Effect of Verapamil on CRF-Induced Abnormalities in Phospholipid Contents of Brain Synaptosomes (43047)

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Abstract. **Chronic renal failure is associated with significant reductions in total phospholipids, phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine of brain synaptosomes. These derangements in synaptosomal phospholipid metabolism were attributed to the state of secondary hyperparathyroidism of chronic renal failure (CRF) and the parathyroid hormone-induced accumulation of calcium in synaptosomes. This study examined whether a calcium channel blocker, verapamil, would prevent this synaptosomal calcium accumulation and correct the abnormalities in synaptosomal phospholipids in CRF. Verapamil treatment of normal rats for 21 days did not affect synaptosomal content of calcium or phospholipids. CRF of 21 days' duration was associated with a significant ($P < 0.01$) increase in synaptosomal calcium (10.2 ± 0.5 vs 7.4 ± 0.6 nmol/mg protein) and a significant reduction ($P < 0.01$) in total phospholipids (397 ± 12 vs 529 ± 19 nmol phospholipid P/mg protein), phosphatidylinositol (2.7 ± 0.22 vs 4.6 ± 0.27 nmol phospholipid P/mg protein), and phosphatidylserine (37 ± 1.9 vs 83 ± 5.2 nmol phospholipid P/mg protein). Simultaneous treatment of CRF rats with verapamil for 21 days reversed the synaptosomal abnormalities in calcium and phospholipid contents. Our data support the notion that the effect of excess parathyroid hormone of CRF on synaptosomal phospholipids is mainly due to the parathyroid hormone-induced calcium accumulation.** [P.S.E.B.M. 1990, Vol 194]

Previous studies from our laboratory have shown that chronic renal failure (CRF) in rats is associated with significant reductions in total phospholipids, phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidylethanolamine (PE) contents of brain synaptosomes (1). These abnormalities were attributed (1) to the state of secondary hyperparathyroidism of CRF (2–4). Indeed, parathyroidectomized CRF rats maintained normocalcemic had normal synaptosomal phospholipid contents, and treatment of rats with normal renal function for 21 days with parathyroid hormone (PTH) produced changes in synaptosomal phospholipid contents similar to those in CRF (1).

PTH is known to augment entry of calcium into many tissues (5–8) including the brain (4, 9), and exposure to excess PTH was associated with accumulation of calcium in brain synaptosomes (10, 11). It has been suggested that the effect of excess PTH in CRF on synaptosomal phospholipids is due to the ability of the hormone to increase calcium content of the synaptosomes (1).

It is possible, therefore, that a calcium channel blocker such as verapamil may prevent the PTH-induced rise in synaptosomal calcium in CRF and hence correct the derangements in phospholipid contents. This study examined the effect of treatment of CRF rats with verapamil on phospholipid contents of brain synaptosomes.

Materials and Methods

Male Sprague-Dawley rats weighing 275–320 g were studied. The animals were fed normal rat chow (ICN Nutritional Biochemical, Cleveland, OH) throughout the study. Experiments were performed in four groups of rats including normal rats, normal rats receiving verapamil (Isoptin; Knoll AG, Ludwigschafen, Germany), rats with CRF, and rats with CRF treated with verapamil. CRF was produced by $^5/_6$ nephrectomy (12). Verapamil (0.1 μg/g body wt) was given to normal and CRF rats twice daily via subcuta-

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neous injections. Other groups of normal and CRF rats received subcutaneous injections of vehicle only. Animals were studied after 21 days of CRF or verapamil treatment.

The animals were sacrificed at Day 22 of the study by decapitation. Blood was collected in heparinized tubes for the measurements of creatinine, calcium, and phosphorus. The forebrains of the rats were immediately removed, placed in ice-cold (4°C) isolation medium (320 m*M* sucrose, 0.2 m*M* potassium-EDTA, 5 m*M* Tris-HCl, pH 7.4), and chopped into small pieces with scissors. The synaptosomes were prepared according to the method of Booth and Clark (13). The details of the technique for the separation of synaptosomes as well as the various approaches to evaluate the intactness of the synaptosomes, their viability, and the degree of contamination with mitochondria and endoplasmic reticulum have been previously reported from our laboratory (1, 10). The calculated mitochondrial contamination of our synaptosomal preparation was 7.6 ± 0.64%.

