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Beneficial Effect of Verapamil in Ischemic Acute Renal Failure in the Rat (41576)

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Abstract. To investigate the possible protective effect of Ca^{2+} blockers in ischemic acute renal failure (ARF), verapamil, in a dose of 10 μg/kg body wt/min was administered for 100 min, starting 15 min before the total occlusion of the left renal artery after right nephrectomy in rats. Mean 24-hr creatinine clearance, blood urea, and serum creatinine levels, 24 hr after declamping, were used as a measure of kidney function. These values which were 135 ± 1.9 μliter/min, 231 ± 22 mg%, and 2.25 ± 0.22 mg%, respectively, in the untreated rats, were found to be significantly different, i.e., 326.3 ± 33.2 μliter/min, $P < 0.001$, 112 ± 25 mg%, $P < 0.001$, and 1.26 ± 0.28 mg%, $P < 0.01$, respectively, in the verapamil-treated animals. Increased 24-hr total urine creatinine, sodium, osmolality, and a lower fractional excretion of sodium were also observed in the verapamil-treated rats with ARF. The combination of propranolol 1 mg/kg body wt/min and verapamil 10 μg/kg body wt/min for 100 min had no additive effect on renal function. In another group of ARF rats in which verapamil was started after declamping, no alleviating effect was observed. It is concluded that verapamil, an inhibitor of cellular membrane transport, when given prior to the renal ischemia, offers a partial but significant protection in this model of ischemic ARF.

Ischemia is associated with high intracellular cytosolic Ca^{2+} concentration as well as with decreased mitochondrial respiration (1). Schrier *et al.* observed that altered mitochondrial Ca^{2+} metabolism occurs in the norepinephrine model of experimental ARF (2). Burke *et al.* (3) recently used verapamil, an inhibitor of membrane transport of calcium, as an agent for the alleviation of norepinephrine-induced ARF in dogs. Following these observations, we have studied the effect of verapamil in another model of ischemic experimental ARF, namely uninephrectomy and contralateral renal artery clamping in rats. These two models share at least one common pathogenetic mechanism—tubular obstruction (4, 5). Because of our previous experience that beta-adrenergic blockade with propranolol alleviates this form of ARF (6, 7), a combination of both drugs was also used.

Materials and Methods. Ischemic acute renal failure (ARF) was induced in female Charles River rats (Yokneam, Israel), weighing 280–300 g each, as previously described (6, 7). In short, during ether anesthesia, immediately after right nephrectomy, the left renal artery was clamped for 70 min. A femoral artery was cannulated with a PE50 catheter for fluid and drug administration. An infusion of 0.9% sodium chloride, with or without drugs, (at the rate of 6 ml/rat/hr) was started in all animals 15 min before clamping, continued during the 70-min clamping period, and for 15 min after declamping. The abdominal wall was closed and the rats were placed in individual metabolic cages. Urine was collected under mineral oil. Twenty-four hours later, blood was drawn for analyses.

Five groups of rats were studied:

Group 1. Sham operated rats.

Group 2. Untreated control animals with ischemic ARF.

Group 3. Ischemic ARF, treated by the intravascular administration of verapamil (Ikakor, Ikapharm, Israel), 10 μg/kg body wt/min. The administration of verapamil was started 15 min before clamping and lasted for a total period of 100 min.

Group 4. As in Group 3, but the administration of verapamil was started immediately after declamping and continued for 15 min only.

Group 5. Rats with ischemic ARF received verapamil 10 μg/kg body wt/min together with *dl*-propranolol, 1 mg/kg/hr. The drugs were administered 15 min before clamping, 70 min during clamping, and 15 min after declamping.

