Physiol. Res. 45: 475–477, 1996

RAPID COMMUNICATION

Blockade of AT_1 Receptors by Specific Antibody Attenuated Hypertension Development in Young Spontaneously Hypertensive Rats

B. ŽELEZNÁ[1], J. VELEK[2], L. VESELSKÝ[1], J. ZICHA, Z. DOBEŠOVÁ, J. KUNEŠ

[1]Institute of Molecular Genetics, [2]Institute of Organic Chemistry and Biochemistry, and Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Received September 3, 1996
Accepted October 16, 1996

Summary

The influence of chronic angiotensin AT_1 receptor blockade by specific antibody on the development of genetic hypertension was studied in young spontaneously hypertensive rats (SHR). The immunization of 4-week-old SHR with a small part of the angiotensin AT_1 receptor molecule attenuated the development of hypertension in these animals. After five subcutaneous injections of the antigen both systolic and diastolic blood pressures were significantly lower ($p<0.005$) in immunized SHR compared to sham-immunized SHR. No effect on blood pressure was seen in immunized Wistar-Kyoto control rats. We conclude that renin-angiotensin system might be partially involved in the development of hypertension in young spontaneously hypertensive rats because it can be attenuated by a specific antibody raised against a part of the angiotensin AT_1 receptor.

Key words

Spontaneously hypertensive rat – Blood pressure – Renin-angiotensin system – Angiotensin II – AT_1 receptor

It has been demonstrated that angiotensin II participated in the development and maintenance of hypertension in spontaneously hypertensive rats (SHR) because the blockade of the renin-angiotensin system is clearly an effective antihypertensive strategy in both young and adult SHR (McLennan *et al.* 1991, Kline and Mercer 1987). The effects of angiotensin II on virtually every cellular, tissue, and organ systems have been defined and there is no doubt that specific angiotensin II receptors are involved in these processes (Timmermans *et al.* 1993). These receptors have recently been classified into several subtypes and specific non-peptide antagonists were developed. Losartan (DuP 753) and other related substances are widely used to block angiotensin AT_1 receptor subtype. It has been demonstrated that even short-term treatment of SHR with losartan decreased blood pressure to normotensive levels (Soltis 1993) and the same effect was also seen in patients with essential hypertension (Tsunoda *et al.* 1993).

A new strategy for the blockade of AT_1 receptors was recently proposed. A polyclonal rabbit antibody was prepared against a synthetic decapeptide corresponding to amino acids 14-23 of the extracellular part of angiotensin II receptor molecule (Železná *et al.* 1992). This antibody is highly specific for angiotensin AT_1 receptor and its intracerebroventricular injection blocked the dipsogenic and blood pressure response induced by centrally administered angiotensin II (Richards *et al.* 1993). Moreover, we have recently demonstrated that the pre-immunization of rats with this decapeptide can completely prevent the development of two-kidney, one-clip renal hypertension in the rat (Železná *et al.* 1994). Therefore

we have studied the influence of chronic AT_1 receptor blockade by this specific antibody on blood pressure of adult SHR rats immunized from youth.

Weanling 4-week-old male SHR rats, bred in the Institute of Physiology AS CR, were immunized as described previously (Železná *et al.* 1994). Briefly, a synthetic peptide corresponding to amino acids 14-23 of the angiotensin AT_1 receptor molecule conjugated with bovine gamma globulin was used as antigen. One hundred μg of antigen in 100 μl PBS suspended in 100 μl Freund's adjuvant was used as one dose for subcutaneous injection. Five immunizations in one-month intervals were performed and antibody production was measured by ELISA and compared with the sera of non-immunized animals. Five weeks after the last antigen injection, systolic, mean arterial and diastolic blood pressures were measured directly in the carotid artery and the organ weight was also determined. The results are expressed as means ± S.E.M. and evaluated by Student's t-test.

After the second antigen injection, the production of antibody against angiotensin AT_1 receptor was detected in 8 of 10 immunized SHR animals and in all immunized Wistar-Kyoto (WKY) rats. The titer of antibody in immunized sera was rather low, approximately 1:200 to 1:400 in comparison with non-immunized animals. After the third antigen injection, the production of antibody was observed in all immunized animals and the titer of antibody rose above 1:1600 in all immunized animals. The immunization did not influence body weight of experimental animals (Table 1).

Table 1
Body weight, relative organ weights and blood pressure in non-immunized (C) and immunized (IM) SHR and WKY rats.

