Changes in Renal Cyclic Nucleotides as a Trigger to the Onset of Compensatory Renal Hypertrophy

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In adult male Wistar rats contralateral nephrectomy was followed, within 10 minutes, by a nearly twofold rise of the content of cGMP in renal tissue. 20 and 40 minutes after contralateral nephrectomy cGMP fell to one half its control level to rise again to its normal level within 90 minutes. The initial rise of the concentration of cGMP was accompanied by a simultaneous fall of the concentration of cAMP by about 30 percent: the cAMP concentration remained 10–20 percent below control level for approximately two hours and rose again to its initial level after three hours. Cross-circulation of a nephrectomized rat with an intact animal led to a sharp increase of cGMP in the kidneys of the latter with a peak at 10 minutes after initiating cross-circulation and also to a fall of the cAMP concentration. When the same nephrectomized donor rat was subsequently cross-circulated with one, or even two, intact receiver animals, similar short-lasting changes of cyclic nucleotide concentrations were recorded in the kidneys of all the receivers. When a normal kidney was transplanted to the neck of a rat, subsequent removal of one of its own kidneys did not result in any change in cyclic nucleotide content in either the remaining or the transplanted kidney. The data are interpreted to indicate that renal tissue produces a factor inhibiting renal growth which counteracts a circulating humoral kidney growth stimulating factor of unknown origin. An initial rise of cGMP and a fall of cAMP may trigger the subsequent stimulation of protein synthesis responsible for hypertrophy.

It is generally thought that following unilateral nephrectomy compensatory renal hypertrophy is produced by a humoral stimulating agent activated by the loss of a renal inhibitory factor. Recent experiments by several groups of investigators [1,2,3] all support the existence of a humoral stimulating agent. On the other hand, work by other investigators [4,5,6,7,8,9] indicates the possible existence in the kidney of an inhibitory factor. Attempts to isolate the latter have been partially successful only. Recently Dicker, Morris and Shipolini [10] have managed to partially purify a substance extracted from the cortex, but not from the medulla, of rats' kidneys, which when injected into a unilaterally nephrectomized animal temporarily inhibited the rate of compensatory hypertrophy. This compound proved to be tissue, but not species specific [11]. The same authors also showed that a transplant of cells from the renal cortex, but not from the medulla, had the same inhibiting activity as the partially purified cortical extract [12]. It is thus possible with the experimental evidence available to speculate that in a normal animal the humoral stimulating agent is in a state of dynamic equilibrium with an inhibiting compound of renal origin. It must be recognized, however, that until such a compound has been completely isolated, purified, and identified, some doubts about its existence and its mode of action will remain.

As for the humoral stimulating agent, it has to be admitted that though so far nothing at all is known as to its identity, its existence does not appear to be in doubt [1,3]. Its mode of action is under active investigation. But what does trigger compensatory renal hypertrophy? If it is a chain of reactions which eventually leads
to cellular hypertrophy and if as it has been suggested cyclic nucleotides may be involved in the regulation of cellular growth, what is the first step? Because any stimulation (from the humoral factor) must of necessity affect the permeability of the cells we decided to estimate cyclic nucleotides in the kidneys of normal and unilaterally nephrectomized rats. This work done in collaboration with A.L. Greenbaum was started quite independently and in complete ignorance of a similar investigation by Schlondorff and Weber [13]. Reading Schlondorff's chapter in this symposium you will realize then that our and his results do both agree and disagree.

Both cAMP and cGMP were estimated by a radio-immunoassay based on the competition between unlabelled cyclic adenosine 3',5'-monophosphate (cAMP) or cyclic guanosine 3',5'-monophosphate (cGMP) and a fixed quantity of the tritium-labelled compound for binding to a protein which has a high specificity and affinity for either of the nucleotides [14]. The kidneys were clamped and frozen in liquid nitrogen immediately after they had been removed. They were homogenized in a cold solution of perchloric acid. The supernatant fluid was neutralized to pH 6.8 and then deep frozen until used for the estimation of the nucleotides. All estimations were made in duplicate and the experiments to be described were repeated several times. Adult litter mate male Wistar rats (bw 200–220 g) anesthetized with Inactin® (Na-ethyl-methyl-propyl-malonylthiourea), 100 mg/kg bw were used. For unilateral nephrectomy the kidney was freed of its pericapsular fat, leaving the adrenal gland intact. The operation lasted between 1–2 minutes.

