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Effect of Verapamil Treatment on Compensatory Renal Growth in Mice

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Summary

Calcium has been shown to control the proliferation of various cells *in vitro* and *in vivo*. In this study we have attempted to modify compensatory renal growth by pharmacological interventions in mice who have undergone uninephrectomy. The effect of a calcium channel blocker verapamil was investigated. Unilateral nephrectomy of intact male mice produced the expected increase in weight of the remaining kidney by 67.5±8.1 %. This rise was accompanied by a proportional increase in RNA. In mice, cell hypertrophy was found to be a major factor in compensatory renal growth. Verapamil given in a i.p. dose of 1.0 or 2.0 mg/day/mouse attenuated the growth of the remaining kidney so that its weight rose by only 48.2±6 % and 28.2±4.4 %, respectively. *In vivo* administration of verapamil decreased the degree of compensatory renal growth and this growth inhibiting effect was directly proportional to the dose.

Key words
Unilateral nephrectomy – Compensatory hypertrophy – Verapamil

Introduction

The loss of renal mass is followed by compensatory growth of the remaining kidney. In mice, the increase in kidney weight has been shown to be due to hypertrophy rather than hyperplasia, since RNA levels increased while DNA content remained constant (Fine 1986). The exact stimuli that trigger these adaptive changes are largely unknown. However, number of factors have been shown to modulate the extent of the growth response: age, growth hormone, testosterone, thyroid hormones or adrenal steroids have been reported to stimulate the degree of compensatory renal growth (Nomura *et al.* 1985). Calcium ions and hormones (calcitonin, parathyroid hormone and calcitriol) are major regulators of DNA synthesis and mitotic activity in the bone marrow, liver and thymus of rats (Whitfield *et al.* 1979). It has not yet been established whether the calcium ion itself or calcium regulatory hormones such as parathormone play a role in renal compensatory growth.

A factor that may play a modulatory role in renal compensatory growth is the calcium ion. Verapamil, a calcium channel blocker given either *in vivo* or *in vitro*, has been shown to inhibit calcium uptake by kidney slices (Goligorsky *et al.* 1985).

The aim of the present study was to define further the mechanisms by which the calcium channel blocker verapamil affects compensatory renal growth.

Materials and Methods

Male mice of the H strain (Velaz, Prague) weighing 32 g were used. They were fed a standard laboratory diet (Velaz, Prague) containing 23 % protein, 0.8 % phosphorus and 1 % calcium, were offered water *ad libitum*, and were kept in an indirectly illuminated room with controlled temperature (24±2 °C). The animals were divided into three groups of 10 animals each. Intact controls were compared with two groups of intact mice to which verapamil hydrochloride (Isoptin Knoll) was given in the food twice daily in a daily dose of 1 or 2 mg. The animals were weighed before and after experiment and their food consumption was checked daily. No food was left so that the amount of food eaten corresponded to the dose of the given drug.

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Animals underwent unilateral nephrectomy on the right side. The excised kidney was immediately weighed, placed in ice-cold 0.4 N perchloric acid and homogenized in a Potter-Elvehjem type homogenizer.

Pair-feeding with or without verapamil started on the day of surgery and continued for 7 days. Because pharmacokinetic data are not available for verapamil administration in mice, we have used the dose that was given in rats by Jobin *et al.* (1986) This dose is considered to be comparable to the dose of verapamil used in man. The mice were sacrificed eight days after nephrectomy. At the end of experiment, mice were anaesthetized by pentobarbital and a blood sample for the determination of plasma calcium, phosphorus and creatinine was withdrawn from the heart. The left kidney was processed in exactly the same way as the right one.

The total content of nucleic acids was determined using the method of De Deken and De Deken (1959). The DNA content of the homogenate was assessed by diphenylamine reaction as described by Burton (1956) using calf thymus DNA as standard. RNA was determined by subtraction of the DNA value from the total nucleic acid value.

Compensatory renal growth was evaluated by comparing the right kidney wet weight at nephrectomy to that of the left kidney when the animals had been killed.

In a separate experiment, the kidneys from control and verapamil-treated mice were removed, weighed, placed in a crucible and incinerated at 60 °C for 24 h. The residue was dissolved in 0.6 N HCl. Calcium was determined by the method of Gitelman (1967) and phosphorus according to Kraml (1966).

In a preliminary experiment we administered verapamil in a dose of 2.0 mg/day/mouse to intact mice by i.p. injection. Mice were sacrificed 10 days later and the kidneys were removed, cleaned and weighed.

The means and the 95 % confidence limits were calculated for all the values obtained and the results were evaluated statistically by the analysis of variance and Duncan's test (1955). The results are expressed as means ± S.D.

Results

Nine days of verapamil administration had no effect on the growth of mice and no effect on plasma calcium, phosphate or creatinine concentration (Table 1).