One forebrain yielded synaptosomes containing ~12–16 mg of protein. Synaptosomal (2–3 mg of protein) phospholipids were extracted twice as described by Bligh and Dyer (14). The measurements of the total and various phospholipid compounds were made by a modification of the method of a combined system of high-pressure liquid chromatography and automated phosphorus analyzer described by Kaitaranta and Bessman (15). The details of the method used in this study were already reported from our laboratory (1, 16). Recoveries of the standard compounds were as follows: 78–80% for PI, 75–77% for PS, 71–73% for PE, and 75–78% for phosphatidylcholine (PC). The recovery for phospholipid phosphorus of the synaptosomes as a percentage of total phosphorus injected into the column was 66%.

Calcium content of brain synaptosomes was estimated according to the method of Goldfarb and Rodnight (17) for the measurement of calcium in membrane fragments of cerebral cortex. These authors reported a value for calcium from normal rats of 8 ± 1 nmol/mg protein, which is not different from our value in normal synaptosomes (1).

The measurements of calcium concentration in plasma and synaptosomes were done with atomic absorption spectrophotometer (model 505; Perkin Elmer Corporation, Norwalk, CT) and of plasma phosphorus and creatinine with an autoanalyzer (Technicon Corporation, Tarrytown, NY). Synaptosomal protein was determined according to the method of Lowry *et al.* (18). Data are expressed as mean ± SE and statistical significance was evaluated by one-way analysis of variance and Bonferroni *t* test for multiple comparison between the groups.

Results

The results are presented in Tables I and II and Figure 1. There were no significant differences in the body weight and in the plasma concentrations of calcium and phosphorus among the various groups of animals. The $^5/_6$ nephrectomy was associated with a significant ($P < 0.01$) rise in the serum concentration of creatinine. Indeed, the latter was three to four times higher in the CRF rats than in the normal animals. Treatment of CRF rats with verapamil did not produce a significant difference in the concentration of serum creatinine (Table I).

The treatment of normal rats with verapamil did not cause significant changes in the total phospholipid contents of brain synaptosomes or in the content of PI, PS, PE, and PC (Tables I and II and Fig. 1). CRF was associated with significant ($P < 0.01$) reductions in the synaptosomal content of total phospholipids, PI, PS, and PE. The treatment of CRF rats with verapamil reversed these abnormalities, and total phospholipids, PI, PS, and PE contents of synaptosomes of these rats were significantly ($P < 0.01$) higher than those of CRF and not different from those of normal rats and normal rats treated with verapamil (Tables I and II and Fig. 1). The various experimental procedures did not cause significant changes in the percentages of PS, PE, and PC (Table II). However, there was a modest but significant decrease in the percentage of PI in the CRF rats.

The calcium content of brain synaptosomes was significantly ($P < 0.01$) higher in CRF rats than in normal animals or normal rats treated with verapamil (Table I). The treatment of CRF rats with verapamil prevented the increase in synaptosomal calcium.

Discussion

The results of this study demonstrate that treatment of CRF rats with the calcium channel blocker verapamil reversed the abnormalities in the phospholipid contents of brain synaptosomes. We have previously shown that the reduction in the synaptosomal contents of phospholipids in CRF is due to the state of secondary hyperparathyroidism associated with CRF (1). This conclusion was based on two observations showing that synaptosomal phospholipid contents were normal in normocalcemic parathyrodectomized rats and were markedly reduced in synaptosomes of rats with normal renal function treated with 1–84 PTH for 21 days. Therefore, the beneficial effect of verapamil could be due either to amelioration of the secondary hyperparathyroidism or to the interference with the action of PTH on brain synaptosomes, or both.

