A Comparison of the Uricosuric Effects in Rats of Diltiazem and Derivatives of Dihydropyridine (Nicardipine and Nifedipine)

Haruko Sugino and Hideyo Shimada

Department of Clinical Pharmacology, School of Pharmaceutical Sciences, Kitasato University, 5–9–1 Shirokane, Minato-ku, Tokyo 108, Japan

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ABSTRACT—The effects of nicardipine and nifedipine on the urinary excretion of urate were examined in rats and compared with those of diltiazem. Test drugs were administered to urethane-anesthetized oxonate-loaded rats by continuous i.v. infusion. Diltiazem (10 μg/rat/min), nicardipine (0.3 μg/rat/min) and nifedipine (1.0 μg/rat/min) caused similar reductions of systemic blood pressure and increased total renal blood flow. Diltiazem did not increase urine volume significantly. However, this drug produced obvious uricosuria, with a significant increase in the ratio of urate clearance to inulin clearance (Cua/Cin), which resulted from an increase in Cua, but not from changes in the glomerular filtration rate (GFR). Nicardipine had clear diuretic and uricosuric effects, with similar increases in Cua and GFR and, thus, no change in Cua/Cin. On the other hand, nifedipine did not have any significant effect on the renal handling of urate. These results suggest that nicardipine produces uricosuria in rats via alterations in renal hemodynamics, while the uricosuric effect of diltiazem involves the tubules, as well as alterations in renal hemodynamics.

Keywords: Diltiazem, Nicardipine, Nifedipine, Urate excretion, Renal hemodynamics

In recent years evidence has accumulated to indicate that calcium channel blockers have uricosuric and hypouricemic effects in addition to their diuretic and natriuretic effects. These actions on the metabolism of urate are considered to be advantageous in the treatment of hypertension complicated by hyperuricemia.

Clinical studies have demonstrated that derivatives of dihydropyridine, including nifedipine (1, 2), isradipine (3), nitrendipine (4), manidipine (5) and, most recently, amlodipine (6) increase the renal clearance of urate, and/or reduce plasma urate levels in patients with hypertension. In addition, ferodipine is effective both in healthy normotensive volunteers (7) and in hypertensive subjects (8). In such studies, nifedipine-induced uricosuria was shown to be related to an increase in the glomerular filtration rate (GFR) and in renal plasma flow (1). In contrast, it was suggested that the uricosuric effects of ferodipine (8) and manidipine (5) might be mediated mainly via a tubular action because of the absence of any change in GFR.

Diltiazem, a derivative of benzothiazepine, is a different type of calcium channel blocker that is used as an anti-hypertensive agent, having obvious diuretic and natriuretic effects. In a previous study with the experimental hyperuricemic rat model (9), we found that diltiazem also produces uricosuria and hypouricemia, and we suggested that this uricosuria was probably due to a tubular action of the drug and also, in part, to alterations in renal hemodynamics that included GFR and renal blood flow.

As mentioned above, the available information about uricosuria that is induced by derivatives of dihydropyridine is based exclusively on data from clinical studies, while information about the derivative of benzothiazepine is derived from animal studies. In addition, reports of some previous clinical studies mentioned only the changes in levels of urate in the plasma or its handling in the kidney. Thus, the available information is incomplete. However, the findings obtained from our own and other studies suggest that the details of the uricosuric actions of calcium channel blockers might differ among animal species and/or among drugs.

The aim of the present study was to examine the uricosuric effects in rats of derivatives of dihydropyridine and to compare them with that of a derivative of benzothiazepine, diltiazem.

MATERIALS AND METHODS

Animals and drugs

Male Wistar rats weighing 270–280 g were used. These
animals were housed in a temperature-controlled (23 ± 1°C) and humidity-controlled (55 ± 5%) room with free access to food and water.

The drugs used and their sources were as follows: diltiazem, nicardipine and nifedipine (Sigma, St. Louis, MO, USA) and potassium oxonate (Aldrich, Milwaukee, WI, USA). In the present study, two series of clearance experiments were performed. In one set of experiments, in which the uricosuric effect of nicardipine was compared with that of diltiazem, the drugs were dissolved in saline. In another series of experiments, in which the uricosuric effects of nifedipine and nicardipine were examined, the drugs were dissolved in a solution of 82% (w/w) polyethylene glycol 400 and 5% (w/w) glycerol in water and then diluted 20-fold with saline.

