Prevention by blockade of angiotensin subtype₁-receptors of the development of genetic hypertension but not its heritability

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1 We determined whether early inhibition of angiotensin II subtype₁ (AT₁) receptors by the newly synthesized nonpeptidic antagonist, A-81988, can attenuate the development of hypertension in spontaneously hypertensive rats (SHR) and if the altered blood pressure phenotype can be passed on to the subsequent generation, not exposed to the antagonist.

2 Pairs of SHR were mated while drinking tap water or A-81988 in tap water, and the progeny was maintained on the parental regimen until 14 weeks of age. At this stage, A-81988-treated rats showed lower systolic blood pressure and body weight values (136 ± 5 versus 185 ± 4 mmHg and 247 ± 4 versus 283 ± 4 g in controls, $P < 0.01$), while heart rate was similar. In addition, mean blood pressure was reduced (101 ± 7 versus 170 ± 7 mmHg in controls, $P < 0.01$), and the pressor responses to intravenous or intracerebroventricular angiotensin II were inhibited by 27 and 59%, respectively. Heart/body weight ratio was smaller in A-81988-treated rats (3.2 ± 0.1 versus 3.8 ± 0.1 in controls, $P < 0.01$).

3 The antihypertensive and antihypertrophic effect of A-81988 persisted in rats removed from therapy for 7 weeks (systolic blood pressure: 173 ± 4 versus 220 ± 4 mmHg, heart/body weight ratio: 3.4 ± 0.1 versus 4.1 ± 0.1 in controls at 21 weeks of age, $P < 0.01$ for both comparisons), whereas the cardiovascular hypertensive phenotype was fully expressed in the subsequent generation that was maintained without treatment.

4 These results indicate that chronic blockade of angiotensin AT₁-receptors attenuates the development of hypertension in SHR but it does not prevent the transmission of hypertension to the following generation. Thus, heritability of the SHR's hypertensive trait is not affected by pharmacological manipulation of the cardiovascular phenotype.

Keywords: Renin; angiotensin; blood pressure; genetic hypertension; angiotensin converting enzyme

Introduction

Early phases of life are crucial to the expression of genes encoding for proteins involved in the regulation of cardiovascular and renal function. The finding that the renin-angiotensin system (RAS) is activated in the new-born period, suggests a pivotal role of this system during early developmental phases (Wallace *et al.*, 1980; Gomez *et al.*, 1989; Carbone *et al.*, 1993). Angiotensin II (AII) not only causes vasoconstriction and antinatriuresis but also promotes vascular smooth muscle hypertrophy (Geisterfer *et al.*, 1988), and angiogenesis in the maturing kidney (Robilard *et al.*, 1983; Fogo *et al.*, 1990). In addition, angiotensin-converting enzyme (ACE), a protease that converts AI to AII, is upregulated in the new-born rat as a result of enhanced gene expression at the pretranscriptional level (Yoipiv *et al.*, 1994). The increased ACE activity could influence renal excretory function and haemodynamics in the developing animal by enhancing the rate of AII formation. Therefore, it is not surprising that environmental or pharmacological influences on the activity of the RAS during this early phase of the life can alter the adult blood pressure phenotype (Unger & Retting, 1990). Indeed, lifetime oral administration of ACE inhibitors prevents the development of arterial hypertension in rats genetically predisposed to this disease (Wu & Berecek, 1993), the latter effect persisting even after drug discontinuation (Giudicelli *et al.*, 1980; Harrap *et al.*, 1990). This property seems to be peculiar to ACE inhibitors since the antihypertensive protection exerted by other classes of drugs, such as β -blockers or calcium antagonists, tends to be lost after their discontinuation in young spontaneously hypertensive rats (SHR) (Giudicelli *et al.*, 1980; Nyborg & Mulvany, 1985).