Available data from *in vivo* studies in rats have shown that the treatment with verapamil either did not affect the blood levels of PTH (19) or higher doses of the drug produced a significant rise in PTH levels both in normal rats (20) or in those with moderate renal

Table I. Blood Chemistry and Phospholipids and Calcium Content in Brain Synaptosomes of Various Groups of Animals

	Normal	Normal-verapamil	CRF	CRF-verapamil
Weight (g)	310 ± 19[a]	311 ± 13	296 ± 6	302 ± 9
Plasma (mg/dl)				
Creatinine	0.40 ± 0.02	0.33 ± 0.04	1.30 ± 0.17[b]	1.54 ± 0.17[b]
Calcium	10.4 ± 0.20	9.9 ± 0.04	10.2 ± 0.30	10.0 ± 0.30
Phosphorus	9.0 ± 0.51	8.3 ± 0.35	8.3 ± 0.36	8.0 ± 0.50
Synaptosomal phospholipids (nmol phospholipid P/mg protein)				
PI	4.6 ± 0.27	5.1 ± 0.31	2.7 ± 0.22[c]	4.5 ± 0.34
PS	51 ± 4.8	48 ± 3.6	37 ± 1.9[c]	50 ± 3.7
PE	83 ± 5.2	81 ± 4.9	59 ± 1.6[c]	83 ± 3.4
PC	203 ± 6.5	209 ± 10.5	179 ± 13.3	202 ± 9.6
Synaptosomal calcium (nmol/mg protein)	7.4 ± 0.6	7.0 ± 0.5	10.2 ± 0.5[c]	7.8 ± 0.5

[a] Data are presented as mean ± SE.
[b] $P < 0.01$ versus normal and normal-verapamil.
[c] $P < 0.01$ versus all other groups.

Table II. Percentage of Molar Composition of Various Phospholipid Components of Brain Synaptosomes

	Total phospholipid[a] (nmol phospholipid P/mg protein)	Individual phospholipid[a] (%)			
		PI	PS	PE	PC
Normal ($n = 8$)	529 ± 19	0.90 ± 0.07	9.3 ± 0.98	15.8 ± 1.31	39 ± 2.7
Normal-verapamil ($n = 6$)	495 ± 19	1.07 ± 0.06	10.0 ± 0.62	16.4 ± 0.76	43.9 ± 2.1
CRF ($n = 6$)	397 ± 12[b]	0.68 ± 0.06[b]	9.3 ± 0.23	15.1 ± 0.24	44.5 ± 1.8
CRF-verapamil ($n = 6$)	480 ± 7.7	0.93 ± 0.06	10.3 ± 0.71	17.3 ± 0.71	42.1 ± 1.5

[a] Data are presented as mean ± SE. The sum of individual phospholipids (PI, PS, PE, PC) does not add up to the total phospholipid content. This is probably due to other phospholipids such as sphingomeylin, ethanolamineplasmalogen, phosphatidic acid, and lysophosphatidylcholine which were not measured.
[b] $P < 0.01$ versus all other groups.