Blood urea, serum creatinine and urine creatinine, sodium, and osmolality were deter-

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TABLE I. BIOCHEMICAL DATA IN THE DIFFERENT EXPERIMENTAL GROUPS

Experimental group	Bl urea (mg/dl)	Ser Cr (mg/dl)	Ser Na (meq/liter)	Ser osmol (mOsm/kg H_2O)	Urine V (ml/24 hr)	UCrV (mg/24 hr)	UNaV (meq/24 hr)	UOsm (mOsm/kg H_2O)	Cr clear (μl/min)	FE Na (%)
Sham	63.2 ± 3.2	1.05 ± 0.1	138.5 ± 2.2	302.0 ± 6	10.2 ± 0.7	10.72 ± 1.07	0.29 ± 0.05	1524 ± 104	675 ± 37	0.215 ± 0.02
Untreated ARF (n = 13)	231 ± 22[a] (c)	2.25 ± 0.22 (c)	139.2 ± 1.1	325.4 ± 3 (c)	15.7 ± 1.72 (c)	4.37 ± 0.46 (c)	0.604 ± 0.13 (c)	584 ± 60 (c)	135 ± 1.9 (c)	2.23 ± 0.02 (c)
ARF + Verap[b] (n = 12)	112 ± 25 (c, z)	1.26 ± 0.28 (y)	139 ± 1.4	314.2 ± 6.4 (c, z)	11.9 ± 1.9	5.92 ± 0.58 (c, y)	1.16 ± 0.2 (c, y)	1167 ± 131 (c, z)	326.3 ± 33.2 (c, z)	1.78 ± 0.13 (c, x)
ARF + Verap[c] (n = 8)	260 ± 41 (c)	3.2 ± 0.75 (c, x)	138 ± 0.7	339 ± 7.7 (c, z)	11.9 ± 4.1	3.87 ± 1.12 (c)	1.08 ± 0.16 (c)	702 ± 138 (c, z)	121 ± 36 (c)	4.48 ± 2.3 (c, y)
ARF + Verap + Propr.[d] (n = 8)	145 ± 18 (c, z)	1.5 ± 0.19 (a, x)	141.8 ± 1.2	319.5 ± 3.2 (c, x)	13.6 ± 1.4 (b)	6.36 ± 0.9 (b, y)	1.12 ± 0.14 (c, y)	1022 ± 183 (b, y)	294.4 ± 5 (c, z)	1.86 ± 0.19 (c, y)

[a] a = $P < 0.05$ versus sham. b = $P < 0.01$ versus sham. c = $P < 0.001$ versus sham. x = $P < 0.05$ versus untreated ARF. y = $P < 0.01$ versus untreated ARF. z = $P < 0.001$ versus untreated ARF.
[b] Verapamil 10 μg/min/kg BW for 100 min.
[c] Verapamil 10 μg/min/kg BW for 15 min after declamping.
[d] Verapamil 10 μg/min/kg BW + propranolol 1 mg/hr/kg BW for 100 min.

mined by standard methods as previously described (6–8). Creatinine clearance and fractional excretion of sodium were calculated with standard formulas.

The data are expressed as means ± SEM. Unpaired Student's t test were used to assess significance. A P value less than 0.05 was considered significant.

Results. Twenty-four hours after the ischemic insult, the blood urea, serum creatinine, and creatinine clearance values in the untreated rats were 231 ± 22 mg%, 2.25 ± 0.22 mg%, and 135 ± 1.9 μl/min, respectively (Table I), these values being significantly different in sham-operated animals in which the corresponding values were 63.2 ± 3.2 mg% ($P < 0.001$), 1.05 ± 0.1 mg% ($P < 0.001$), and 675 ± 37 μl/min ($P < 0.001$). The untreated rats with acute renal failure had a significantly higher 24-hr urine volume and fractional excretion (FE) Na% and lower 24-hr urinary osmolality, as compared with the sham-operated group.

The rats with acute renal failure which were treated with verapamil 10 μg/kg body wt/min, started prior to the clamping and given for 100 min, had significantly lower blood urea, 112 ± 25 mg% ($P < 0.001$), serum creatinine 1.26 ± 0.28 mg% ($P < 0.01$), and higher creatinine clearance 326.3 ± 33.2 μl/min ($P < 0.001$) as compared with the untreated ARF rats. This group had significantly higher 24-hr urinary creatinine, Na volume (UNaV), and osmolality (UOsm) than in the control ARF group. However, the verapamil-treated rats had lower FE Na% 1.78 ± 0.13 ($P < 0.05$) as compared with 2.23 ± 0.02 in ARF without verapamil. FE Na in the verapamil-treated rats (for 100 min) was still much higher than in the sham operated rats, 0.215 ± 0.02 ($P < 0.001$). The addition of propranolol 1 mg/kg body wt/hr to the 10 μg/kg body wt/min verapamil had no additional alleviating effect. The severity of ARF rats in which the verapamil administration started after declamping was similar to that of the untreated animals.