Recently, Wu & Berecek (1993) extended the former concepts by showing that the antihypertensive effect induced by early oral administration of captopril, an ACE inhibitor, is accompanied by a failure in the offspring (that were never exposed to antihypertensive therapy) to express a full hypertensive phenotype. Although these interesting results may be consistent with an alteration of the brain RAS in the progeny of captopril-treated rats (as suggested by a blunted drinking response to intracerebroventricular administration of AI), the exact mechanism resulting in the persistence of the antihypertensive effect in the progeny remains unclear. In addition, as ACE degrades many other endogenous peptides, namely bradykinin, enkephalins and substance P (Erdos, 1974), some of the effects of ACE-inhibitors may also be unrelated to the RAS. Thus, studies using antihypertensive compounds more specific than ACE-inhibitors may help clarify how blood pressure phenotype, once altered by a pharmacological manipulation, can be passed on to subsequent generations.

Recently, nonpeptidic AII receptor antagonists, exemplified by DuP-753 (Timmermans *et al.*, 1992), have been used as a more direct approach to address the role of the RAS in blood pressure regulation, and among AII receptor subtypes recognised so far, the AT₁-receptor subtype is responsible for the majority of the cardiovascular, renal, and central nervous system actions of AII (Duncia *et al.*, 1992).

In the present study, the compound A-81988, a newly synthesized AT₁-receptor antagonist (De *et al.*, 1992), approximately 10 times more potent than DuP-753, was used to test the hypothesis that blockade of the RAS not only prevents the development of genetic hypertension, but also alters the cardiovascular phenotype of the following generation. In particular, we wished to address the following questions: (1) Can early chronic blockade of AT₁ receptors alter the development

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of hypertension and target organ damage in SHR? (2) Is the protective effect associated with blunted central and peripheral responses to AII? (3) Does the antihypertensive effect persist after therapy is stopped? and (4) Can the protective effect be passed on to the progeny despite absence of treatment in these animals?

Methods

Wistar Kyoto rats (WKY) and SHR (Charles River, Milan, Italy) were housed at a constant room temperature ($24 \pm 1^\circ\text{C}$) and humidity ($60 \pm 3\%$) with a 12 h light/dark cycle. They had free access to rat chow (sodium, 0.12 mmol g^{-1} , Mucedola, Milan, Italy) and tap water for the duration of the experiment. The experimental protocol was approved by the local animal care and use committee. All procedures complied with the standards for the care and use of animals as stated in *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academy of Science, Bethesda, MD, U.S.A.). All surgical procedures (except implantation of cerebroventricular cannula) were performed with rats under ether anaesthesia using disappearance of corneal reflex to adjust the depth of anaesthesia.

Experiment 1 Effect of oral short-term administration of A-81988 on the pressor response to AII in adult rats

Fourteen-week old, male WKY rats and SHR were given a solution containing 0.6 mg A-81988, 2-(N-propyl-N(2'-[1H-tetrazol-5-yl]biphenyl-4-yl)methyl) amino) pyridine-3-carboxylic acid, (Abbott Company, Abbott Park, IL, U.S.A.) in 100 ml tap water ($\sim 1 \text{ mg kg}^{-1}$ per day) to drink. Controls were maintained on tap water. Six days later, a polyethylene catheter (PE-10, Clay-Adams, Parsippany, NJ, U.S.A.) was inserted via the left femoral artery and advanced into the abdominal aorta; another polyethylene catheter was inserted into the left femoral vein and advanced into the vena cava. Both catheters were tunnelled under the skin and exteriorized at the back of the neck. On the morning of the following day, the mean blood pressure (MBP) of unanaesthetized A-81988- or vehicle-treated rats was measured by connecting a Statham transducer (Gould, Oxford, CA, U.S.A.) to the femoral artery catheter. After 20 min stabilization, the pressor responses to i.v. boluses of AII (from 100 to 500 ng in 20 μl saline, Sigma Chemical Company) were tested. Doses of AII were given in random order and sufficient time (30 min) was allowed between the injections for MBP to return to basal levels. Each group consisted of 6 rats.

