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Renin–Angiotensin Activation in Hyperacute Renal Xenograft Rejection[1] (37979)

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Although the renal nerves appear to play a role in the normal secretion of renin (1, 2), the transplanted kidney, which is of course denervated, is capable of fine regulation of renin secretion in response to acute stimuli (3). Indeed, increases in renin secretion by transplanted kidneys have been incriminated by some in postrenal transplant hypertension associated with allograft rejection (4–6).

The role of the renin–angiotensin system (RAS) in the process of hyperacute rejection (HAR) has not, however, been previously studied. Hyperacute rejection of a renal heterograft can be observed immediately after transplantation of a pig kidney into a dog, for example. Within minutes, the kidney becomes mottled and cyanotic, loses its turgor, and renal blood flow (RBF) ceases. Since HAR has been under study in our laboratory (7), we have had the opportunity to analyze a number of the elements in the RAS in the HAR model of pig-to-dog renal xenografting. It was also of interest to study the RAS when HAR was delayed by the infusion of disodium-ethylenediaminetetraacetate (EDTA) which also prevents the conversion of angiotensin I to II (8), and the effect of the infusion of potassium which reduces renin secretion *in vivo* (9) and *in vitro* (10).

Materials and Methods. The surgical animal experiments have been previously described (7). In brief, the recipient animal was bilaterally nephrectomized about 1 hr prior to the transplant. The donor pig organs were perfused with a cold heparinized dextran solution (Perfudex) until the venous effluent was clear and then implanted into the iliac fossa of the host: the same pig in control experiments and a dog in experiments to study HAR or its prevention. Catheters were placed to sample "peripheral" blood high in the aorta as well as renal venous blood. Renal blood flow was measured by a previously published method (11). Blood samples were obtained pre- and postnephrectomy as well as at short intervals after the anastomosis of the recipient renal artery to the donor organ. The test substances were infused into the donor renal artery in Ringer's lactate solution beginning immediately before its opening to the recipient circulation.

Plasma renin activity (PRA), angiotensin I (AI), and renin substrate (RS) were measured by the radioimmunoassay methods previously outlined for these determinations on human blood (12). The pH optimum for dog renin was 5.5, for pig renin 6.5, and for the action of pig renin (Nutritional Biochemicals Corp.) on dog plasma renin substrate, such as would occur when a nephrectomized dog was given a pig xenograft, 5.5. The incubations for PRA

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and RS were carried out at their appropriate pH, depending on the specie. Angiotensin II (AII) was measured by the method of Page *et al.* (13). Plasma angiotensinase activity was measured by incubating the plasma in question, after the removal of all angiotensins by fuller's earth (14), with and without 5 ng AI/ml at 37° for 2 hr. The net destruction of AI was then measured by radioimmunoassay. For all angiotensin radioimmunoassays, the standards were prepared in angiotensin-free (fuller's earth-extracted) plasma from the specie concerned.

Results. Control values, obtained after placement of the sampling catheters and before nephrectomy, for systemic and renal vein levels of AI and PRA varied over a very wide range in the animals studied, as shown in Table I. These differences might perhaps be ascribable to variations in manipulation of the kidneys during catheter insertion and other unknown causes. Therefore, the results are presented in terms of the alterations from the baseline of these values in typical experiments. In order to study the secretion of renin and production of AI most proximally, only the renal vein values are generally presented.

Since HAR is complete within 15 min, in three experiments AI and PRA were measured for that period of time in pigs receiving autografts. In each case, such as the example in Fig. 1, there was a substantial increase in renin secretion and angiotensin production (after a prior drop in the case of a high pretransplant level). The increase over the lowest PRA in each study averaged 15.3 ± 11.3 SD ng/ml/hr and that for AI 28.5 ± 8.7 ng/ml.

In seven pig-to-dog renal xenografts, HAR always occurred within a few minutes (7). In these transplants there were also considerable increases in renal vein AI and PRA levels. However, the increases were greater than those in the autografts, averaging 69.6 ±59.2 ng/ml for AI and 32.1 ± 34.3 ng/ml/hr for PRA. These peak values were seen at an average of 5.2 and 5.8 min. A typical experiment is shown in Fig. 2. This figure also illustrates another phenomenon observed in 3 of the

TABLE I. Prenephrectomy Values of AI and PRA.

