Transmission of Hypertension in Rats by Cross Circulation

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Cross circulation was performed in 54 couples of spontaneously hypertensive and normotensive rats. Blood was pumped through two anastomoses between the carotid arteries and external jugular veins in both directions with equal flow rate. In normotensive rats cross-circulated with untreated spontaneously hypertensive rats mean arterial pressure increased by 20.9±12.2 mm Hg (p<0.01). Administration of digoxin antibody in a dose binding 0.25 mg digoxin to the spontaneously hypertensive rats before cross circulation prevented the transmission of hypertension to the normotensive rat, whereas chemical sympathectomy with 6-hydroxydopamine and intravenous injection of inactive Fab fragments had no inhibitory effect. It is concluded that, in this strain of spontaneously hypertensive rats, a circulating hypertensive factor exists. The factor binds to digoxin antibody and is not produced in sympathetic nervous tissue. (Hypertension 1989;14:61-65)

There are several indications for the role of a circulating vasopressor agent in primary hypertension. First, hypertensive plasma enhances the vasoconstrictor effect of noradrenaline and angiotensin II.1,2 Second, parabiosis experiments with spontaneously hypertensive rats demonstrated the transmission of hypertension.3-5 Third, injection of hypertensive plasma or other blood components increased blood pressure in normotensive rats.6-8 Likewise, a transmission of hypertension from spontaneously hypertensive to normotensive rats has been demonstrated by cross circulation.9

Early experiments with parabiosis10,11 and recent cross circulation studies pointed to an important role of kidneys and adrenal glands for the production of the hypertensive factor. Injection of plasma from nephrectomized hypertensive rats failed to increase blood pressure in normotensive rats.5 The transmission of hypertension by cross circulation can be prevented by nephrectomy and adrenalectomy in the spontaneously hypertensive rat.9 Although the production of the hypertensive factor in the spontaneously hypertensive rat was localized by several studies in the kidneys or adrenal glands, the structure of the hypertensive factor is yet unknown. Therefore, in the present study, cross circulation was used as a bioassay of the circulating hypertensive factor.

As to the nature of the yet unidentified agent, several lines of evidence point to a circulating sodium-potassium adenosine triphosphatase (Na,K-ATPase) inhibitor. First, in essential hypertensive individuals the ability of plasma to inhibit Na,K-ATPase has been repeatedly demonstrated.12-15 Second, cellular [3H]ouabain binding is decreased after addition of hypertensive plasma, and in hypertensive plasma a substance binding to digoxin antibody is found.15-18 Third, disturbances of cellular Na+ metabolism found by some investigators in essential hypertension19-22 may be compatible with the concept of a circulating Na,K-ATPase inhibitor.

Recently, neuropeptide Y has been described as a vasopressor and natriuretic agent23,24 and may thus fulfill the criteria for the hypothetical natriuretic hormone. Neuropeptide Y has been shown to be produced in sympathetic nerve cells,25 and its synthesis could be suppressed by chemical sympathectomy. In the present study, whether the transmission of hypertension could be influenced by chemical sympathectomy of the spontaneously hypertensive rat and by administration of digoxin antibody was tested.

Materials and Methods

The experiments were performed in 54 male spontaneously hypertensive rats from the Münster strain. The development of hypertension in the Münster strain of spontaneously hypertensive rats does not differ significantly from that in other strains. In the Milan strain a decreased intracellular Na+ concentration was found, but in the Münster strain both elevated intracellular Na+ and Ca2+ concentrations were described.26 Wistar-Kyoto rats were...
used as normotensive controls. The age of the rats was 3–5 months, and systolic blood pressure was 170–200 mm Hg. Cross circulation was performed between 30 untreated spontaneously hypertensive rats and their respective normotensive controls. The rats were anesthetized with 1.5 g/kg body wt urethane i.p.; urethane anesthesia decreased systolic blood pressure, as measured by tail-cuff method, by 21.4±12.7 mm Hg in normotensive and by 29.3±16.4 mm Hg in spontaneously hypertensive rats 15 minutes after injection of urethane. The common carotid artery and the external jugular vein of two rats were connected by a silastic tube and vice versa. Then, by a peristaltic pump, blood was pumped in both directions at a rate of 3–5 ml/min. The tubes through which the blood was pumped were inserted into thick-walled silastic tubes with a larger diameter, so that different flow rates in both directions due to a different distension of the tubes by the blood pressure of either rat could be minimized. The tubing, which had a volume of 2.8 ml for each rat, was filled with saline before cross circulation. The rats were weighed continuously during the experiment with an electronic balance sensitive in the milligram range. Thereby it was ensured that equal volumes were pumped in both directions, so that a net volume shift was avoided. The changes of weight during cross circulation were lower than 1 g.