In the first series of experiments, rats were killed 5, 10, 20, 40, 80 minutes and 2, 4, 6 and 8 hours after unilateral nephrectomy. Sham operated rats were killed after 0, 10, 20 minutes and 2 and 8 hours. Control values were of the order of 1,000 pM and 95 pM/g wet tissue for cAMP and cGMP, respectively. Neither the anesthesia nor the sham operation had an effect on their levels. As shown in Fig. 1, in unilaterally nephrectomized animals, there was an immediate sharp rise of cGMP, which reached its maximum at 10 minutes. Two hours after the operation the level of cGMP was back to preoperative values. As for cAMP, there was an abrupt fall of about 30 percent, which did not last more than 2–3 hours [12]. These results agree with those of Schlondorff and Weber [13] only insofar as the rise of cGMP coincided with a fall of cAMP; they differ, however, in the duration and extent of the changes of these nucleotides.

In the second series of experiments, cross circulation was used. The technique followed was that described by Moolten and Bucher [15], and by van Vroonhoven et al. [1]. One rat was nephrectomized, and the removed kidneys used as controls for nucleotides estimations. About 20 minutes later a cross-circulation with another intact rat was started. The animal was killed 10, 20 or 30 minutes after the onset of the cross-circulation, its kidneys removed as quickly as possible (average 2 minutes) and immediately frozen in liquid nitrogen. The results confirmed the previous experiments: there was a sharp increase of cGMP in the kidneys of the intact animal, which reached its peak 10 minutes after the onset of the cross-circulation. Though these results strongly suggested that the changes in levels of cGMP were due to a stimulation of humoral origin from the anephric animal, the sudden rise of cGMP and quick return to normal level raised the possibility that the stimulus was short acting only. To test this three litter mates were used. One anephric rat was circulated first with one rat for 10 minutes. As soon as its kidneys had been removed and frozen the cross-circulation was switched on to another intact rat. After 10 minutes cross-circulation, the kidneys of the latter were removed and frozen. The kidneys of both
the first and the third rats showed the usual increase of cGMP level with commensurate decrease of cAMP. A similar experiment using 4 rats (i.e., 3 animals being cross-circulated successively by one anephric rat) showed a similar pattern, though, of course, the response became progressively smaller.

These results then support the view that after nephrectomy there is a stimulating agent circulating in the blood which may act, directly or indirectly, on a normal kidney, and that the resulting changes of cyclic nucleotides may be an indication of its action. It is very likely that this humoral factor is tissue specific, as suggested by the fact that partial hepatectomy did not affect the level of cyclic nucleotides in the kidneys. The humoral factor can act, however, only when there is a loss of renal parenchyma.

This could be demonstrated by transplanting a kidney from a litter mate to the neck of another rat. Unilateral nephrectomy in this case had no effect on the levels of cGMP and cAMP which remained similar in the 3 kidneys (i.e., the transplanted, the removed, and the remaining kidneys). However, ten minutes after interrupting the circulation to the transplanted kidney, the level of cGMP in the remaining kidney rose markedly.

Some of the results presented here have not been published yet. More experiments are planned, and obviously the work is not finished. It is, however, possible to draw some conclusions even if some of them may appear somewhat speculative. Since compensatory renal hypertrophy occurs only when there is a loss of renal parenchyma, and since when an hypertrophied kidney (removed from a previously unilaterally nephrectomized animal) is transplanted to another unilaterally nephrectomized rat with an hypertrophied kidney, both kidneys regress [16], it follows that a growth controlling factor must exist in the kidney. Though our efforts to extract and purify such a factor were partially successful only [10], it can be postulated that such
a compound normally released by the kidneys inactivates another substance, whose origin and nature are still unknown, but which in a normal animal circulates in the blood stream. In the normal animal these two compounds, the humoral stimulating one and the renal inhibitor, both tissue specific, would be in a kind of dynamic equilibrium, and together would control the physiological growth of the kidneys. It is likely, though not yet demonstrated, that the physiological growth of other organs may have a similar control [15]. If one kidney is removed, the amount of inhibitor available will be halved and the humoral stimulating agent will be activated. What happens then is not clear. Professor Malt and his collaborators later on will tell us of their investigation on molecular and translational mechanisms, which may lead to RNA accretion, protein synthesis and eventually cellular hypertrophy [17,18,19,20]. In the light of our present work, it is possible to postulate that changes in cyclic nucleotides may act as a trigger to the following molecular events leading ultimately to compensatory renal hypertrophy. What the stimulating factor is, where it is produced, how it can act on the renal cells so as to produce changes in cyclic nucleotides, and whether the latter are the prime mover to other chemical changes are all conjectural questions which remain to be investigated. Clearly much more work is needed before the problem of compensatory renal hypertrophy is solved.

Addendum: Most of the results presented have now been published in J Physiol 273:241–253, 1977.

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