Calcium Entry Blocker Nicardipine Inhibits Sodium and Inorganic Phosphate Reabsorption Independent of Renal Circulation in Dogs

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The effects of nicardipine on renal function were studied in anesthetized dogs. The changes in the tubular sodium (Na) and inorganic phosphate (PO_4) reabsorption caused by the drug infusion into the renal artery without altered systemic and renal circulation were especially evaluated. In dogs receiving a smaller dose of nicardipine (5 $ng \cdot kg^{-1} \cdot min^{-1}$) into the left renal artery the blood pressure and renal circulation did not change, but urine volume and urinary Na and PO_4 excretion increased significantly. In dogs receiving a larger dose of nicardipine (50 $ng \cdot kg^{-1} \cdot min^{-1}$) into the renal artery, renal plasma flow, urine volume and urinary Na and PO_4 excretion increased significantly, but creatinine clearance did not. The fractional distal Na reabsorption did not change with nicardipine infusion in either group. PO_4 reabsorption is considered to occur mainly in the proximal renal tubule, so its appearance in urine in increased quantities without the changes of systemic and renal circulation suggests proximal activity of the drug. (Key words: calcium entry blocker, nicardipine, excretion of inorganic phosphate, phosphaturia, fractional distal sodium reabsorption)

(Goto F, Watanabe I: Calcium entry blocker nicardipine inhibits sodium and inorganic phosphate reabsorption independent of renal circulation in dogs. J Anesth 6:153–160, 1992)

The systemic hemodynamic effects of calcium (Ca) antagonists have generally been thoroughly investigated. During the last decade interest has increased in the renal functions as well as the cardiovascular effects of such drugs. The renal hemodynamic effects of Ca antagonists vary depending on the experimental conditions[1]. A fuller understanding of the hemodynamic action of Ca antagonists requires detailed information on the modulation of specific modes of activation in the renal vasculature.

Previous studies of Ca antagonists show that diltiazem and nifedipine increase the glomerular filtration rate (GFR) and/or effective renal plasma flow (RPF)[2]. On the contrary, the effect of Ca antagonists on the renal tubule distinct from their renal hemodynamic action can be demonstrated by acute administration of Ca antagonists[3]. Abe et al.[4] reported that following the in-

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trarenal administration of nicardipine (2,6-dimethyl-4(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid-3-(2-(N-benzyl-N-methylamino))-ethylester-5-methylester hydrochloride) in dogs, the urine flow and sodium (Na) excretion increased by more than 3 times and 7 times, respectively. Variations in the results of investigations into the effects of Ca antagonists on renal hemodynamics and diuretic functions may result from the type of agent, the dose administered, the experimental conditions, and whether acute or chronic administration was used.

These unresolved problems and clinical experiences have encouraged the study of further research to examine the effects of popular Ca antagonist, nicardipine, on renal hemodynamics and renal tubular electrolytes excretion in dogs. In clinical study we sometimes experienced unexpected brisk diuresis following nicardipine infusion in hypertensive surgical patients[5]. In this animal study changes in the Na and inorganic phosphate (PO_4) reabsorption caused by drug infusion into the renal artery without altered systemic and renal circulation were especially evaluated. PO_4 reabsorption is considered to occur mainly at the early proximal renal tubule, so increased quantities in the urine without changes in systemic hemodynamics and plasma PO_4 suggest proximal activity of the drug[6]. Moreover, fractional distal reabsorption of Na was calculated from fractional excretion of PO_4 ($FEpo_4$) and Na (FENa)[7]. This value allows the difference between proximal and distal tubular transport of electrolytes to be distinguished.

Materials and Methods

Institutional approval of the experimental protocol was obtained and guidelines of the National Institute of Health for the care and use of the laboratory animals were followed.

Mongrel male dogs (body weight, 11 ± 2 kg) were used as the subjects. Dogs were anesthetized with an i.v. bolus dose of pentobarbital (30 $mg \cdot kg^{-1}$). After tracheal intubation, ventilation was controlled with a mechanical ventilator (tidal volume: 15 $ml \cdot kg^{-1}$) and Pa_{CO_2} was maintained at 30–35 mmHg. Anesthesia was maintained with pentobarbital (5 $mg \cdot kg^{-1} \cdot hr^{-1}$) and pancuronium (0.1 $mg \cdot kg^{-1} \cdot hr^{-1}$). After anesthesia was induced, polyethylene catheters were inserted into the femoral artery and femoral vein to measure the blood pressure and for fluid and drug administration and blood sampling.