During the experiment, mean arterial pressure was monitored by a Statham element every 10 minutes after stopping the peristaltic pump, using a plastic catheter inserted into the common carotid artery. In six spontaneously hypertensive rats 10 minutes before cross circulation, 20 mg digoxin antibody with a binding capacity of 0.25 mg digoxin (antigen binding) (Fab) fragments (Fa. Boehringer, Mannheim, West Germany) dissolved in 0.5 ml 0.9% NaCl solution were injected intravenously. Mean arterial pressure decreased thereafter by 13.6±6.7 mm Hg. In another group of six spontaneously hypertensive rats, the same amount of analogous Fab fragments not capable of binding digoxin (Fa. Boehringer) was injected before cross circulation. Blood pressure decreased insignificantly by 2.5±2.1 mm Hg after injection of the inactive Fab fragment. Chemical sympathectomy was performed in 12 spontaneously hypertensive rats by using 6-hydroxydopamine. The hypertensive rats aged 3 months received twice 50 mg/kg 6 and twice 100 mg/kg hydroxydopamine i.p. at day 0 and 7, respectively; the cross circulation was performed at day 14. Systolic blood pressure decreased from 184.7±9.1 to 165.9±10.8 mm Hg after chemical sympathectomy. Six of the chemically sympathectomized rats also received digoxin antibody before cross circulation as described above. The results were statistically evaluated by the Wilcoxon-Mann-Whitney test.

Results

Figure 1A shows the changes of mean blood pressure in normotensive rats, the circulations of which were connected to those of spontaneously hypertensive rats with a peristaltic pump. Blood pressure of the normotensive rats began to increase after about 10 minutes and further increased during the 30 minutes of cross circulation. After disconnection of the rats, blood pressure in normotensive rats decreased again slowly, reaching baseline values after 1–1.5 hours. When two normotensive animals were connected by this method, no rise in blood pressure could be detected. Initially a transient decrease in blood pressure was noted (Figure 1B).

Figure 2A shows the changes of mean arterial pressure during cross circulation between normotensive rats and spontaneously hypertensive rats pretreated with digoxin antibody. There was no increase in mean arterial pressure during and after cross circulation. The initial decrease in blood pressure after starting cross circulation was slightly more pronounced in the normotensive rats. On the other hand, pretreatment with inactive Fab fragments in the spontaneously hypertensive rat did not prevent the transmission of hypertension to normotensive rats during cross circulation (Figure 2B). Cross circulation with spontaneously hypertensive rats pretreated with 6-hydroxydopamine caused a marked increase in mean arterial pressure in the normotensive rats (Figure 3A), which was statistically not significantly different from the changes in
FIGURE 2. Graphs of changes of mean arterial pressure (MAP) during cross circulation (black bar). Panel A, spontaneously hypertensive rats treated with Fab fragments from digoxin antibody. Panel B, spontaneously hypertensive rats treated with inactive Fab fragments. Mean values and standard deviations are noted. •, normotensive rats; ○, spontaneously hypertensive rats.

FIGURE 3. Graphs of changes of mean arterial pressure (MAP) during cross circulation (black bar). Panel A, chemically sympathectomized spontaneously hypertensive rats. Panel B, chemically sympathectomized spontaneously hypertensive rats pretreated with Fab fragments from digoxin antibodies. Mean values and standard deviations are noted. •, normotensive rats; ○, spontaneously hypertensive rats.

血 clears pressure with untreated spontaneously hypertensive rats. Cross circulation of chemically sympathectomized spontaneously hypertensive rats pre-treated with digoxin antibody with normotensive rats did not reveal a transmission of hypertension to the latter (Figure 3B).

Discussion

The results show that cross circulation between spontaneously hypertensive and normotensive rats induces an increase in blood pressure in the latter. In contrast, cross circulation between normotensive rats caused a transient decrease in mean arterial pressure. This may be due to a loss of blood into the tubing after starting cross circulation, which was not adequately compensated by the influx of saline from the tubing into the circulation. The experiments suggest that a circulating hypertensive factor exists in the spontaneously hypertensive rat. As to the role of humoral factors in primary hypertension, interest has focused on a circulating Na,K-ATPase inhibitor. The presence of a circulating ATPase inhibitor as the cause of primary hypertension has been suggested, since cellular ouabain-sensitive Na+ efflux was found to be reduced in primary hypertension. Accordingly, a circulating ATPase inhibitor has been described by several authors.12–17 In some studies, a quite good correla-
tion of Na,K-ATPase inhibition and blood pressure was reported,17 suggesting a causal role of the circulating ATPase inhibitor in the development of hypertension. Furthermore, in numerous studies a digoxinlike immunoreactrvity was described in hypertensive plasma.16–18

On the other hand, some investigators failed to detect a significant influence of hypertensive plasma on ATPase activity,29–31 whereas in uremia,32 as well as after salt and volume loading,33,34 a Na+ transport inhibitor was found. Therefore, the presence of an ATPase inhibitor may not necessarily be linked to hypertension, but could also be secondary to volume expansion per se. In essential hypertension, the circulating ATPase inhibitor may thus occur in patients with beginning expansion of extracellular volume. Since the circulating ATPase inhibitor has been claimed to act as a natriuretic hormone, a secondary secretion of this substance in longstanding essential hypertension could be physiologically appropriate. Plasma concentrations increasing with age have also been reported of the atrial natriuretic factor in essential hypertension35 as well as in the spontaneously hypertensive rat.36 Another argument against a causal role of the
Na,K-ATPase inhibitor is that in secondary hypertension due to renal artery stenosis and deoxycorticosterone salt administration a digoxinlike immunoreactivity was detected in plasma.\(^{37,38}\)

In contrast to these observations suggesting a secondary role of a circulating ATPase inhibitor, the prevention of the increase in blood pressure due to cross circulation rather points to a causal role of a digoxinlike compound. The binding of the hypertensive factor to the digoxin antibody would imply a structural similarity of the hypertensive factor and the digitals glycosides. The digoxin antibody was administered in a dose binding 0.25 mg digoxin, which is likely in excess of the concentration of the hypertensive factor. Therefore, a relatively weak affinity of the antibody to the hypertensive factor is also possible. However, functional analogies between the digitals glycosides and the hypertensive factor cannot be speculated from more or less pronounced structural similarities.

On the other hand, chemical sympathectomy did not abolish, but rather enhanced the transmission of hypertension to the normotensive rat. Neuropeptide Y is produced in sympathetic nerve cells, and consequently, its production can be suppressed by chemical sympathectomy.\(^{24}\) Therefore, neuropeptide Y does not seem to be involved in the transmission of hypertension. Thus neuropeptide Y, which has both natriuretic and vasopressive actions, is likely not identical with the circulating hypertensive factor in the spontaneously hypertensive rat. The blood pressure response in normotensive rats cross-circulated with chemically sympathectomized spontaneously hypertensive rats tended to be more prolonged compared with experiments in untreated spontaneously hypertensive rats. This finding may be compatible with an enhanced production of the hypertensive factor after chemical sympathectomy to maintain blood pressure. A further possible candidate as a humoral vasopressor agent in primary hypertension might be a newly described peptide called endothelin.\(^{39}\) The experiments with the Münster strain of spontaneously hypertensive rats, however, do not support this possibility, since a high affinity binding of this peptide to the digoxin antibody is unlikely.

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References
13. De The H, Devynck MA, Rosenfeld JB, Pernollet MG, Elghozzi JL, Meyer P: Plasma sodium pump inhibition in essential hypertension and normotensive subjects with hypertension.\(^{24}\) Therefore, neuropeptide Y does not seem to be involved in the transmission of hypertension. Thus neuropeptide Y, which has both natriuretic and vasopressive actions, is likely not identical with the circulating hypertensive factor in the spontaneously hypertensive rat. The blood pressure response in normotensive rats cross-circulated with chemically sympathectomized spontaneously hypertensive rats tended to be more prolonged compared with experiments in untreated spontaneously hypertensive rats. This finding may be compatible with an enhanced production of the hypertensive factor after chemical sympathectomy to maintain blood pressure. A further possible candidate as a humoral vasopressor agent in primary hypertension might be a newly described peptide called endothelin.\(^{39}\) The experiments with the Münster strain of spontaneously hypertensive rats, however, do not support this possibility, since a high affinity binding of this peptide to the digoxin antibody is unlikely.

KEY WORDS • digoxin antibody • hypertensive factor • spontaneously hypertensive rat
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