	WKY		SHR	
	C	IM	C	IM
Body weight (g)	327±10	331±3	351±4	354±10
Relative heart weight (mg/100g b.w.)	252±5	247±1	347±5$^{\times}$	337±4$^{\times}$
Relative kidney weight (mg/100g b.w.)	502±6	526±7	559±8$^{\times}$	547±6$^{\times}$
Systolic blood pressure (mm Hg)	146±3	147±4	244±9$^{\times}$	211±4*$^{\times}$
Mean arterial pressure (mm Hg)	128±3	131±3	204±6$^{\times}$	177±4*$^{\times}$
Diastolic blood pressure (mm Hg)	107±3	111±2	165±5$^{\times}$	143±3*$^{\times}$
Pulse pressure (mm Hg)	38±1	36±2	80±5$^{\times}$	68±3*$^{\times}$

*Data are means ± S.E.M ($n=6-8$). Significant differences: * $p<0.005$ compared to non-immunized rats, $^{\times}$ $p<0.001$ compared to appropriate WKY controls.*

Compared to non-immunized animals, systolic, mean arterial and diastolic blood pressures were significantly decreased in immunized SHR rats (Table 1) while this procedure did not influence blood pressure of WKY animals. Regarding of pulse pressure, its reduction in immunized SHR suggested that the blockade of AT_1 receptors by specific antibody influenced the elasticity of conduit arteries.

The major objective of the present study was to determine whether the blockade of angiotensin AT_1 receptors by specific antibody is able to influence blood pressure level of adult SHR immunized from youth. The long-term immunization of SHR which started immediately after weaning leads to the significantly lower blood pressure level measured at the age of 6 months. Our data strongly suggest that this methodological approach to the blockade of AT_1 receptors was successful not only in rats with two-kidney, one-clip Goldblatt hypertension (Železná *et al.* 1994) but also in genetic hypertension. Moreover, the results of this study confirmed the hypothesis that renin-angiotensin system can play a significant role in the development and maintenance of hypertension in SHR. Nevertheless, the decrease of blood pressure in immunized SHR was not large enough to reach the blood pressure level of WKY rats suggesting that other system(s) are also responsible for blood pressure rise in SHR. This is in contrast to the observation that treatment of 24-week-old SHR with losartan for

2 weeks completely normalized their blood pressure (Soltis 1993).

In conclusion, the exact mechanism of blood pressure decrease by the blockade of angiotensin AT_1 receptors by specific antibody is not clear yet. However, it must be due to the blockade of peripheral AT_1 receptors because one can suppose that the antibody does not reach the central AT_1 receptors because of the blood-brain barrier. Another question to be answered is whether this methodological approach which is able to decrease blood pressure to some extent in young SHR, would be also efficient in adult SHR in which hypertension and some organ changes are fully developed. This will require further studies.

Acknowledgement

This work was supported by grant No 306/94/1480 (Grant Agency of the Czech Republic), CIPA CT 94-0239 (EC program COPERNICUS), and by grant No OK 170 (1996) (Ministry of Education CR).

References

KLINE R.L., MERCER P.F.: Effect of captopril and hydralazin on arterial pressure-urinary output relations in spontaneously hypertensive rats. *Hypertension* **10**: 590–594, 1987.

McLENNAN G.P., KLINE R.L., MERCER P.F.: Effect of enalapril treatment on the pressure-natriuresis curve in spontaneously hypertensive rats. *Hypertension* **17**: 54–62, 1991.

RICHARDS E.M., LU D., ŽELEZNÁ B., PHILLIPS M.I., TROLLIET M., SUMNERS C., RAIZADA M.K.: Inhibition of central angiotensin responses by angiotensin type-1 receptor antibody. *Hypertension* **21**: 1062–1065, 1993.

SOLTIS E.E.: Alterations in vascular structure and function after short-term losartan treatment in spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.* **266**: 642–646, 1993.

TIMMERMANS P.B.M.W.M., WONG P.C., CHIU A.T., HERBLIN W.F., BENFIELD P., CARINI D.J., LEE R.J., WEXLER R.R., SAYE J.A.M., SMITH R.D.: Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol. Rev.* **45**: 205–251, 1993.

TSUNODA K., ABE K., HAGINO T., OMATA K., MISAWA S., IMAI Y., YOSHINAGA K.: Hypotensive effect of losartan, a nonpeptide angiotensin II receptor antagonist, in essential hypertension. *Am. J. Hypertens.* **6**: 28–32, 1993.

ŽELEZNÁ B., RICHARDS E.M., TANG W., LU D., SUMNERS C., RAIZADA M.K.: Characterization of a polyclonal anti-peptide antibody to the angiotensin II type-1 (AT_1) receptor. *Biochem. Biophys. Res. Commun.* **183**: 781–788, 1992.

ŽELEZNÁ B., VESELSKÝ L., VELEK J., ZICHA J., KUNEŠ J.: Angiotensin AT_1 receptor blockade by specific antibody prevented two-kidney, one-clip renal hypertension in the rat. *Eur. J. Pharmacol.* **260**: 95–98, 1994.

Reprint Requests

J. Kuneš, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic.