Increased Pressor Responsiveness to Kidney Extracts[1]

In rats daily injections of renin result in the following pattern of pressor responsiveness: after the characteristic response to the first injection there is a period of vascular refractoriness followed by hyperresponsiveness[2]. This observation raises 2 questions. First, is the period of refractoriness essential for the development of hyperresponsiveness? This is suggested by the observation that the hypertension caused by infusion of subpressor doses of angiotensin is preceded by a period of normotension, which may be the equivalent of vascular refractoriness[3] Secondly, is the increase in responsiveness specific for renin and angiotensin?

The purpose of the present experiments was to study the effects of kidney extracts that initially had little or no pressor activity when administered s.c. and to observe responses to pressor agents given before and after injections of kidney extracts.

Methods. Female Sprague-Dawley rats weighing 160 to 200 g were uninephrectomized so that injections of kidney extracts would mimic the pressor function of a clipped kidney in the presence of a contralateral kidney. They received s.c. injections of kidney extracts every 8 h. Tests for pressor responsiveness were carried out in unanesthetized animals by recording arterial pressure with a pressure transducer from an aortic catheter. Another canula was inserted into the jugular vein for injection of pressor agents, other than kidney extracts. Starting with the first injection of kidney extracts, groups of 5 rats were tested for responsiveness to i.v. injection of angiotensin (0.05 μg), vasopressin (5 mU), tyramine (0.01–0.05 mg) or norepinephrine (0.025 μg). Tests were performed before and after the 16.00 h injection of kidney extracts.

Saline extracts prepared from normal rat kidneys had a pressor activity of about 5 Goldblatt units/ml. The daily dose of 15 U corresponded to one and half kidney. Each animal was tested only on 2 consecutive days. The experiment was terminated on the 11th day, at which time the last remaining rats had received 32 injections of kidney extracts.

Results. The first s.c. injection of kidney extracts caused only small elevations in pressure (Figure 1): 2 of the 5 rats tested did not respond. Twenty-four h later, responses to the 4th injection were already markedly elevated; in the 2 rats which had not previously responded, the rise in pressure now amounted to 28 and 35 mmHg. Rats tested during the following days showed further enhancement in pressor responsiveness which was sustained until the end of the experiment. Differences between the first and the subsequent responses were highly significant, with P values less than 0.005 except on the 3rd and 9th days, when they were equal to 0.01 and 0.025 respectively. These injections did not cause sustained elevations in pressure: the initial control pressure averaged 113.2 $\pm$ 3.8 mmHg; in the succeeding days base pressure at the time of each test varied between 108.4 $\pm$ 7.7 and 124.6 $\pm$ 8.8 mmHg.

In tests for responsiveness prior to injection of kidney extract responses to angiotensin were significantly and consistently increased (P values between 0.025 and 0.005) (Figure 1). Responses to vasopressin increased from a normal value of 25.4 $\pm$ 5.1 mmHg, to 39 $\pm$ 11.5 on the 2nd day ($P = 0.05$), 36 $\pm$ 7.03 on the 4th day ($P < 0.025$) and 43.3 $\pm$ 8.08 on the 6th day ($P < 0.005$) but afterwards were within the normal range. Responses to tyramine were significantly increased only on the 3rd and 5th day with P values of 0.025 and 0.05. Responses to norepinephrine remained normal throughout the experiment.

When tests for responsiveness were made during the rise in pressure resulting from the injection of kidney extracts, responses to angiotensin were consistently and significantly smaller ($P < 0.005$) than those obtained before the injection of extracts (Figures 1 and 2). When responses were compared with those in untreated rats, differences were also significant (P between 0.01 and 0.005) except on the 5th day. Responses to vasopressin were consistently lower after than before administration of kidney extracts (Figure 2). There was, however, no significant difference from control responses. Responses to tyramine and norepinephrine were irregular and not significant (Figure 2).

Discussion. These experiments confirm that repeated injections of renin cause an increase in pressor responsiveness to renin and angiotensin[2] and show that this occurs with doses of renin which initially are barely pressor. A

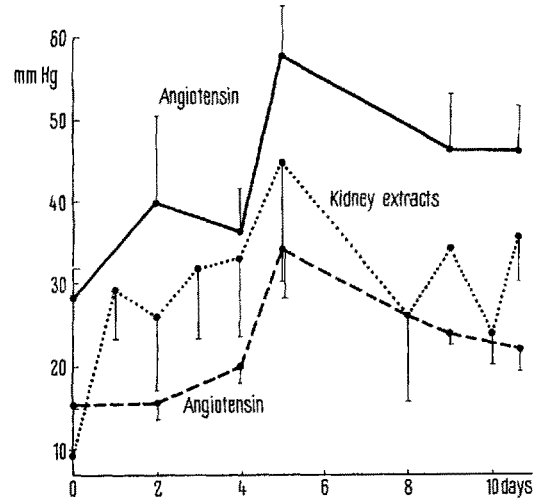

Fig. 1. Pressor responses to repeated injections of kidney extracts (middle curve) and to test doses of angiotensin given before (upper curve) and after (lower curve) injections of kidney extracts. Cross bars indicate standard deviations.