Unilateral nephrectomy of intact male mice produced the expected increase in weight of the remaining kidney (67.5±8 %). The RNA content of the kidneys was affected in a similar way as kidney weight. The content of DNA also increased but the change was not statistically significant (Tables 2 and 3).

Verapamil in a dose of 1.0 mg/day/mouse attenuated renal compensatory growth because the increase in weight of the remaining kidney was 48 % only. This was significantly less as compared with unilaterally nephrectomized control animals ($p<0.01$). Similar changes could be seen in kidney RNA content (Tables 2 and 3).

Table 1. Blood composition and kidney calcium content in unilaterally nephrectomized mice with or without verapamil

	Calcium mmol/l	Phosphorus mmol/l	Creatinine μmol/l	Kidney Calcium mg/100 g w.wt.
Control	2.20±0.09	4.2±0.12	74.3±6.3	6.4±0.06
Verapamil 1 mg	2.24±0.08	4.1±0.11	76.2±6.0	6.3±0.07
Verapamil 2 mg	2.21±0.06	4.2±0.14	75.1±6.3	6.3±0.08

Data are means ± S.D.

Table 2. Body weight and kidney weight in unilaterally nephrectomized mice with or without verapamil

	Final body weight (g)	Right kidney (mg)	Left kidney (mg)
Control	34.0±3.2	233.7±12.0	392.3±18.7
Verapamil 1 mg	33.8±2.2	232.6±10.1	345.0±14.1*
Verapamil 2 mg	33.6±3.1	240.2±14.1	308.0±10.5*

** significantly different ($p<0.01$) from controls (unilaterally nephrectomized mice without verapamil).*

Table 3. RNA and DNA content of the kidney in unilaterally nephrectomized mice with or without verapamil

	Right kidney RNA μg/g	Left kidney RNA μg/g	Right kidney DNA μg/g	Left kidney DNA μg/g
Control	3.55±0.2	6.04±0.1	3.68±0.2	3.93±0.3
Verapamil 1 mg	3.75±0.1	5.48±0.1*	3.77±0.2	4.03±0.3
Verapamil 2 mg	3.53±0.3	4.48±0.2*	3.44±0.2	3.68±0.2

** significantly different ($p<0.01$) from controls (unilaterally nephrectomized mice without verapamil).*

In animals receiving 2.0 mg/day/mouse of verapamil, the remaining kidney weight rose only by 27 %, the gain being smaller as compared to the controls ($p<0.01$). There was also a smaller increase of the RNA content in the remaining kidney.

Verapamil itself did not decrease kidney weight in intact mice (737±23.4 mg) as compared with intact mice without verapamil (712±33.2). Compared to intact mice, values of kidney calcium content were not significantly different after seven days of verapamil administration.

Morphological observations made under a light microscope revealed no structural differences between kidneys of intact and verapamil-treated mice.

Discussion

In our experiments, three parameters were chosen to assess the degree of compensatory renal growth: increase in kidney weight and total kidney RNA and DNA content. We used male mice because the mouse kidney which is particularly responsive to unilateral nephrectomy (Kochakian 1976). Verapamil given *in vivo* decreased the degree of compensatory renal growth observed in control animals and this growth-inhibiting effect was directly proportional to the administered dose. Similar changes were observed in the kidney RNA content suggesting that renal hypertrophy is being inhibited. Verapamil had no effect on the weight of kidneys in intact animals.

Calcium antagonists or calcium channel blockers, such as verapamil, act as ligands, binding strongly to the cellular membranes and thereby inhibiting calcium influx (Bogin *et al.* 1981). One factor that may play a modulating role in renal compensatory growth is the calcium ion. This element and the hormones that regulate its distribution between the extra- and intracellular compartment have been shown to control cell proliferation of various systems or tissues *in vivo* and *in vitro* (Whitfield *et al.* 1979). The total calcium content in the remaining kidney of verapamil-treated mice was not lower than that of intact animals . Problems, which prevent a better understanding of calcium involvement in cell proliferation, arise from the fact that it is rather difficult to measure cytosolic free calcium levels directly. The results of Jobin *et al.* (1984) indicate that calcium restriction can markedly enhance renal compensatory growth without affecting normal kidney growth. However, administration of parathormone (PTH) enhances renal compensatory growth and can thus mimic the effect of dietary calcium restriction (Jobin and Bonjour 1986). The effect of calcium channel antagonists on PTH secretion have been assessed in numerous studies *in vitro* (Seely *et al.* 1989) and *in vivo* (Boesgaard *et al.* 1991, Wynne *et al.* 1995). The data are conflicting. PTH raises cytosolic calcium concentration in renal cells. PTH directly stimulates compensatory renal growth and acts at the cell level as calcium ionophores with a resultant increase in cell calcium (Borle and Uchikawa 1978).

