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Experimental Normocalcaemic Hyperparathyroidism Induced by Renal Venous Congestion

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Summary. Ligation of the inferior vena cava above the renal veins in male Sprague-Dawley rats produces a predominantly transient renal injury with vascular congestion, tubular degeneration and necrosis with calcification. This is accompanied by impaired renal function with temporary azotaemia, hyperkalaemia, phosphate retention but with a normal calcium level. The parathyroid glands on the second postoperative day show cellular hypertrophy and on the third and fourth days signs of rapid cell proliferation with numerous mitoses. Thereafter the glands remain enlarged.

Key words: Renal venous congestion - Hyperparathyroidism.

Primary hyperparathyroidism has been experimentally produced by transplanting parathyroid glands (18) or injecting parathormone (1, 7, 9, 10). Secondary hyperparathyroidism has been commonly studied by producing renal injury, by nephrectomy or by means of the exogenous administration of phosphate (1, 5, 6, 11, 12, 13, 17, 19, 22, 23). The pathogenesis of secondary hyperparathyroidism is not completely known in spite of many different experimental models. In a previous study of predominantly transient renal injury after suprarenal ligation of the inferior vena cava, necrotizing and calcific vessel changes were reported (8). Corresponding changes have previously been described in connection with secondary hyperparathyroidism (16). This has stimulated further systematic studies, both morphological and metabolic. An association between renal injury after acute renal venous congestion and hyperparathyroidism has been determined, and we present a new simple experimental model for inducing secondary hyperparathyroidism.

METHODS

The experiments were performed on male Sprague-Dawley rats fed on pellets (Astra-Ewos) and water ad libitum during the entire experimental period.

The animals were divided into various groups depending on age (3, 4, 8). They were anaesthetized with ether, weighed and labelled. Laparotomy was performed via a midline incision. The vena cava was carefully dissected free from the hepatic ligament and surrounding tissue using a fine curved pincett, and tied above the level of the renal veins by means of a silk ligature (Fig. 1). The abdomen was then closed with simple sutures in the muscles and running sutures in the skin.

Quantitative and qualitative changes in the urine were investigated in a group of rats kept in metabolic cages beginning several days before the operation and during the postoperative experimental period.

The remaining animals were returned to their usual environment postoperatively. Sham-operated and nonoperated animals served as controls. At predetermined times after the operation the animals were again lightly anaesthetized with ether, weighed and laparatomy performed. The aorta was freed and punctured with a needle (diameter 1.2 mm) which was coupled to a pressure transducer (Siemens-Elema EMT 33) and a recorder (Siemens-Elema mingograph 81) for registering blood pressure. Thereafter the animals were exsanguinated through the needle in the aorta and the blood was collected and centrifuged; serum was stored frozen (-20° C) until analysis

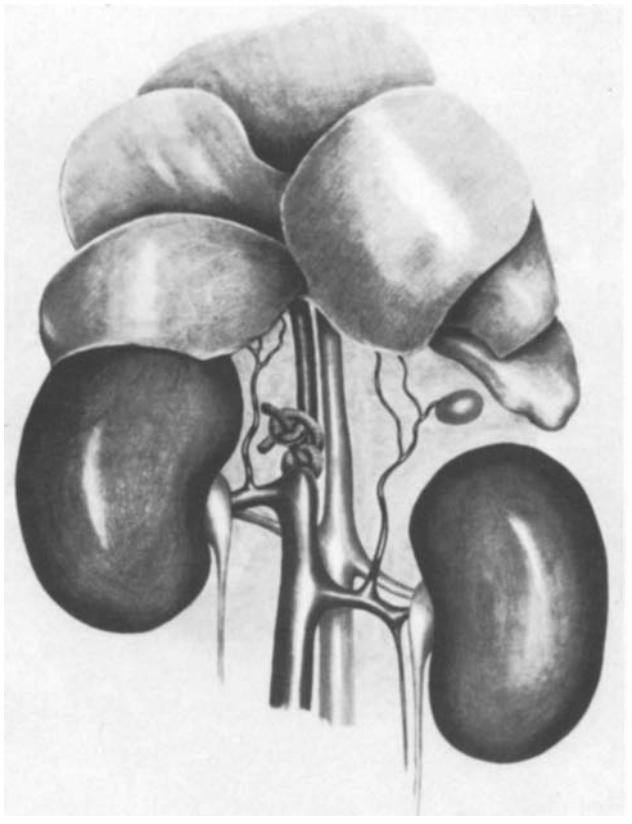

Fig. 1. Schematic drawing of a rat preparation showing suprarenal position of the inferior vena cava ligature