An additional set of rats was used to test the effect of oral administration of A-81988 on the pressor effect induced by intracerebroventricular (i.c.v.) AII. Male SHR and WKY rats were anaesthetized with pentobarbitone (50 mg kg^{-1} , i.p.) and a 22-gauge stainless cannula (15 mm long and bent at 90° angle at midpoint) was implanted stereotactically into the left lateral cerebral ventricle (1.5 mm lateral and 1.0 posterior to the bregma, and 4.5 mm deep from the skull surface). The cannulae were anchored to the skull with screws embedded in dental acrylic cement. The free end of the cannula was attached to a silicone elastomer tube filled with sterile artificial cerebrospinal fluid (aCSF). The tube was tunnelled under the skin, exteriorized between the scapulae, and occluded with a metal pin. The rats were allowed to recover for three days and then they were instrumented with an arterial femoral catheter as described above. The pressor response to an i.c.v. bolus of AII (500 ng in 5 μl saline) or vehicle was determined in 14-week old male WKY rats and SHR, one week after A-81988 or vehicle was started. Injections were made with a 50 μl Hamilton syringe (Reno, NE, U.S.A.). Each group consisted of 6 rats. At the end of the experiments, the correct placement of the cannula in the lateral cerebral ventricle was tested by injecting 5 μl of 1% fast green dye and then by determining the presence of dye in the cerebroventricular system.

Experiment 2. Effect of early oral administration of A-81988 on the development and heritability of hypertension in SHR

SHR breeders were given a solution containing 0.6 mg A-81988 in 100 ml tap water ($\sim 1 \text{ mg kg}^{-1}$ per day) to drink. Dams were maintained on A-81988 during pregnancy and lactation. The control group consisted of breeders given tap water. The pups (2nd generation, 2nd G) were weaned at 4 weeks of age and maintained on the same treatment (A-81988 or vehicle) as their parents until the 14th week of age. After weaning, dosage of A-81988 (1 mg kg^{-1} per day) was kept constant throughout the duration of the experiment by adjusting the concentration of the antagonist in the drinking water.

At 14 weeks of age, 12 rats (6 males) given A-81988 had the treatment withdrawn (Off-A-81988 rats). Then, they were mated either immediately or 3 weeks after A-81988 discontinuation. This was done to discover whether duration in the time elapsed from A-81988 withdrawal is crucial in determining the blood pressure phenotype of the following generation. Since no difference was observed among rats conceived either immediately or 3 weeks after antagonist discontinuation, data relevant to these groups were combined (see Results). The progeny (third generation, 3rd G) of A-81988-treated rats was maintained on tap water (without A-81988) throughout the experiment.

Systolic blood pressure (SBP) and heart rate (HR) of rats of the 2nd G (A-81988-treated and control groups, $n=44$ [22 males] each) and 3rd G ($n=44$ [22 males]) were measured by tail-cuff plethysmography (Recorder 8002, Ugo Basile, Biological Research Apparatus, Comerio, Italy). Briefly, unanaesthetized rats were warmed for 10 min at 35°C in a thermostatically controlled heating cabinet. Then, measurements were performed with the rat gently wrapped in a cotton hand towel. Each pressure value was obtained by averaging 8 to 10 individual readings. At 14 weeks of age, 24 h urine collections were obtained from rats in individual metabolic cages. Then, cerebroventricular and femoral catheters were inserted (as described above). Three days later, the vasopressor responses to i.v. or i.c.v. injections of 500 ng AII were determined. During the following 24 h, rats that received i.c.v. AII were maintained in individual metabolic cages, which allowed for a high degree of accuracy in the measurement of water intake by the inclusion of spill catches. At the end of the experiment, with rats under anaesthesia, both kidneys and the heart were excised, washed three times in saline, blotted and weighed.

Analytical procedures

Urinary volume (UV) was determined gravimetrically. Urinary sodium (U_{NaV}) and potassium (U_{KV}) were determined by flame photometry. Urinary creatinine and osmolality were measured with an automatic analyser (Hitachi 704, Kyoto, Japan). Kallikrein activity in urine was measured by using the synthetic substrate H-D-Val-Leu-Arg-p-nitroanilide (S2266, Kabi Diagnostica, Molndal, Sweden) in the presence of soybean trypsin inhibitor (Sigma Chemical Company, St. Louis, MI, U.S.A.) and expressed in nanokatal (1 nkat represents the enzyme activity able to cleave 1 nmol p-nitroaniline s^{-1} from substrate).