The dog's abdomen was opened through a midline incision and the right kidney removed. The left ureter was cannulated and urine collected for every 20 min throughout the study. A curved 22-gauge needle, attached to polyvinyl tubing, was inserted into the left renal artery and patency secured by 4 $ml \cdot hr^{-1}$ infusion of 0.9% saline. At the end of surgery, the abdominal cavity was closed and 40–60 min were allowed for equilibration before starting the experiment. Lactated Ringer's solution (containing 0.1% paraaminohippuric acid (PAH)) was administered intravenously at 10 $ml \cdot kg^{-1} \cdot hr^{-1}$ throughout the study.

The 18 dogs were divided into three groups. In Group 1 dogs saline was infused into the left renal artery at 5 $ml \cdot hr^{-1}$ throughout the study. In Groups 2 and 3, after two 20 min control periods due to saline infusion into the renal artery, nicardipine (Yamanouchi Pharmaceutical Co. Ltd.) was infused into the artery at 5 $ng \cdot kg^{-1} \cdot min^{-1}$ and 50 $ng \cdot kg^{-1} \cdot min^{-1}$, respectively. After allowing 20 min for equilibration, two 20 min clearance periods during nicardipine infusion were obtained.

In each group of dogs, urine was col-

Table 1. Effects of nicardipine infusion (5 ng·kg ^{1}·min^{-1}: Group 2, 50 ng·kg^{-1}·min ^{1}: Group 3) into the renal artery on the renal function in dogs (Mean + SD, n=6)

Period	Group 1	Group 2	Group 3
UV (ml·min^{-1})			
control	0.274 ± 0.167	0.258 ± 0.207	0.183 ± 0.092
during	0.305 ± 0.167	0.380 ± 0.281*	0.533 ± 0.158*‡
C_{PAH} (ml·min^{-1})			
control	54.7 ± 27.1	61.5 ± 29.0	57.6 ± 23.1
during	66.1 ± 30.2	72.3 ± 33.4	74.1 ± 32.3
Ccr (ml·min^{-1})			
control	19.4 ± 5.2	18.4 ± 5.4	17.6 ± 5.5
during	18.9 ± 5.3	18.9 ± 5.3	18.8 ± 4.8
Upo_4V (μg·min^{-1})			
control	132.4 ± 130.2	147.6 ± 132.2	139.8 ± 140.4
during	148.0 ± 125.6	201.5 ± 143.3*	239.9 ± 167.5*
$FEpo_4$ (%)			
control	11.6 ± 8.4	14.9 ± 11.4	13.9 ± 8.9
during	13.1 ± 8.0	21.6 ± 11.7*	21.0 ± 9.8*
UNaV (μEq·min^{-1})			
control	44.0 ± 21.8	51.4 ± 38.7	35.4 ± 21.8
during	50.0 ± 17.4	71.0 ± 46.3*	95.1 ± 19.3*‡
FENa (%)			
control	1.92 ± 1.24	1.68 ± 1.29	1.40 ± 0.92
during	1.83 ± 0.56	2.48 ± 1.59*	3.49 ± 1.01*‡
FDRNa (%)			
control	82.7 ± 21.5	83.7 ± 28.0	82.7 ± 16.9
during	84.9 ± 19.4	87.5 ± 19.5	79.6 ± 18.7
DTRFNa (%)			
control	18.1 ± 3.8	16.3 ± 2.9	19.0 ± 3.6
during	14.8 ± 5.2	14.5 ± 3.2	19.8 ± 4.1
UkV (μEq·min^{-1})			
control	16.0 ± 9.2	17.2 ± 9.1	17.6 ± 8.1
during	18.1 ± 9.0	18.0 ± 7.0	19.5 ± 6.6
FEk (%)			
control	25.0 ± 9.2	23.8 ± 7.7	26.0 ± 8.2
during	26.4 ± 8.8	25.2 ± 5.5	28.0 ± 3.4
CH_2O (ml·min^{-1})			
control	−0.43 ± 0.13	−0.48 ± 0.18	−0.34 ± 0.12
during	−0.46 ± 0.12	−0.51 ± 0.09	−0.51 ± 0.15
Cosm (ml·min^{-1})			
control	0.65 ± 0.18	0.74 ± 0.35	0.59 ± 0.14
during	0.71 ± 0.19	0.89 ± 0.32	1.05 ± 0.13*

*$P < 0.05$; compared with the values in the control period in each group.
‡$P < 0.05$; compared with the value of Group 1.