	Systemic					Renal vein				
		AI (ng/ml)		PRA (ng AI/ml/hr)			AI (ng/ml)		PRA (ng AI/ml/hr)	
	N	Range	Mean ± SD	Range	Mean ± SD	*N*	Range	Mean ± SD	Range	Mean ± SD
Dog	18	0– 4.31	0.94 ± 1.15	0.75–23.4	6.5 ± 7.2	17	0– 17.6	3.0 ± 4.3	0.59–97.8	22.2 ± 24.0
Pig	5	0.11–47.5	12.3 ± 18.2	0.13–63.9	26.2 ± 30.1	5	0–295	69.2 ± 114	0.05–73.3	26.1 ± 25.9

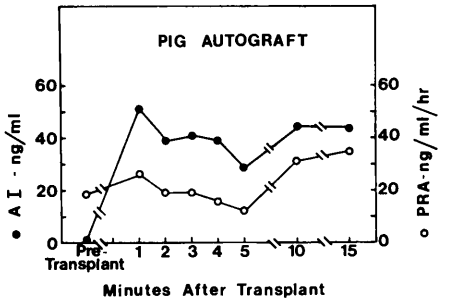

FIG. 1. Levels of renin activity and angiotensin I in renal-vein plasma of a pig kidney autotransplanted into the iliac fossa from its normal location. The "0" time sample was obtained before the normal circulation to the kidney was interrupted prior to perfusion with dextran solution. The kidney functioned normally in its transplanted site.

7 experiments, namely, a drop in PRA to a negative value at a time when astronomical levels of AI were observed as HAR was becoming complete and RBF reduced to 5–10% of baseline. In other words, there was no net AI generation during the *in vitro* incubation for PRA determination. As can also be seen in Fig. 2, the renal vein levels of AII and the AII produced during the PRA incubations also rose significantly as HAR proceeded. Systemic AI, AII, and PRA values at these times were within the control range for each animal, presumably due to the low rate of venous return from the graft.

As previously described (7), the administration of Na_2EDTA solution, 250 mg/100 ml, at the rate of 5 ml/min into two dogs postponed the first gross changes of HAR until 10 min after revascularization, with maintenance of good RBF until 15 min. However, by 20 min, rejection was complete. Maximum AI levels of 63 and 93 ng/ml occurred at 15 and 20 min respectively, and PRA peaks of 70 and 85 ng/ml/hr at 10 min in these dogs. The renal vein levels of AII never exceeded 1.1 ng/ml nor were significant amounts of AII generated in the PRA incubations.

Table II shows the systemic and renal vein AI, PRA, and renin substrate levels in such an experiment and demonstrates that the substrate is consumed in the kidney.

Figure 3 shows the renal vein AI and PRA values in one of the three dogs in

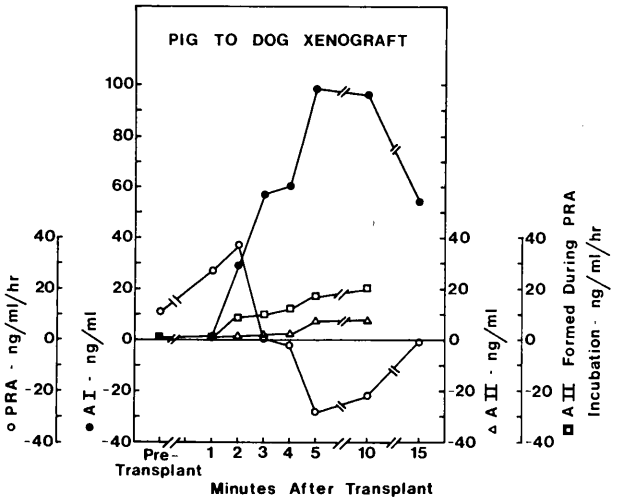

FIG. 2. Levels of angiotensin I (AI), angiotensin II (AII), and renin activity (PRA) as measured by angiotensin I generation during incubation and angiotensin II formed during the same incubation, in renal-vein plasma of a pig kidney prior to ("0" time sample) and following transplantation to the iliac fossa of a nephrectomized mongrel dog. Patchy cyanotic mottling had its onset within 2 or 3 min and the organ had been rejected by 10 min after opening of the circulation. No urine was formed. PRA was the difference between plasma AI concentration before and after incubation. The negative values thus represent net AI destruction during the incubation.

TABLE II. AI, PRA, and Renin Substrate During HAR Modified by Na_2EDTA 25 mg/min.