Statistical analysis

All data are expressed as mean $\pm$ s.e.mean. Multivariate repeated-measures ANOVA was performed to test for interaction between time and grouping factor. Univariate ANOVA then was used to test for differences among groups and over time. Differences within or between groups were determined using Student's paired or unpaired *t* test, respectively, with the Bonferroni multiple-comparison adjustment. Mathematical

and statistical analysis were performed with as STATVIEW II package (Brain Power, Los Angeles, CA, U.S.A.) on an Apple Macintosh IICX computer.

Results

Experiment 1. Effect of oral short-term administration of A-81988 on the basal blood pressure and on the pressor response to AII in adult rats

Rats given A-81988 in their drinking water for one week showed lower MBP values compared to controls (WKY rats: 96 ± 1 versus 106 ± 2 mmHg; SHR: 149 ± 3 versus 175 ± 4 mmHg, $P < 0.05$ for both comparisons).

As shown in Figure 1a, b the pressor response to i.v. AII was significantly reduced in rats given A-81988 compared to controls. Figure 1c, d shows that the pressor response induced in SHR by 500 ng i.c.v. AII was greater than that observed in WKY rats. However, in both strains the response to i.c.v. AII was reduced in magnitude and duration by oral administration of A-81988.

Experiment 2. Effect of early oral administration of A-81988 on the development and heritability of hypertension in SHR

There was no significant difference between control and experimental groups of the 2nd G as far as the size of litters at birth were concerned (5 to 10 pups per litter).

As shown in Figure 2 (a, b), development of hypertension was attenuated by early oral administration of A-81988 in

SHR (males: 136 ± 5 versus 185 ± 4 mmHg in controls; females: 136 ± 6 versus 184 ± 3 mmHg at 14 weeks, $P < 0.01$ for both comparisons). In male SHR removed from therapy (Off-A-81988), attenuation of the hypertensive condition was still evident 7 weeks after discontinuation of the antagonist (173 ± 4 versus 220 ± 4 mmHg in age-matched never-treated controls, $P < 0.05$). By contrast, the hypertensive phenotype was fully expressed in rats of the 3rd G. In particular, the trend of SBP to increase with ageing was similar in 3rd G female SHR and controls, whereas it was steeper in 3rd G male SHR so that a plateau was already reached at 11 weeks of age. Direct measurement of MBP confirmed the difference between A-81988-treated male rats, their offspring and controls at 14 weeks of age (101 ± 7 , 169 ± 5 and 170 ± 7 mmHg, respectively, $P < 0.05$).

As shown in Figure 2c, d, no significant difference was observed between groups regarding HR. Body weight (Figure 2e, f) was lower in A-81988-treated rats compared to controls at 7 weeks of age and this difference persisted throughout the duration of the experimental period (247 ± 4 versus 283 ± 4 g in controls at 14 weeks, $P < 0.01$). Body weight was not altered in the offspring of A-81988-treated rats.

As shown in Figure 3, A-81988-treated rats showed reduced pressor (17 ± 2 versus 41 ± 3 mmHg, $P < 0.05$) and dipsogenic (29 ± 6 versus 53 ± 4 ml day⁻¹, $P < 0.05$) responses to i.c.v. AII compared to those observed in controls. The pressor response to i.v. AII was also reduced (33 ± 6 versus 45 ± 4 mmHg in controls, $P < 0.05$). By contrast, the effects of AII were not altered in Off-A-81988 SHR (i.c.v. AII: 36 ± 2 mmHg and 53 ± 2 ml day⁻¹; i.v. AII: 47 ± 3 mmHg) as well as in their offspring (i.c.v. AII: 43 ± 2 mmHg and 54 ± 2 ml day⁻¹; i.v. AII: 45 ± 3 mmHg).