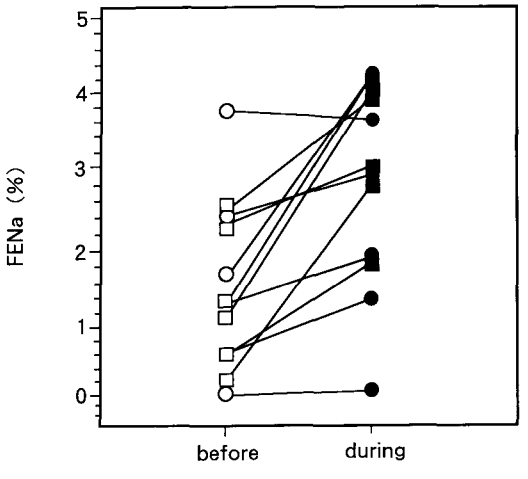

Fig. 1. Fractional excretion of sodium (FENa) increased significantly due to nicardipine infusion (○-●; 5 $ng \cdot kg^{-1} \cdot min^{-1}$, □-■; 50 $ng \cdot kg^{-1} \cdot min^{-1}$) into renal artery in anesthetized dogs.

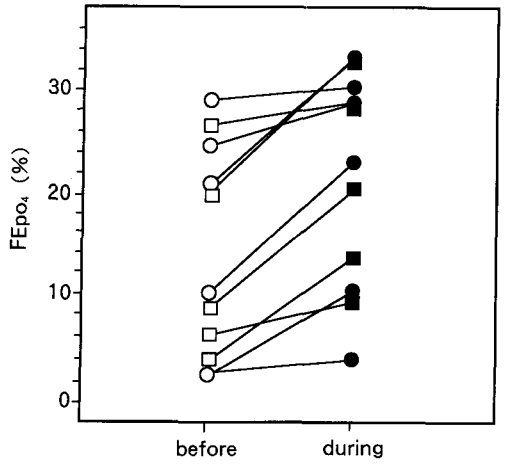

Fig. 2. Fractional excretion of inorganic phosphate ($FEpo_4$) increased significantly due to nicardipine infusion (○-●; 5 $ng \cdot kg^{-1} \cdot min^{-1}$, □-■; 50 $ng \cdot kg^{-1} \cdot min^{-1}$) into renal artery in anesthetized dogs.

lected for each two 20 min periods and 5 ml of blood was drawn at the mid point of each urine collection period. Each blood sample was replaced by twice the volume of saline. The mean values of each two control data and two nicardipine infusion period data were used for statistical analysis.

The urine and plasma concentrations of PAH, creatinine (Cr) and PO_4 were determined colorimetrically. The Na, Ca and potassium (K) concentrations in plasma and urine were determined by flame-photometry. The plasma osmolarity was measured by a freezing-point technique using a Knauer osmometer. The free water clearance (CH_2O), fractional excretion of Na (FENa), K (FEk) and PO_4 ($FEpo_4$) were calculated from the formulae:

$$CH_2O = UV\ (1 - Uosm/Posm)$$
$$FENa = (UV \times UNa)/(Ccr \times PNa)$$
$$FEk = (UV \times Uk)/(Ccr \times Pk)$$
$$FEpo_4 = (UV \times Upo_4)/(Ccr \times Ppo_4)$$

where UV is the urine volume in $ml \cdot min^{-1}$, and Uosm and Posm are urine and plasma osmolarities, respectively. UNa, Uk, and Upo_4 are the concentrations of Na, K and PO_4 in urine and PNa, Pk and Ppo_4 are the plasma concentrations of Na, K and PO_4, respectively. Ccr is the creatinine clearance in $ml \cdot min^{-1}$.

The fractional Na reabsorption in the distal (post-proximal) tubule (FDRNa) and the distal tubular rejection fraction of Na (DTRFNa) were estimated by $FDRNa(\%) = ((FEpo_4 - FENa)/FEpo_4) \times 100$, and $DTRFNa(\%) = 100 - FDRNa$[7–8].

The significance of changes in values was assessed by the Wilcoxon test for paired data. The non-parametric unpaired data was analysed by the Mann-Whitney test. The Spearman rank correlation was used to calculate the correlation coefficient (r). The regression lines were obtained by the method of least squares. Values are presented as mean ± SD, and a value of $P < 0.05$ was taken as significant.

Results

The blood pressure and heart rate did not change significantly during the study without nicardipine in Group 1 or with nicardipine in Groups 2 and 3.