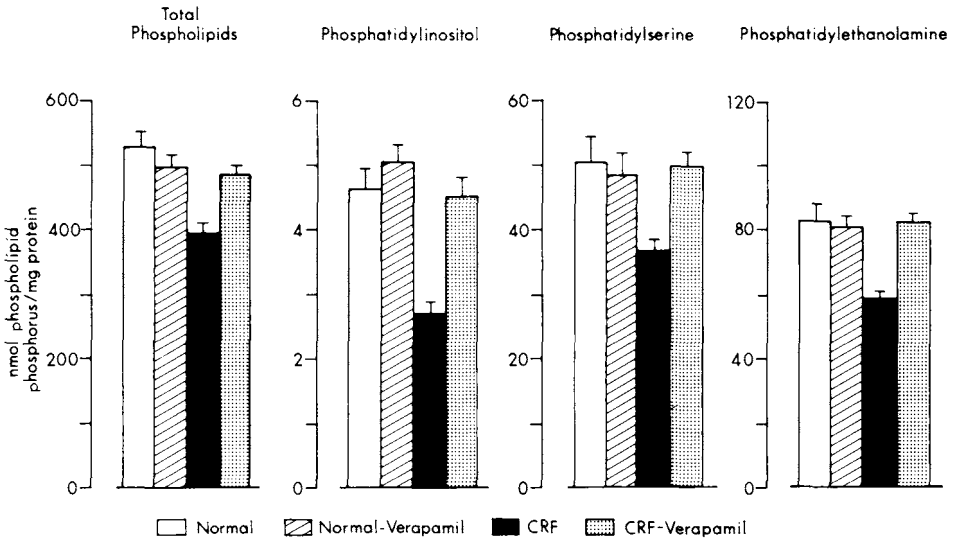

Figure 1. Total phospholipid, phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine contents of brain synaptosomes in the various groups of animals studied. Each column represents mean value and the brackets denote 1 SE.

failure (20, 21). It is, therefore, reasonable to assume that the treatment of our CRF rats with verapamil did not reduce the blood levels of PTH in these animals. Thus, the normalization of the CRF-induced derangement in synaptosomal contents of phospholipids by verapamil is most likely due to the blocking of the effect of PTH on the synaptosomes. Further support for the notion that verapamil blocks calcium entery in synaptosomes is found in the report of Ichida *et al.* (22) who reported that verapamil inhibits potassium-stimulated ^{45}Ca uptake by brain synaptosomes of rats.

The mechanisms through which increased calcium burden of brain synaptosomes affects their phospholipid content are not clear. Synaptosomal phospholipids are determined by the rate of their synthesis and degradation. It is possible that the increased synaptosomal calcium content affects either or both of these processes. Increased calcium content of myocardium and skeletal muscle was associated with inhibition of mitochondrial and myofibrillar creatine phosphokinase (23, 24) and mitochondrial carnitine palmitoyl transferase (25, 26) and with inhibition of synaptosomal Na^+-K^+-ATPase of brain synaptosomes (10). It is possible that the increased calcium content of synaptosomes may also inhibit the activity of the enzymes involved in the synthesis of phospholipids in brain synaptosomes. It is of interest that two of the pathways responsible for the synthesis of PE involves the synthesis of PS (27). Indeed, the predominant pathway of PS synthesis in animals is the base exchange reaction with PE. Thus, it is not surprising to find that the synaptosomal contents of both PS and PE are decreased in the synaptosomes of CRF rats, if the increased calcium burden in these synaptosomes reduces the synthesis of either compound.

It has been shown that CRF is associated with reduced norepinephrine (NE) release and uptake by brain synaptosomes (10). Certain data suggest that the reduction in synaptosomal phospholipids may be responsible, at least, in part for the derangements in NE metabolism of synaptosomes in CRF. For example, PS is critical for the aggregation of synaptosomal vesicles, an important step for the fusion of these vesicles with synaptosomal membrane and for subsequent release of their content (27, 28). Thus, a reduction in PS may provide an explanation for the reduced NE release from synaptosomes in CRF. Also, a reduction in synaptosomal phospholipids may be associated with decreased activity of synaptosomal Na^+-K^+-ATPase, an enzyme that modulates NE uptake by these structures (29). Indeed, synaptosomal Na^+-K^+-ATPase is reduced in CRF (10). Therefore, one may expect that the correction of the CRF-induced derangement in synaptosomal phospholipids should reverse or ameliorate the abnormalities in Na^+-K^+-ATPase activity and in NE uptake and release. Indeed, we found that treatment of CRF rats with verapamil normalized Na^+-K^+-ATPase activity and NE release and markedly (30) improved NE uptake.

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