-PCR

To confirm the ethanol-induced increase in ventricular BNP mRNA content in the SHR but not WKY rats, which was demonstrated by Northern blot analysis, the content of BNP mRNA in the ventricles of water- and ethanol-treated SHR and WKY rats was also estimated by RT-PCR. Results from the RT-PCR analysis demonstrated that long-term ethanol treatment induced a significant increase in the BNP mRNA content in the ventricles of SHR rats, a 91% increase from the SHR-water group (Fig. 8). In agreement with the Northern blot analysis, chronic ethanol treatment failed to induce a significant increase of the BNP mRNA content in the ventricles of WKY rats.

4. Discussion

Although the hypertensive effect of the long-term consumption of high quantities of alcohol has been well documented (Puddey et al., 1985; Lian, 1915), the effect of chronic low-to-moderate ethanol consumption remains controversial (MacMahon, 1987). Indeed, it has been previously reported by Howe et al. (1989) and confirmed by us that chronic administration of moderate quantities of ethanol delays or even prevents the age-dependent increase

in blood pressure not only in hypertensive animals, but in normotensive animals as well.

Since BNP has hypotensive properties, the objective of the present studies was to investigate the effect of chronic moderate ethanol consumption on the heart BNP system. The present studies demonstrated that chronic ethanol treatment induced a decrease in circulating BNP levels of both SHR and WKY rats. The low plasma BNP levels are associated with elevated atrial BNP content and concentration, without any alterations in atrial BNP mRNA. In the ventricles, the BNP concentration and the BNP mRNA content are significantly increased by the ethanol treatment in SHR, but not WKY rats.

Chronic moderate ethanol consumption significantly decreased plasma BNP levels and significantly increased the content and concentration of BNP in the right and left atria of WKY animals and in the left atria of SHR rats. It has been demonstrated that BNP is cosecreted with ANP by the atrial tissue (Thibault et al., 1992; Iida et al., 1990; Aburaya et al., 1989). Indeed, 2 types of atrial granules have been reported, type I with ANP alone, and type II with both ANP and BNP (Hasegawa et al., 1991). This may explain the similar variations of ANP (Guillaume et al., 1995) and BNP in the atrial tissue following chronic ethanol treatment. A differential regulation of left and right rat atrial cardiocytes has also been reported (Marie et al., 1976). It is, therefore, possible that ethanol may have a different effect on the right than left atrial cardiocytes, as observed in SHR rats where left atrial BNP content is increased by ethanol while right atrial BNP levels remain unchanged. The reason for this strain difference in right atrial BNP content is unclear, but since numerous genetic and hormonal deviations from normal rats have been observed in SHR animals (Kreutz et al., 1992; Lang et al., 1981; Nagaoka and Lovenberg, 1977), it may be possible that the BNP system and its sensitivity to ethanol are also different in the hypertensive strain. The lower circulating BNP levels may be secondary to the hemodynamic changes induced by the ethanol treatment. However, since atrial BNP mRNA contents in ethanol-treated rats remained at the same levels as those of the water-treated controls, which displayed significantly higher blood pressure and plasma BNP levels, it may be suggested that the ethanol treatment maintained the atrial BNP synthesis at the same level as that of the water-treated rats by a different mechanism than secondary hemodynamic changes due to reduced blood pressure. This mechanism may be either a direct or indirect effect of ethanol. Thus, it seems that in water-treated controls the BNP activity is stimulated by the higher blood pressure whereas in ethanol-treated rats the BNP activity is stimulated by ethanol. It is possible then that, when the blood alcohol content is high (immediately after drinking), there is an increase in atrial BNP release whereas in the absence or with low levels of alcohol the release of BNP is not stimulated and BNP accumulation in the atria occurs. Indeed, at the time of killing (between 09:00 and 12:00 h), the blood alcohol content was very low for both strains of rats.

In contrast to ANP, in the heart the major site of BNP synthesis and release is the ventricle (Lang et al., 1992; Ogawa et al., 1991). Therefore, the BNP concentration in circulation depends more on the ventricular BNP release which is controlled by the blood pressure and the ventricular load than on atrial distention pressure (Richards et al., 1993). Ventricular granules are also practically non-existent, forcing BNP to be released in a constitutive manner (Davidson and Struthers, 1994). Moreover, it appears that in pathophysiological conditions, like congestive heart failure or hypertension, a shift occurs in the production of natriuretic peptides (BNP, but also ANP) from atria to ventricles (Lang et al., 1992). In consequence, the release of BNP (and ANP) from the hypertrophied ventricles of SHR rats is significantly higher than from the ventricles of WKY rats (Kinnunen et al., 1993; Kohno et al., 1992a,1994; Roy et al., 1992; Yokota et al., 1990, 1993; and this report).