Preparation of animals for the clearance study
Animals were anesthetized with urethane (1.2 g/kg, s.c.) and placed on a warm plate (34°C). The left femoral vein and urinary bladder were catheterized for infusion of the loading solution and the collection of urine, respectively. The loading solution, containing 0.1% oxonate, 4% mannitol, 1.5% inulin, 0.03% sodium bicarbonate and 0.85% sodium chloride (w/v), was infused at a rate of 2.6 ml/rat/hr. A needle connected to a tube filled with the appropriate vehicle, either saline or the solution of polyethylene glycol 400 and glycerol in saline (PGS), was placed in the jugular vein for continuous infusion of the vehicle or a solution of a drug. This infusion was begun at a rate of 3.0 ml/rat/hr at the time when the needle was inserted. After equilibration for 60–90 min, a clearance study was carried out to examine the effects of each test drug.

Blood pressure and heart rate were monitored in all animals throughout the experiments. Two hemodynamic parameters were determined as follows: The right femoral artery was cannulated for measurements of systemic mean blood pressure with a pressure transducer (P23XL; Ohmeda, Liberty Corner, NJ, USA) that was connected to a blood pressure amplifier (AP-641G; Nihon Kohden, Tokyo). Heart rate was measured with a heart rate counter (AT601G, Nihon Kohden). Blood pressure and heart rate were recorded simultaneously.

Clearance study
The uricosuric effects of diltiazem at a dose of 10 μg/rat/min, nicardipine at doses of 0.2 and 0.3 μg/rat/min and nifedipine at a dose of 1.0 μg/rat/min were examined according to the previously described experimental schedule (9). A 10-min urine sample was collected five times: once 20 to 10 min prior to drug administration and then during four consecutive 10-min periods after the administration of the drug. Drugs were administered by changing the infusing solution from the vehicle to a solution of the drug at time 0, and the drug was infused throughout the experiment. Control animals received vehicle only.

A blood sample was taken from the right jugular vein at the mid-point of the urine-collection periods (15 min before, 15 min after and 35 min after administration of the drug), and plasma was obtained by centrifugation.

Measurement of total renal blood flow
At the end of the clearance experiments, total renal blood flow (RBF) of rats that had been treated with diltiazem (10 μg/rat/min), nicardipine (0.3 μg/rat/min) or nifedipine (1.0 μg/rat/min) was measured by a colored-microsphere method. In brief, a bolus injection into the left ventricle of one million microspheres (EZ-Trac, Los Angeles, CA, USA) of 15 μm in diameter was given by cardiac puncture (10). A reference blood sample was withdrawn over the course of 1 min at a rate of 0.2 ml/min with a peristaltic pump (MP201; Tokyo Rikakikai Corp., Tokyo) during the injection of microspheres. Subsequently, the left kidney was removed and weighed. Extraction of the microspheres from the kidney and blood, counting of the extracted microspheres in a counting chamber and calculation of RBF were performed by the standard method.

Quantitation of urate and inulin
Urate was quantitated by the phosphotungstate colorimetric assay with a Uric Acid-Test Wako kit (Wako Pure Chemical Co., Osaka) since the animals had received oxonate, an inhibitor of uricase. Inulin was quantitated by the fluorometric method of Vurek and Pegram (11). GFR was calculated as the rate of inulin clearance (Cin).

Statistical analyses
The results were expressed as means ± S.E. The data from the control groups were analyzed by two-way ANOVA in order to evaluate the time-dependent changes throughout the experiments. Comparisons among the control and drug-treated groups were performed at each time by Dunnett’s multiple range test after one-way ANOVA.

RESULTS
Effects of diltiazem and nicardipine on urine volume and the renal clearance of urate
In the experiment in which the uricosuric effects of diltiazem (10 μg/rat/min) and nicardipine (0.2 μg/rat/min and 0.3 μg/rat/min) were examined, the animals in the control group and those in the drug-treated groups received saline and a test drug dissolved in saline, respec-
tively (n=5 for each group).

The time-dependent effects of diltiazem and nicardipine on urine volume (Uvol) and the rate of urinary excretion of urate (Uua) are shown in Fig. 1. Mean pre-treatment values for all rats (n=20) were 19.4±1.1 µl/min for Uvol and 33.7±1.4 µg/min for Uua. In the vehicle (saline) control group, Uvol and Uua did not change significantly throughout the experiment. Diltiazem at 10 µg/rat/min had no significant effect on Uvol, but it increased Uua significantly during the first 10-min period after administration of the drug. Nicardipine at 0.2 and 0.3 µg/rat/min was diuretic, and a significant increase in Uua was observed at the higher dose of 0.3 µg/rat/min. Maximum increases in Uvol and Uua above the pre-treatment values at both doses were observed during the first 10-min period.