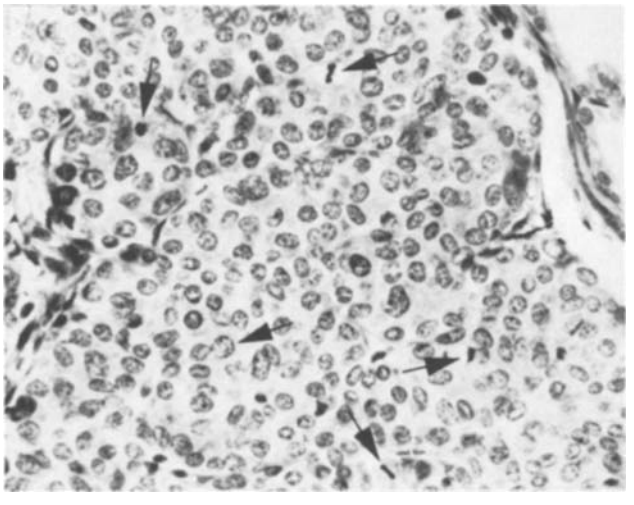

Fig. 2. Parathyroid gland of a rat 4 days after suprarenal caval ligation. The cells are hyperplastic and numerous mitoses (arrows) are seen. Haematoxylin and erythrosin. x 325

was carried out. An autopsy was performed immediately after death.

The thyroid and parathyroid glands, trachea, oesophagus and cervical vessels with surrounding muscle tissue were removed en block. The kidneys, adrenals, liver, spleen and heart were weighed. Samples from these organs and from the aorta, lungs, stomach and thigh muscles were fixed in Histofix^{R} (Histo-lab). The tissues were embedded in paraffin, sectioned and stained routinely with haematoxylin and erythrosin and by the von Kóssa method. Van Gieson, Periodic-Acid-Shiff (PAS) and toluidine blue staining were performed on selected tissues.

Whereas the kidneys and adrenals were studied in various age groups the remaining organs including the parathyroid were investigated sequentially in only one weight group (250-350 g). Many sections at various depths were studied from the parathyroid and thyroid glands. The sections were projected with a Zeiss microprojector onto a table by means of a mirror and the contours of the parathyroids were drawn on paper and the size was estimated.

The serum concentrations of the following substances were measured; urea, creatinine, sodium, potassium, chloride, lactate, H^{+}, calcium, phosphate and albumin. The urinary concentrations of urea, creatinine and protein together with the urinary volume and osmolality were recorded. The analytical methods are described in previous publications from this group (3, 4) except for calcium and phosphate which are described elsewhere (14, 24).

The distribution of intravenously injected rubidium, an alkali metal closely related to potassium, was determined before and on several occasions during the first two days following caval ligation (20).

RESULTS

The mortality, mainly restricted to the first days after the operation, varied in the different studies from 10 % of young rats weighing about 130 g to 90 % of rats weighing over 500 g. The mortality correlated well with the degree of morphologically demonstrable renal injury and reduction of function (3, 8).

The morphological lesions in the kidneys were considerable in the first two to three days after caval ligation with vascular congestion, tubular degeneration and necrosis and hyaline and granular casts. Calcification of necrotic tubules and intraluminal casts was often seen. The lesions were characteristically most extensive in the inner stripe of the outer medul-

la and the adjacent inner medulla. Necrosis of short segments of proximal convoluted tubules was often conspicous. On the second or third day there was regeneration and repair of the epithelium. Renal morphology returned to near normal after one to two months though slight interstitial fibrosis and scattered calcified atrophic tubules were seen. Older rats recovered at a slower pace and with more sequelae.