In the present studies, chronic ethanol consumption augmented significantly BNP concentration and BNP mRNA in the ventricular tissue of SHR, but not WKY rats, suggesting an increase in the rate of BNP synthesis by the ventricles. Interestingly, chronic moderate ethanol consumption prevented ventricular hypertrophy in the SHR rats. Since ventricular hypertrophy is produced by high blood pressure and cardiac overload (Kinnunen et al., 1993; Kohno et al., 1992a), the absence of elevated blood pressure in ethanol-treated SHR rats may have prevented the development of the hypertrophy. Therefore, the elevated ventricular BNP mRNA levels and BNP concentration, but not total BNP content, following chronic alcohol treatment suggests a direct stimulatory effect of ethanol on the ventricular BNP system of SHR rats, despite a lower ventricular mass and, thus, a lower basal ventricular total BNP content than the water-treated SHR rats. Again, genetic differences between SHR and WKY rats may explain this variation in ventricular BNP sensitivity to chronic ethanol consumption. Although the effects of acute moderate ethanol administration on the BNP system are still unknown, a recent study reported a significant increase in circulating BNP levels (with no variations in ANP or heart BNP content and BNP mRNA) following a short-term (6 weeks) chronic ethanol treatment in Sprague-Dawley rats (Wigle et al., 1993), indicating an increased activity of the BNP system as was also suggested in the present studies by the increased content of ventricular BNP mRNA in SHR rats. However, even though both ventricular BNP mRNA and BNP concentration are increased in SHR rats following ethanol treatment, plasma BNP content is decreased. The reason for the discrepancy between plasma BNP levels and ventricular BNP concentration and mRNA is unclear. It may be expected that the lower blood pressure in ethanol-treated rats should have led to a decrease in ventricular BNP

synthesis and release and eventually to lower ventricular BNP mRNA content. Instead, chronic ethanol treatment prevented the expected decrease in ventricular BNP activity in WKY rats and even enhanced the potential BNP activity in SHR rats. Therefore, it is possible that a transient increase in plasma BNP levels is produced after each ethanol drink, during the period of elevated blood alcohol content, so that the activity of the ventricular BNP system is maintained (WKY rats) or even enhanced (SHR rats). Alternatively, it has been proposed that BNP mRNA is more unstable than ANP mRNA, requiring larger amounts to maintain basal tissue levels and secretion by the ventricles (Lang et al., 1992). Thus, one would need to verify (by pulse-chase experiments) whether this increase in ventricular BNP mRNA in ethanol-treated SHR rats reflects substantial elevations in BNP synthesis and release.

Interestingly, acute and short-term chronic ethanol experiments have reported significant increases in plasma BNP and ANP levels (Guillaume et al., 1994; Wigle et al., 1993). In chronic ethanol experiments, the blood pressure and circulating ANP (Guillaume et al., 1995) and BNP levels are decreased. Nevertheless, the potential biosynthetic activity of the atrial and ventricular natriuretic BNP system appears to be maintained or even enhanced compared to the potential biosynthetic activity of the corresponding water-treated controls, despite the decrease in blood pressure observed following moderate ethanol consumption. This maintenance of the potential biosynthetic BNP activity in the atria and ventricles of the ethanol-treated rats may be due to the repetitive stimulatory effects of the daily ethanol consumption. Future studies estimating plasma BNP levels following acute ethanol administration in rats chronically exposed to moderate amounts of alcohol or the determination of circulating BNP levels at various intervals during the chronic ethanol treatment should provide a better insight on the exact pattern of changes in the activity of the heart BNP system, its possible correlation with the changes in the blood pressure and the possible contribution of the natriuretic peptides in the prevention of the age-dependent hypertension by moderate ethanol consumption. Conversely, it is possible that these ethanol-induced increases in plasma BNP and ANP levels following acute and short-term chronic ethanol exposure (Guillaume et al., 1994; Wigle et al., 1993) may have no long-term effects on the blood pressure. In such a case, the lower plasma levels of BNP and ANP in rats treated chronically with ethanol could represent only a secondary response to the ethanol-induced 'hypotensive' effect, which could have been mediated by other physiological systems, such as the ethanol-induced changes in lipoproteins (Hojnacki et al., 1988).

In summary, the present experiments have demonstrated that chronic ethanol exposure for 8 months alters significantly the heart BNP levels in both normotensive and hypertensive rats. Circulating BNP levels are lowered by ethanol in both strains. Moreover, BNP mRNA levels are significantly elevated in the ventricles but not atria of ethanol-treated SHR but not WKY rats. Further studies should be performed to determine the significance of the ethanol-induced changes in the activity of the heart BNP system on the prevention of the age-dependent increase in blood pressure by low-to-moderate chronic ethanol consumption.

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