Discussion. In the myocardium, ischemia was shown to disturb the mechanisms which maintain normal intracellular calcium ion concentration, leading to increased cytosolic calcium as well as to mitochondrial calcium overload. An increased intracellular calcium

concentration is therefore an important factor in the development of the myocardial injury which follows ischemia (9, 10). Parallel changes have also been found in the kidneys. In noradrenaline-induced ischemic ARF, impaired renal cortical mitochondrial respiration and impaired mitochondrial calcium handling have been found, which seem to contribute to the intracellular ion elevation (2). Furthermore, it has been clearly shown that pretreatment with verapamil or nifedipine, inhibitors of slow-channel calcium transport, protect the myocardium from loss of structure and function after prolonged periods of ischemia (1). Likewise, the administration of verapamil in noradrenaline-induced ARF corrected the disturbed mitochondrial respiration and calcium transport and was associated with a less severe form of ARF (3).

The present results demonstrate that verapamil, as a blocker of cellular calcium uptake, is effective in reducing the severity of another form of ARF, namely that produced by complete renal artery occlusion for 70 min after contralateral nephrectomy. This was shown by lower blood urea and serum creatinine concentrations 24 hr after the ischemic insult and higher creatinine clearance calculated from the 24-hr urinary and serum creatinine. The higher urinary osmolality and lower fractional excretion of sodium of the partially protected verapamil-treated rats document a less severe compromised tubular function compared with the untreated ARF group. It must be emphasized that verapamil administration offers its protective effect only when given prior to the ischemic insult. It was completely ineffective when given after declamping. This shows that, in our model, it seems to be effective only when used as a preventive measure. This result is in contrast to the beneficial effect of verapamil given after the norepinephrine administration in dogs (3) where it can be justifiably considered as a therapeutic measure. Burke *et al.* infused verapamil for 2 hr postischemia; thus, 15 min of infusion, postclamp in our model, may not have been sufficient to permit improvement in ARF. In addition, total verapamil levels were probably much higher in the rats infused for 100 min compared to those infused for just 15 min. Certainly the amounts given over time were different and the concentrations reaching the kidney may have been much higher in the fourth group compared with that of the third group. It is only speculative that this difference may be due to the different ways of verapamil administration, i.e., systemically in the clamping model and directly into the renal artery in the norepinephrine-induced ARF.

It would seem logical to attribute the initial vasoconstriction in the ischemic model of ARF to the high cytosolic Ca^{2+} concentration, which is known to occur following an ischemic insult and is known to be associated with increased smooth muscle tone. Thus the beneficial effect of verapamil as a calcium ion blocker would seem to occur through the decrease of the said vasoconstriction after the calcium ion blockade.

However, Burke *et al.* (3) presented evidence that although the renal vasodilatation obtained by verapamil was associated with improvement in the severity of ARF, a similar renal vasodilatation obtained by acetylcholine was not accompanied by improved renal function. They concluded that the effect of verapamil could not be attributed to renal or systemic hemodynamic changes. These procedures were demonstrated in the norepinephrine model of ARF. In the clamping model of ARF similar results were obtained. The administration of alpha adrenergic blockers in the clamping model resulted in significant vasodilation which was not accompanied by improved renal function (11). Furthermore, the main pathogenic mechanism in both the clamping and the norepinephrine models of ARF is renal tubular obstruction, (4, 5). These facts do not favor the possibility that the verapamil is acting through renal vascular mechanisms.