Figure 1 (a,b) Line graphs show changes in mean blood pressure (ΔMBP) induced by intravenous (i.v.) bolus injections of angiotensin II (AII) in Wistar Kyoto (WKY) rats and in spontaneously hypertensive rats (SHR) pretreated with oral A-81988 (●) or vehicle (○) for 1 week. Each group consisted of 6 rats. (c,d) Line graphs show changes in mean blood pressure (ΔMBP) induced by intracerebroventricular (i.c.v.) injection of 500 ng AII in WKY rats and SHR pretreated with oral A-81988 (●) or vehicle (○) for 1 week. Each group consisted of 6 rats. Data are mean $\pm$ s.e.mean. * $P < 0.05$ versus control group that received vehicle.

Figure 2 Line graphs show systolic blood pressure (SBP) heart rate and body weight values from 7 to 14 weeks of age in male and female spontaneously hypertensive rats (SHR). The following groups are shown: rats given early oral administration of A-81988 in tap water (□), their offspring (△) and controls (●). The last two groups drank tap water, instead of A-81988 solution. Each group consisted of 22 male and 22 female rats. Data are mean $\pm$ s.e.mean. * $P < 0.05$ versus control group that received vehicle.

No significant difference was observed among groups at 14 weeks of age regarding $U_{Na}V$, U_KV , urinary kallikrein, urinary creatinine and urinary osmolality (Table 1).

A-81988-treated SHR, but not their offspring, had lower heart/body weight ratios compared to controls at 14 weeks of age (3.21 ± 0.14 , 3.82 ± 0.14 and 3.76 ± 0.07 , respectively, $P < 0.01$). Heart/body weight ratio was also reduced in Off-A-81988 SHR compared to age-matched never-treated controls (3.44 ± 0.10 versus 4.06 ± 0.08 at 21 weeks of age, $P < 0.05$). Kidney/body weight ratios did not differ among groups (data not shown).

Discussion

A-81988 is a selective nonpeptidic AT_1 -receptor antagonist, the specificity of which is suggested by the lack of affinity for adrenoceptors cholinceptors, endothelin, vasopressin, bradykinin or PAF receptors (De *et al.*, 1992; Pollock *et al.*, 1993a; Lee *et al.*, 1994). Oral administration of A-81988, at doses ranging from 0.01 to 1 mg kg⁻¹ per day, reportedly prevents hypertension caused by long-term inhibition of nitric oxide synthase (Pollock *et al.*, 1993b) or by severe reduction in renal mass (Pollock *et al.*, 1993a). Similar blood pressure lowering effects were observed by Lee *et al.* (1994) in 18-weeks old SHR, following the acute oral administration of the antagonist at 1 or 3 mg kg⁻¹. In the same model, oral administration of the antagonist for 4 weeks was able to normalize SBP and to prevent left ventricular hypertrophy. In the present study, we demonstrated that in adult SHR the antihypertensive

effect of administration of A-81988 for 1 week is associated with inhibition of the vasopressor responses to i.c.v. or i.v. AII.

Previous studies showed that the magnitude of the antihypertensive action of ACE-inhibitors strongly depends on the duration of the treatment and on how early it is started. Indeed, these compounds are modestly effective in adult SHR, while the blood pressure lowering effect reportedly averages some 30 mmHg if treatment is started at weaning (Harrap *et al.*, 1990; Giudicelli *et al.*, 1993). Furthermore, administration of captopril since the foetal stage of life, completely prevents the development of hypertension and myocardial hypertrophy in SHR (Wu & Berecek, 1993). Our finding that early oral administration of an antagonist of AT_1 -receptors was as effective as captopril confirms that the RAS plays an important role in the pathogenesis of arterial hypertension in this genetic model. Persistence of the antihypertensive effects after discontinuation of treatment indicates that early pharmacological inhibition of the RAS affects the adult cardiovascular phenotype, permanently. Not surprisingly, A-81988 prevents myocardial hypertrophy in SHR as does ACE-inhibition (Sen *et al.*, 1980). Indeed, AII is able to stimulate DNA turnover, and RNA and protein synthesis in cardiovascular tissues by activating various growth factors and oncogenes (Baker *et al.*, 1990). The possibility that remodelling of cardiovascular tissues contributes to the ability of A-81988 to prevent the development of hypertension, or, alternatively, is the consequence of blood pressure normalisation, remains to be elucidated.