The effects of diltiazem and nicardipine on the plasma level of urate (Pua), GFR, urate clearance (Cua) and the ratio of Cua to Cin (Cua/Cin) during two periods, from 10 to 20 min and from 30 to 40 min after administration of each drug, are shown in Fig. 2. Mean pre-treatment values for all rats (n=20) were 1.94±0.10 mg/dl for Pua, 1.84±0.13 ml/min for Cua, 2.43±0.13 ml/min for Cin and 0.761±0.023 for Cua/Cin. These parameters in the vehicle (saline) control group did not change significantly during both periods.

Diltiazem affected neither Pua nor GFR significantly during the early period after its administration, but it clearly increased Cua and Cua/Cin. Cua/Cin was sig-

![Uvol and Uua changes during infusion of diltiazem and nicardipine](image1)

![Urosecretion changes during infusion of diltiazem and nicardipine](image2)
significantly elevated even during the later period. In contrast, nicardipine at 0.3 μg/rat/min increased Cua and GFR to the same extent during the early period after its administration, but there were no changes in Pua or in Cua/Cin.

Effects of nifedipine on urine volume and the renal clearance of urate

We examined the uricosuric effects of nifedipine (1.0 μg/rat/min) and nicardipine (0.3 μg/rat/min). The animals in the control group and in the drug-treated groups received PGS and test drugs dissolved in PGS, respectively (n=6 for each group).

The time-dependent effects of nifedipine and nicardipine on Uvol and Uua are shown in Fig. 3. Mean pre-treatment values for all rats (n=18) were 23.0±0.9 μl/min for Uvol and 41.8±1.3 μg/min for Uua. The vehicle, PGS, changed neither Uvol nor Uua throughout the experiment. Nifedipine caused a moderate increase in Uvol, but it did not affect Uua significantly. Nicardipine clearly increased both Uvol and Uua. Maximum increases above the pre-treatment value of Uvol were observed during the period from 10 to 20 min after the administration of nicardipine, and those in Uua were observed during the period from 0 to 10 min after its administration.

The effects of the two drugs on Pua, Cua and Cua/Cin during two periods, namely, from 10 to 20 min and from 30 to 40 min after drug administration are shown in Fig. 4. Mean pre-treatment values for all rats (n=18) were 1.96±0.10 mg/dl for Pua, 2.22±0.11 ml/min for Cua, 2.79±0.10 ml/min for Cin and

![Fig. 4. Changes in Pua, Cua, GFR and Cua/Cin during infusion of nicardipine (NIC) at 0.3 μg/rat/min and nifedipine (NIF) at 1.0 μg/rat/min. □, Control; □, NIF (1.0 μg/rat/min); ■, NIF (0.3 μg/rat/min). Upper panel: changes during the early period, from 10 to 20 min after administration of each drug. Lower panel: changes during the late period, from 30 to 40 min after administration of each drug. Data are expressed as percentage changes relative to pre-treatment values and are given as means±S.E. (n=6 for each group). *P<0.05 vs the control group.](image-url)
0.794±0.031 for Cua/Cin. The vehicle, PGS, did not affect these parameters. Nifedipine had no significant effects on Cua and GFR. In contrast, nicardipine tended to increase Cua and GFR to the same extent during the early period after its administration, whereas changes in Cua were not significant. Moreover, neither nifedipine nor nicardipine affected Pua or Cua/Cin.

**Effects of diltiazem, nicardipine and nifedipine on blood pressure and heart rate**

The effects on blood pressure and heart rate of diltiazem and nicardipine, dissolved in saline, are shown in Fig. 5. Mean pre-treatment values for all rats (n=20) were 103±2 mmHg for blood pressure and 458±8 beats/min for heart rate. Blood pressure in the vehicle-treated (saline) control group did not change throughout the experiment, whereas diltiazem at 10 µg/rat/min and nicardipine at 0.2 and 0.3 µg/rat/min reduced blood pressure by 10.1±1.1%, 4.3±0.8% and 7.4±2.0%, respectively, as determined 14 min after administration of the drugs and by 12.5±1.3%, 8.7±1.5% and 12.9±1.2%, respectively, as determined 34 min after administration of the drugs. The heart rate was unchanged in the control group; it was reduced by diltiazem and enhanced by both tested doses of nicardipine.