Some investigations have focused attention on the modulation of intracellular calcium by cyclic AMP-mediated process. It was suggested that, in smooth muscle, the cyclic AMP may enhance calcium uptake by membrane fractions, accelerate calcium efflux from the cell, and prevent transmembrane influx of calcium (12, 13), mechanisms leading to results similar to those obtained after calcium blockers. In a previous work (14), we found that in the ischemic ARF model studied by us, in the first 2 min following induction of

ischemia there was an increase in renal intracellular cyclic AMP which was not prevented by the administration of propranolol. On the contrary, beta-adrenergic blockade was followed by an accumulation of cyclic AMP. The propranolol-treated ARF had improved renal function when compared with the untreated animals (6, 7). In the present work the combination of propranolol and verapamil had no additive effect on renal function. In the excellent recent review on the interaction of calcium channel blockers and the beta-adrenergic receptor blocking agents in the transcellular calcium ion transport (15), it was emphasized that propranolol inhibits calcium ion transport and total ATPase activity of both cardiac and skeletal sarcoplasmic reticulum. These did not occur through a direct effect of beta-adrenergic receptor blockade. It is very attractive to combine these findings and to attribute to both drugs a similar mechanism, namely, through the normalization of intracellular calcium ion transport, for reducing the severity of ischemic acute renal failure.

1. Nayler WG. Cardioprotective effects of calcium ion antagonists in myocardial ischemia. Clin Invest Med **3**:91–99, 1980.
2. Schrier RW, Arnold PE, Burke TJ. Alterations in mitochondrial respiration and calcium movements in norepinephrine-induced acute renal failure: modification by mannitol. In Eliahou HE, ed. Acute Renal Failure. London, John Libbey, pp21–22, 1982.
3. Burke TJ, Arnold PE, Grossfeld PD, Schrier RW. Effect of calcium membrane inhibition on norepinephrine-induced acute renal failure. In Eliahou HE, ed. Acute Renal Failure. London, John Libbey, pp239–240, 1982.
4. Cronin RF, De Torente A, Miller PD, Bulgar RE, Burke TJ, Schrier RW. Pathogenetic mechanisms in early norepinephrine induced acute renal failure. Functional and histological correlates of protection. Kidney Int **14**:115–125, 1978.
5. Finn WF, Arendshorst WJ, Gottschalk CW. Pathogenesis of oliguria in acute renal failure. Circ Res **36**:675–681, 1975.
6. Iaina A, Solomon S, Eliahou HE. Reduction in severity of acute renal failure in rats by beta adrenergic blockade. Lancet **2**:157–159, 1975.
7. Eliahou HE, Iaina A, Solomon S, Gavendo S. Alleviation of anoxic experimental acute renal failure in rats by beta adrenergic blockade. Nephron **19**:158–166, 1977.
8. Iaina A, Orndorff M, Gavendo S, Solomon S. ADH effects in development of ischemic acute renal failure. Proc Soc Exp Biol Med **163**:206–211, 1980.
9. Lown B. Introduction: Symposium on nifedipine and calcium flux inhibition in the treatment of coronary arterial spasm and myocardial ischemia. Amer J Cardiol **44**:780–782, 1979.
10. Clark RE, Christlieb IJ, Henry PD, Fisher AE, Nora JD, Williamson JR, Sobel BE. A myocardial protective agent. Amer J Cardiol **44**:825–831, 1979.
11. Eliahou HE, Brodman RR, Friedman EA. Adrenergic blockers in ischemic acute renal failure in the rat. In Proceedings of the Conference on Acute Renal Failure, DHEW Publication No. (NIH)74-608, New York, pp265–280, 1973.
12. Anderson RGG, Nielsson KB. Role of cyclic nucleotide metabolism and mechanical activity in smooth muscle. In Stephens NL, ed. The Biochemistry of Smooth Muscle. Baltimore, Univ. Park press, pp261–291, 1977.
13. Edelstein RS, Hathaway DR. Role of calcium and cyclic adenosine 3′5′ monophosphate in smooth muscle. Amer J Cardiol **44**:783–786, 1979.
14. Iaina A, Goldfarb D, Serban I, Gavendo S, Kapuler S, Eliahou HE, Thurau K. Renal cellular responses to ischemia, In Eliahou HE, ed. Acute Renal Failure. London, John Libbey, pp50–54, 1982.
15. Herbette L, Messineo FC, Katz AM. The interaction of drugs with the sarcoplasmic reticulum. Annu Rev Pharmacol Toxicol **22**:413–434, 1982.

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