Interestingly, the antihypertensive effect of A-81988 was associated with lower body weight values, an effect that reportedly occurs also in SHR given chronic treatment with ACE-inhibitors and that could reflect interference with sodium-fluid homeostasis (Harrap *et al.*, 1990). Indeed, reduction in total body fluid volume reportedly contributes to the antihypertensive effect that occurs during chronic blockade of the RAS (Harrap *et al.*, 1990).

Evidence for an enhanced activity of the RAS in the brain of SHR has been provided by a series of immunohistochemical and functional studies (Ganten *et al.*, 1983; Hermann *et al.*, 1984; Saavedra *et al.*, 1986). This alteration may play a role in the pathogenesis of genetic hypertension as suggested by the finding that early administration of ACE-inhibitors not only has antihypertensive effects in SHR but also inhibits their enhanced pressor and drinking responses to i.c.v. AI or AII (McDonald *et al.*, 1980; Unger *et al.*, 1981; Okuno *et al.*, 1983; Wu & Berecek, 1983). We found that the pressor response to i.c.v. AII was effectively inhibited by chronic oral administration of A-81988, and this inhibitory effect was greater than that exerted on the pressor response to i.v. AII (59 versus 27%). These findings favour the possibility that the antagonist can pass the blood-brain barrier and eventually exert its antihypertensive effect through a central mechanism. However, since A-81988 levels in the central nervous system were not determined in this study, a direct demonstration that the antihypertensive effect of the antagonist is mediated by a central, angiotensin-dependent mechanism is lacking. It is equally possible that normalization of blood pressure in the A-81988-treated rats alters the balance of factors regulating blood

Figure 3 (a) Changes in mean blood pressure (Δ MBP) induced by i.c.v. bolus injection of 500 ng angiotensin II (AII) in spontaneously hypertensive rats (SHR) of the 2nd generation [controls (●), A-81988-treated (□), Off-A-81988 (◇), $n=10$ in each group], and 3rd generation (△, $n=6$). Data are mean $\pm$ s.e.mean. * $P < 0.05$ versus control group, (b) Twenty-four hour water intake after intracerebroventricular bolus injection of 500 ng AII in spontaneously hypertensive rats (SHR) of the 2nd generation [A-81988-treated (□), controls (●), Off-A-81988 (◇), $n=10$ in each group], and 3rd generation, offspring of Off-A-81988 rats (△, $n=6$). Data are mean $\pm$ s.e.mean. * $P < 0.05$ versus control group.

Table 1 Effect of early oral administration of A-81988 on the urinary excretion of creatinine, sodium, potassium, kallikrein and osmolality in 14 week old male SHR

	$UCreat$	$U_{Na}V$	U_KV	$UKall$	$U_{Osm}V$
Controls ($n=22$)	11.1 ± 0.7	0.77 ± 0.11	1.11 ± 0.06	16.6 ± 1.6	11.24 ± 0.62
A-81988 ($n=22$)	11.3 ± 0.6	0.71 ± 0.05	1.06 ± 0.05	13.4 ± 0.4	11.06 ± 0.48
3rd G ($n=22$)	11.2 ± 0.5	0.60 ± 0.06	1.15 ± 0.05	16.6 ± 0.3	11.08 ± 0.42

Urine collections were obtained with rats in individual metabolic cages. No significant difference was observed among A-81988-treated rats, their offspring (3rd G), and controls as far as urinary creatinine ($UCreat$, μg day⁻¹), sodium ($U_{Na}V$, μmol day⁻¹), potassium (U_KV , μmol day⁻¹), and kallikrein ($UKall$, $nkat$ day⁻¹) excretion and urinary osmolality ($U_{Osm}V$, $mosmol$ day⁻¹) are concerned.