		Systemic			Renal vein		
	Min	AI (ng/ml)	PRA (ng/ml/hr)	Substrate (ng AI/ml)	AI (ng/ml)	PRA (ng/ml/hr)	Substrate (ng AI/ml)
Prenephrectomy		4.3	23.4	339	17.6	97.8	370
Postnephrectomy	20	1.4	1.8	316			
	40	0.9	2.3	258			
Posttransplant	1	1.6	4.8	262	12.8	65.3	260
	2	1.5	5.7	268	17.1	26.3	111
	3	1.0	5.5	295	16.0	18.7	69
	4	.8	3.9	249	19.3	49.0	82
	5	.9	2.7	314	17.5	27.2	85
	10	.8	2.6	151	28.3	70.4	79
	15	.22	2.2	172	47.5	62.5	72
	20	.03	1.9	270	92.3	60.6	0

whom a normal gross appearance and the circulation of the xenograft were maintained for an hour or more by the infusion of Na_2EDTA at 10–12 ml/min. Following the usual early spurts of AI and renin, the values of these parameters tended to level off until the infusion of the chelator was discontinued. Then HAR and AI generation proceeded as before. In these experiments, too, renal-vein renin substrate dropped to negligible levels. In an experiment in which the Na_2EDTA infusion was modified by the addition of $CaCl_2$ after 30 min, typical HAR changes developed which were essentially complete by 10 min. Although ionized calcium measurements in renal-vein plasma were not altered from their severely depressed levels, renal-vein AI rose and PRA became negative.

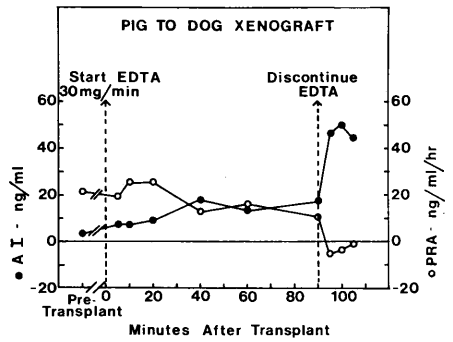

FIG. 3. Postponement of the posttransplantation spike in angiotensin I concentration and negative turn in renin activity levels (cf. Fig. 2) in renal-vein plasma of a pig kidney heterotransplanted into a mongrel dog, during a 90-min perfusion with disodium EDTA. During the 90-min infusion, the kidney remained pink and excreted an average of 1 ml urine/min. Hyperacute rejection, as in the previous experiment, proceeded when the EDTA was discontinued.

In an experiment in which KCl at a rate of 2 mEq/min was infused into the xenograft, the typical morphological changes of HAR proceeded, although RBF continued unchanged. AI also rose and PRA became negative.

A plasma sample with negative PRA destroyed 20% of the added AI during 3 hr of incubation, the identical value found to disappear when pooled normal dog plasma was used in the incubation.

Discussion. These studies have demonstrated that during the placement of kidney transplants, even autografts, there is considerable release of renin and production of AI in the kidney. If the graft is a xenograft, which is then hyperacutely rejected, these alterations in AI and PRA levels are more pronounced to the point that renin substrate is exhausted in the renal-vein blood. Not only is AI produced in large amounts in these heterografts, but conversion to AII also proceeds *in vivo* and *in vitro,* despite the presence of EDTA. The high intrarenal levels of the angiotensins could be instrumental (15) in the marked reduction in RBF seen in HAR. However,

the continuation at control levels of RBF in the experiment in which HAR and high levels of AI generation proceeded despite the infusion of KCl makes it seem unlikely that angiotensin-induced reduction of RBF is the sole etiology of HAR.

HAR can be delayed by the infusion of large amounts of Na_2EDTA. During the period of infusion, the jolt of renin secretion and AI production is also delayed. Since calcium ions are needed for renin release (16) and EDTA is a good inhibitor of the conversion of AI to AII, it is not surprising that AII levels in renal-vein blood also remain low. Thus, although delay in HAR is associated with delay in superactivation of the RAS, the identification of the RAS as the etiologic basis of HAR is far from certain. A better candidate for this role, as discussed elsewhere (7), is complement activation which is inhibited by calcium chelation. Complement activation can aid in platelet aggregation and coagulation, both of which are prominent components of HAR. The angiotensins could be responsible for severe vasospasm, but, as demonstrated in the experiment with KCl infusion, normal RBF may be maintained in the face of HAR with high angiotensin levels. Alterations in regional perfusion in the kidney caused by angiotensin could of course play a role here (17).

Summary. The role of the renin–angiotensin system in pig-to-dog renal xenograft rejection has been studied. Both autograft and xenograft kidneys show an acute increase in renin and angiotensin I and II levels in renal-vein blood, but the increases are higher in the xenografts. The period of maximum secretion of renin and angiotensin is delayed when the hyperacute rejection is delayed by the infusion of sodium EDTA into the graft. The chelating agent also prevents the *in vivo* conversion of angiotensin I to II. During the rejection process, the generation of angiotensin by the secreted renin is so massive that the renin substrate is exhausted and therefore probably a limiting factor. These studies thus demonstrate that the renin–angiotensin system is markedly activated during hyperacute heterograft rejection.

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