The hypothesis that takes all these observations into account may further be strengthened by the fact that verapamil inhibits cellular calcium entry without a resulting increase in plasma PTH and thus cannot act as calcium ionophores.

The other pathway, whereby verapamil may exert its effect on compensatory renal growth, is by altering intrarenal haemodynamics. However, calcium antagonists are not selective renal vasodilators: they fail to modify the fraction of cardiac output perfusing the kidney (Kobrin *et al.* 1984, Hof 1983).

In our experiments, verapamil administered *in vivo* was found to have a growth-inhibiting effect on compensatory renal growth. Further experiments are needed to assess the precise physiological meaning of our findings and of the exact role of ionized calcium and PTH.

References

BOESGAARD S., HYLDSTRUP L., FELDSTED H.: Changes in calcium homeostasis and bone formation in patients recovering from acute myocardial infarction: effect of verapamil treatment. *Eur. J. Clin. Pharmacol.* **41**: 521–523, 1991.

BOGIN E., MASSRY S.G., HARARY I.: Effect of parathyroid hormone on rat heart cells. *J. Clin. Invest.* **67**: 1215–1227, 1981.

BORLE A.B., UCHIKAWA T.: Effects of parathyroid hormone on the distribution and transport of calcium in cultured kidney cells. *Endocrinology* **102**: 1725–1732, 1978.

BURTON K.: A study of the conditions and mechanism of diphenylamine reaction for the colorimetric estimation of DNA. *Biochem. J.* **62:** 315–323, 1956.
DE DEKEN-GRENSON M., DE DEKEN R.H.: Elimination of substances interfering with nucleic acid estimation. *Biochem Biophys. Acta* **31:** 195–206, 1959.
DUNCAN D.B.: Multiple range and multiple F tests. *Biometrics* **11:** 1–4, 1955.
FINE L.: The biology of renal hypertrophy. *Kidney Int.* **29:** 619–634, 1986.
GITELMAN H.: An improved automated procedure for the determination of calcium in biological specimens. *Anal. Biochem.* **18:** 521–531, 1967.
GOLIGORSKY M.S., CHAIMOVITZ C., RAPOPORT J., GOLDSTEIN J., KOL R.: Calcium metabolism in uremic nephrocalcinosis. Preventive effect of verapamil. *Kidney Int.* **27:** 774–779, 1985.
HOF R.P.: Calcium antagonist and the peripheral circulation: differences and similarities between PY 108-068, nicardipine, verapamil and diltiazem. *Br. J. Pharmacol.* **78:** 375–394, 1983..
JOBIN J.R., TAYLOR C.M., CAVERZASIO J., BONJOUR J.P.: Calcium restriction and parathyroid hormone enhance renal compensatory growth. *Am. J. Physiol.* **246:** F685–F690, 1984.
JOBIN J.R., BONJOUR J.P.: Compensatory renal growth: modulation by calcium, PTH a $1,25(OH)_2D_3$. *Kidney Int.* **29:** 1124–1130, 1986.
KOBRIN I., SESOKO S., PEGRAM B.L., FROHLICH E.D.: Reduced cardiac mass by nitrendipine is dissociated from systemic or regional hemodynamic changes in rats. *Cardiovasc. Res.* **18:** 158–162, 1984.
KOCHAKIAN C.D.: *Anabolic Androgenic Steroids*. Springer, Berlin 1976.
KRAML M.: A semiautomated determination of phospholipids. *Clin. Chim. Acta* **13:** 442–448, 1966.
NOMURA K., DEMURA H., SHIZUME K.: Stimulation of renal deoxyribonucleic acid synthesis by a pituitary derived renotropin and its inhibition by testosterone and thyroxine. *Endocrinology* **116:** 616–621, 1985.
SEELY E.W., LEBOFF M.S., BROWN E.M., CHEN C., POSILLICO J.T., HOLLppENBERG N.K., WILLIAMS G.H.: The calcium channel blocker diltiazem lowers serum parathyroid hormone levels in vivo and in vitro. *J. Clin. Endocrinol. Metab.* **68:** 1007–1112, 1989.
WHITFIELD J.F., BOYNTON A.L., MACMANUS J.P., SIKORSKA M., TSANG B.K.: The regulation of cell proliferation by calcium and cyclic AMP. *Mol. Cell. Biochem.* **27:** 155–179, 1979.
WYNNE A.G., ROMANSKI S.A., KLEE G.G., ORY S.J.O., FALLON W.H., FITZPATRICK L.A.: Nifedipine but not verapamil acutely elevates parathyroid hormone levels in premenopausal women. *Clin. Endocrinol.* **42:** 9–15, 1995.

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