pressure, resulting in a depressed response to i.c.v. administration of AII. In preliminary experiments, we observed that acute i.c.v. administration of 10 µg A-81988 (a dose able to inhibit the pressor response to 500 ng i.c.v. AII by more than 80%) does not alter the blood pressure of SHR (Paolo Madeddu, unpublished observations 1994). Similarly, DePasquale *et al.* (1992) and Kawano *et al.* (1994) showed that neither acute nor chronic i.c.v. administration of DuP-753 lowers blood pressure of SHR, whereas the antagonist was effective by oral route. They concluded that, in this experimental model, DuP-753 decreases blood pressure by blockade of peripheral, not central, AT₁ receptors. However, a mild antihypertensive effect reportedly occurs in salt-sensitive SHR after the injection of DuP-753 into the anterior hypothalamic area of the brain (Yang *et al.*, 1992). Thus, brain AII may participate in the exacerbation of hypertension in conditions of sodium loading. On the other hand, failure of AII antagonists to affect basal blood pressure, when given i.c.v., might be attributable to their inability to reach receptors located in deep areas of the brain.

The antagonist A-81988 and captopril, though similar in many aspects (namely, the antihypertensive potency, the ability to alter the pressor and drinking responses to AII during treatment and the persistence of blood pressure effects after discontinuation), differ in the fact that responses to i.c.v. application of AI or AII were persistently decreased even after captopril withdrawal, whereas this effect was not seen in the Off-A-81988 SHR. Persistent alteration of the responses to i.c.v. AI after captopril withdrawal suggests that inhibition of central ACE, if it occurs in a critical phase, could lead to a permanent reduction in central content or activity of the enzyme and therefore to decreased local formation of AII. In addition, the reduced responses to i.c.v. AII in rats removed from captopril may be due to concomitant changes in central receptor number, affinity, and/or second messenger systems (Wu & Berecek, 1993). By contrast, antagonism of AT₁ receptors could reflexly increase renin release and plasma/tissue AII levels and stimulate angiotensin receptor expression (Lee *et al.*, 1994). These important differences should be taken into account when trying to explain why parental treatment with captopril attenuates the expression of the hypertensive genotype in the offspring never exposed to treatment (Wu & Berecek, 1993), whereas A-81988 does not. On the contrary, the rise in blood pressure was even accelerated in male SHR of the

third generation (offspring of Off-A-81988 rats) possibly because they were exposed to elevated parental levels of renin and AII during foetal development. The findings that the drinking response to i.c.v. AI is blunted in the progeny of captopril-treated rats (Wu & Berecek, 1993) but not in the offspring of A-81988 SHR suggest that functional integrity of central angiotensinergic mechanism is essential for the maintenance of heritable hypertension.

One might speculate that failure of A-81988 to alter the phenotype of the following generation is due to activation of AT₂-receptors. However, this possibility appears unlikely since most of the cardiovascular effects of AII are mediated by AT₁-receptors.

Since, ACE inhibitors interfere with the metabolism of various vasoactive hormones such as bradykinin, enkephalins and substance P (Erdos, 1975), and since endogenous kinins could modulate the vasopressor action of AII (Madeddu *et al.*, 1994), mechanisms other than the RAS could be, at least in part, responsible for the effects of captopril on the blood pressure of the following generation. However, evidence for participation of endogenous kinins in the blood pressure effect of ACE-inhibitors in SHR is scant (Bao *et al.*, 1992). In addition, early pharmacological manipulation of the rat blood pressure phenotype with a receptor-antagonist of bradykinin does not alter the blood pressure levels of the following generation (Madeddu *et al.*, 1995).

Other possible, though only hypothetical, explanations for the difference between A-81988 and captopril include ability of the latter to produce psychobehavioural changes (nursing behaviour) and/or chemical modifications of parental DNA (methylation patterns), that could be a source of heritability (Lindpainter, 1994).

In conclusion, our results indicate that the RAS plays an important role in the development of hypertension in SHR. Furthermore, in contrast to results with captopril (Wu & Berecek, 1993), the protective effect of AII AT₁ receptors blockade does not attenuate the hypertensive phenotype of following generation.

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