The adrenals of several animals showed focal necrosis or small haemorrhagic infarcts in the early postoperative period.

In the majority of the animals from the second day onwards arteritis - periarteritis, most consistently in the skeletal and cardiac muscle; and calcification in the lung, gastric mucosa and thymus were seen. Calcific lesions in the media of the aorta and the large arteries varied from minimal to extensive and were regularly observed from the third day onwards. No changes in blood pressure were observed.

The parathyroid glands on the second day showed cellular hypertrophy and on the third and fourth days signs of rapid cell proliferation with numerous mitoses (Fig. 2). The glands thereafter remained enlarged compared to glands of sham operated animals.

Urine production ceased during the first 24-48 hrs after the ligation of vena cava. At the same time, a twenty-fold rise in serum creatinine and a two- to three-fold increase in serum phosphate were noted. Serum calcium levels remained normal.

During the same period hyperkalaemia with serum values up to 10 mmol/l was observed and interpreted as the immediate cause of death. Studies with rubidium suggested that potassium was released from the kidneys. Surviving animals developed polyuria after two days and in the older animals this persisted for more than one month.

DISCUSSION

The serum calcium in our experiments remained within normal limits, which is in agreement with most studies regarding the serum calcium level after acute renal injury or nephrectomy (5, 6, 12). This does not, however, exclude the possibility of a disturbance in calcium metabolism.

A change in the phosphate concentration in blood does not directly influence the parathyroid glands. However there is a reciprocal relationship between the blood levels of phosphate and calcium and a reduced level of ionized calcium is considered to be the only known stimulus for parathyroid hyperplasia (15). It is probable that the high phosphate levels recorded in our experiments have reduced the levels of ionized calcium, thereby inducing hyperparathyroidism in the experimental animals.

The disturbances in electrolyte balance following renal venous congestion may be the cause of tissue necrosis and calcification in various organs. However experimentally induced cardiovascular injury after various renal manipulations does not arise in previously parathyroidectomized animals (16). This indicates that a functioning parathyroid gland is necessary for such injury to occur.

As the turn-over of parathormone is known to be rapid and since the elimination of parathormone to some degree occurs via the kidneys (21), its serum level can change within hours following a renal lesion. Administration of parathormone causes morphological renal changes, predominantly in the form of tubular injury (7, 9, 10). The possibility that hyperparathyroidism may have contributed to the renal injuries after suprarenal ligation of the vena cava can therefore not be completely ruled out.

The renal tubule, which is that component of the nephron which is affected most by congestion, is not only the effector organ for parathormone but also the site of vitamin D conversion to its active metabolite (1, 25 - dihydroxycholecalciferol). It is known that the interaction between vitamin D and parathormone is of significance for the turn-over of calcium and phosphorus in chronic renal insufficiency. The role of vitamin D in acute renal injury is, however, unclear (2, 18).

When choosing an experimental model for inducing secondary hyperparathyroidism, one is forced to take into account several factors. The model should have a certain degree of clinical relevance. It should be simple, easily reproducible, give reliable results and not be too costly. Many previously used experimental models have obvious draw-backs. Bilateral nephrectomy can only be used for short term experiments (6, 11, 12). Partial nephrectomy is an uncertain method which requires the experimental animal to become chronically uraemic (15, 23). So-called obstructive nephropathy induced by intraperitoneal injections of sodium sulphacetylthiazole also produces chronic uraemia with secondary hyperparathyroidism (17, 19).

However, even relatively benign renal injuries have been seen to influence parathyroid function (2, 5) and lead to secondary hyperparathyroidism without uraemia. This has been demonstrated in experimental pyelonephritis in the rat (13).

Our method for producing venous congestion and renal injury by means of ligating the vena

cava above the kidneys, thereby inducing hyperplasia of the parathyroid glands, is a new method which appears to fulfil the requirements noted above.

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