In Group 2 C_{PAH} and Ccr did

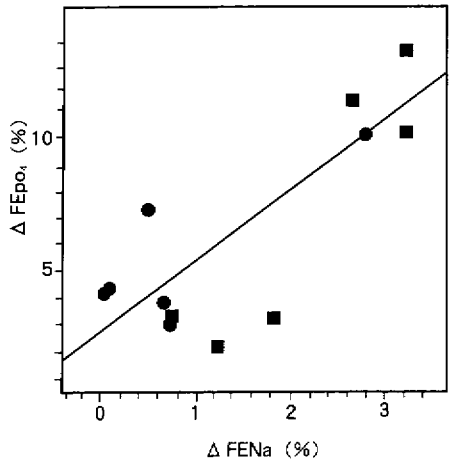

Fig. 3. There is a significant correlationship between the increase of fractional excretion of sodium (△ FENa) and inorganic phosphate (△ $FEpo_4$) that were calculated from the value difference before and during nicardipine infusion in Groups 2 and 3. (y=2.670x+2.777, r=0.854, $P < 0.01$). (●; Group 2, ■; Group 3).

not change due to the smaller volume of nicardipine. However, urine volume (UV) and urinary Na and PO_4 excretion volume (UNaV, Upo_4V) increased significantly (table 1). The urinary K excretion and CH_2O did not change in all three Groups throughout the study.

In Group 3 UV increased significantly, and UNaV and Upo_4V increased significantly from 35.4 ± 21.8 $\mu Eq \cdot min^{-1}$ to 95.1 ± 19.3 $\mu Eq \cdot min^{-1}$, 139.8 ± 140.4 $\mu g \cdot min^{-1}$ to 239.9 ± 167.5 $\mu g \cdot min^{-1}$, respectively (table 1).

FENa and $FEpo_4$ increased due to nicardipine infusion in Groups 2 and 3 (fig. 1, fig. 2). The correlationship between the increase of FENa (△ FENa) and $FEpo_4$ (△ $FEpo_4$) that were calculated from the value difference before and during nicardipine infusion in Groups 2 and 3 was shown in figure 3 (y=2.670x+2.777, r=0.854, $P < 0.01$).

The calculated distal fractional reabsorption of Na (FDRNa) remained stable at a mean value of 83.7 ± 28.0% to 87.5 ± 19.5% in Group 2 and 82.7 ± 16.9% to 79.6 ± 18.7% in Group 3 during the infusion of nicardipine (table 1). There was no significant change in any of the renal function data in Group 1.

Table 2. Plasma electrolytes and osmotic pressure levels in control period (control) and during nicardipine infusion (during) in Groups 2 and 3

	Group 2	Group 3
Na ($mEq \cdot l^{-1}$)		
Control	150.9 ± 7.7	148.5 ± 7.4
During	149.9 ± 8.1	149.1 ± 6.8
PO_4 ($mg \cdot dl^{-1}$)		
Control	4.47 ± 0.83	4.35 ± 0.67
During	4.18 ± 0.90	4.31 ± 0.81
K ($mEq \cdot l^{-1}$)		
Control	3.9 ± 0.4	3.8 ± 0.3
During	3.6 ± 0.3	3.8 ± 0.5
Cl ($mEq \cdot l^{-1}$)		
Control	120.4 ± 6.8	118.5 ± 5.4
During	119.9 ± 8.0	118.1 ± 4.9
Ca ($mg \cdot dl^{-1}$)		
Control	4.2 ± 0.6	4.0 ± 0.9
During	4.1 ± 0.4	4.4 ± 0.5
Posm ($mOsm \cdot l^{-1}$)		
Control	294.0 ± 8.1	291.3 ± 7.9
During	289.8 ± 4.1	291.5 ± 5.8

(Mean ± SD, n=6)

There were no significant changes in the values of plasma Na, K, PO_4 and Ca levels before and during the infusion of nicardipine in Groups 2 and 3 (table 2).

Discussion

The main finding of our study was that diuresis and phosphaturia were observed in all dogs regardless of the systemic and renal circulation and plasma PO_4 levels. Moreover, the calculated distal tubular rejection fraction of Na (DTRFNa) was not changed by nicardipine infusion. These results suggest that nicardipine inhibits proximal electrolyte reabsorption regardless of the renal circulation. In clinical studies

we showed that the systemic infusion of nicardipine in hypertensive surgical patients induced phosphaturia without changes in renal circulation, plasma PO_4 and parathyroid hormone levels[5,9]. However, the systemic administration of nicardipine will induce circulatory changes affecting the renal circulation and release of endogenous diuretic or antidiuretic substances. Therefore, smaller doses of nicardipine were infused into the renal artery to eliminate systemic influences in this animal study.