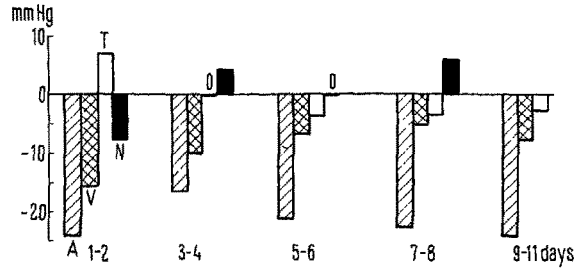

Fig. 2. Changes in responses to various pressor agents. Values represent differences between responses to pressor agents given before and after injections of kidney extract. A, angiotensin; V, vasopressin; T, tyramine and N, norepinephrine.

[1] Supported by NIH Grant No. HE-6835.

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period of vascular refractoriness does not seem to be a prerequisite.

Although hyperresponsiveness seems to develop whenever renin or angiotensin is administered chronically, its demonstration has been possible only under certain conditions: by showing that subpressor doses of angiotensin caused a sustained rise in pressure[3], or that the hypertension resulting from infusion of pressor doses of angiotensin remains unchanged when infusion rate is reduced[4]. In our experiment hyperresponsiveness was demonstrable when the rise in pressure caused by the renin contained in kidney extracts had subsided. Whenever angiotensin was injected during the response to an injection of renin, pressor responses instead of being increased were decreased thus suggesting that elevated amounts of circulating renin and angiotensin were masking the hyperresponsiveness.

As observed in dogs[5], we found that responses to another polypeptide vasopressin were decreased when superimposed on the response from kidney extracts. Results were however different concerning tyramine. The enhancement of tyramine responses by angiotensin or renin in dogs[5,6] contrasts with its absence in the rat. Responses to norepinephrine in rats and dogs were not affected by the administration of kidney extracts or angiotensin[5].

The fact that renal hypertension at least during its chronic phase is not consistently associated with an increase in plasma renin or angiotensin has been considered against the sustained participation of the renal pressor system. Presently available analytical procedures may not be sufficiently sensitive. Indirect evidence suggests that chronic renal hypertension is associated with small elevations in plasma angiotensin[5], which may be sufficient to maintain hypertension because of hyperresponsiveness.

Résumé. La réponse hypertensive à des injections s.c. d'extrait de rein n'ayant initialement que peu ou pas d'effet sur la tension artérielle s'accroit progressivement avec la durée du traitement.

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An Analysis of Distal Dominance in the Regenerating Limb of the Axolotl

The concepts developed to explain morphogenetic control of axial polarity of regenerating invertebrate organs, have recently been used in an attempt to explain the development of polarity during urodele limb regeneration[1]. It has been proposed that the distal-most regenerating organs in a linear system permit the morphogenesis of only those organs proximal to them. For example, in coelenterates the distal portion of a regenerating hydranth, when transplanted homoplastically to the distal end of a regenerating host hydranth, suppresses the morphogenesis of a similar distal organ but not of more proximal ones[2]. This inhibitory action is thought to be mediated by a proximally flowing substance which originates in the distal organ. Since, however, specific evidence for distal inhibition in urodele limb regeneration has not been reported, the purpose of this communication is to describe experiments designed to determine what, if any, influence a grafted distal organ has on the development of an homologous distal organ during urodele limb regeneration.

Materials and methods. The axolotl *Ambystoma mexicanum*, measuring 14–16 cm in overall length, was used exclusively. The animals were divided into 6 groups. Following anesthesia with MS222 (Sandoz), the right hind limbs of the axolotyles were amputated through the midfemoral region. In all but 1 of the 6 groups, the ventral $^2/_3$ of the wound surface was closed with sutures to prevent the initiation of host stump regeneration. In groups I, II, IV and V a distal organ (a small differentially pigmented donor foot amputated through the tarsal region) was homoplastically transplanted to the remaining wound surface. In group III, a donor limb segment, amputated through the distal end of the femur and again midway through the femur (a level similar to that of the host stump amputation surface), was transplanted in normal axial orientation, to the open dorsal wound surface as in groups I, II, IV and V and served as a control. To aid healing of the graft to the stump, the operated larvae were placed for 48 h in a refrigerator at 6°C, after which they were placed in 20 °C incubators for the duration of the experiment. By 10 days all grafts were reinnervated and revascularized, and the sutured amputation surfaces of all groups but V were reopened at this time. To determine the effect of a fully differentiated distal organ on a freshly reamputated proximal limb stump (group I), the unobstructed area of the host limb's amputation surface was reopened by excising the sutured skin, but the foot graft was not amputated. To determine the effect of a freshly amputated distal organ on a freshly reamputated proximal limb stump (group II), the foot graft was amputated through the metatarsals at the same time that the unobstructed wound surface of the host stump was reopened. In the controls (group III), the host amputation surface was reopened at the same time that the distal surface of the limb segment graft was reopened. To determine the effect of a freshly amputated distal organ on a regenerating proximal limb stump (group IV), a mound blastema was allowed to form on the unobstructed wound surface of the host stump before the grafted foot was amputated through the metatarsals. To determine the effect of a regenerating distal organ on a freshly reamputated proximal limb stump (group V), the

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