Studies investigating the mechanism of diuretics have measured the excretion rate of Na most frequently. However, Na is not a good indicator for identifying the active site of a drug, because Na is reabsorbed at many sites in the renal tubule. Therefore we investigated the renal handling of PO_4[10-11], because phosphate reabsorption is considered to occur mainly at the proximal renal tubule. An increased level of PO_4 appearing in the urine then suggests proximal activity of the drug[12]. Nicardipine infusion produced a dramatic increase in PO_4 excretion in surgical patients, even in a man with decreased Ccr caused by the administration of th drug[5]. However, the plasma PO_4 and parathyroid hormone levels did not change due to nicardipine infusion[9].

Under normal diet and parathyroid activity conditions, the mammalian kidneys reabsorb more than 80% of the filtered load of PO_4[12] in the proximal renal tubule. The appearance of increased quantities of PO_4 in the urine therefore indicate the proximal activity of the drug, as neither the GFR nor the plasma PO_4 concentration was affected. PO_4 reabsorption averaging perhaps 5% of the filtered load may occur in other portions of the nephron if the PO_4 delivery to this region is augmented. PO_4 reabsorption in the loop of Henle can be demonstrated more convincingly in the acute absence of parathyroid hormone; however, the physiological impact of this small contribution on the overall rate of PO_4 excretion is uncertain[12].

Drug action in the ascending limb of Henle's loop was assumed if CH_2O was impaired, either alone or together with an impairment of free-water reabsorption[13]. However, CH_2O did not change significantly in the nicardipine group. Moreover, a loop diuretic, furosemide, did not change $FEpo_4$ in our clinical study[5]. These findings essentially rule out any effect of nicardipine on the loop of Henle. The absence of change in FEk, CH_2O and DTRFNa also suggests that the action site of nicardipine is in the proximal tubules other than the distal nephron.

The significant correlation between the changes in FENa and $FEpo_4$ due to nicardipine infusion suggests that the movement of PO_4 may be associated with Na excretion in the proximal renal tubule. Some investigators reported that PO_4 reabsorption was highly dependent on the presence of Na transport[14]. Little or no detectable PO_4 reabsorption occurred in the proximal tubule in the absence of Na transport[15]. Eliminating net Na transport by inhibiting Na-K ATPase activity with either ouabain or by removal of K from the bath does eliminate PO_4 reabsorption. These results suggest that the mechanism of proximal PO_4 reabsorption being simple chemical coupling to Na transport may be an over-simplification. However, at least three different cellular control systems seem to participate in this regulation and are exemplified by parathyroid hormone-dependent inhibition, PO_4 deprivation-dependent increase, and insulin-like growth factor I-dependent increase in Na-PO_4 cotransport[16]. Moreover, recent evidence suggests a role of the phospho-

lipase C/protein kinase C-dependent regulatory cascade in inhibition of Na-PO_4 cotransport[17]. But little is known of the cellular mechanism in PO_4 reabsorption in proximal renal tubule.

In micropuncture experiments, MacLaughlin et al.[18] observed that 5–10 mol verapamil added to the luminal perfusate of normal Wistar rats caused a 36% decrease of Na reabsorption. When verapamil was infused into the peritubular capillaries a greater reduction of 61% of Na reabsorption occurred. Similarly, Figueiredo et al.[19] found a significant decrease of Na reabsorption in the isolated perfused proximal tubule of rabbits when verapamil was added to the bath solution. These investigators agree that the inhibitory effect of verapamil on the tubular transport of Na is not due to a decrease in cytosolic Ca but to the effect on some other transport mechanism. Taylor et al.[20] emphasized the decrease in Na reabsorption when the intracellular concentration of Ca is increased in the renal tubular epithelium by means of quinidine, Ca ionophore, or low peritubular Na concentration. These observations suggest that cytosolic Ca is a major factor in regulating the entry of Na through the apical border of the tubular epithelial cell. In this scheme, an increase in intracellular Ca reduces Na reabsorption and presumably causes natriuresis. However, this mechanism is difficult to reconcile with the preconceived notion that Ca antagonists produce natriuresis by decreasing the level of cytosolic Ca in tubular epithelial cells.

In summary, nicardipine was infused into the renal artery in order to reduce the changes in systemic circulation and endogenous substances. However, PO_4 reabsorption was inhibited by smaller doses of nicardipine without changes in systemic and renal circulation. These findings show the primary site of diuretic action by nicardipine is the proximal renal tubule, because PO_4 reabsorption was considerably inhibited regardless of RPF, Ccr and plasma PO_4 levels. But the precise cellular mechanism of phosphaturia remains unclear.

The data was presented in part at the 38th Meeting of Japan Society of Anesthesiology (Osaka, 1991).

This research was supported by a Grant-in-Aid for Scientific Research (No. 03670718) from the Ministry of Education, Science and Culture of Japan.

(Received Jul. 17, 1991, accepted for publication Sep. 24, 1991)

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