The effects of nifedipine and nicardipine dissolved in PGS on blood pressure and heart rate are shown in Fig. 6. Mean pre-treatment values for all rats (n=18) were 106±2 mmHg for blood pressure and 464±7 beats/min for heart rate. Blood pressure in the vehicle-treated (saline) control group did not change throughout the experiment. However, nifedipine at 1.0 µg/rat/min and...
nicardipine at 0.3 μg/rat/min reduced the blood pressure by 4.7% and 9.4%, respectively, as determined 14 min after administration of the drugs and by 12.6% and 15.6%, respectively, as determined 34 min after the administration. In addition, nifedipine also increased the heart rate.

**Effects of diltiazem, nicardipine and nifedipine on renal blood flow**

As shown in Fig. 7, RBF at the end of the experiment was increased by all the test drugs. In the experiment with saline as the vehicle, values of RBF in the diltiazem-treated (10 μg/rat/min) and nicardipine-treated (0.3 μg/rat/min) rats were 45.9% and 51.1% greater than that in the vehicle-treated (saline) rats, respectively. In the experiment with PGS as the vehicle, values of RBF in the nifedipine-treated (1.0 μg/rat/min) and the nicardipine-treated (0.3 μg/rat/min) groups were 30.5% and 46.3% greater than that in the vehicle-treated (PGS) control group, respectively.

![Fig. 7. Renal blood flow (RBF) after infusion of diltiazem, nicardipine and nifedipine. Total RBF was measured by the colored-microsphere method, as described in the text, at the end of each experiment. Upper panel: RBF after infusion of diltiazem (10 μg/rat/min), nicardipine (0.3 μg/rat/min) or vehicle (saline). Lower panel: RBF after infusion of nicardipine (0.3 μg/rat/min), nifedipine (1.0 μg/rat/min) or vehicle (PGS). Data are given as means±S.E. (n=5–6). *P<0.05 vs the control group.](image)

**DISCUSSION**

When we re-examined the effects of diltiazem, a derivative of benzothiazepine, on plasma urate levels and the renal excretion of urate in a manner similar to that described in a previous report (9), we obtained results that were in close agreement with those obtained previously. The i.v. infusion of diltiazem at 10 μg/rat/min caused clear increases in Uua, Cua and Cua/Cin during the period from 10 to 20 min after its administration, whereas changes in Uvol, Pua and GFR during this period were not significant.

In the present study, in order to compare the uricosuric action of diltiazem with those of derivatives of dihydropyridine, we first examined the effects of nicardipine. At a dose of 0.3 μg/rat/min, which caused a reduction in mean systemic blood pressure almost equivalent to that caused by diltiazem, nicardipine increased Uvol and Uua. The pattern of the increase in Uua resembled that induced by diltiazem, in that peak responses were obtained within 10 min after the start of administration of each drug. In contrast, the clearance data clearly differed between the two drugs. For example, the uricosuric effect of nicardipine lacked any change in Cua/Cin, as a consequence of similar increases in Cua and Cin.

Although various effects of nicardipine on GFR have been reported, the different results seem to result from differences in experimental conditions (probably in doses of the drug rather than in animal species) (12–14). As described above, we observed synchronous changes in Cua and GFR upon treatment of rats with nicardipine. This result implies the significant contribution of the increased GFR to the nicardipine-induced uricosuria, as contrasted to the negligible contribution in the case of diltiazem.

In the second experiment, we examined the uricosuric effect of another derivative of dihydropyridine, nifedipine, and, for comparison with and confirmation of previous results, we tested nicardipine again. When administered at 1.0 μg/rat/min, nifedipine moderately increased Uvol, but it had no significant effect on the renal excretion of urate, unlike the results of a clinical study by Christensen et al. (1) who reported that this drug had a uricosuric effect in hypertensive patients. We could not examine the effect of nifedipine at doses above 1.0 μg/rat/min because at higher concentrations, the drug did not dissolve in dilute PGS.

PGS as the vehicle had no effect, as indicated by the following observations. The basal levels of Uvol, Uua and blood pressure were almost the same in the two control groups (saline-treated controls and PGS-treated controls). Moreover, nicardipine in PGS had a similar uricosuric effect to that caused by the same drug in saline.
Thus, no uricosuric effect of nifedipine seems to have resulted from no change in GFR, but not from the influence of PGS.

The absence of a change in Cua/Cin after administration of nicardipine suggests that its uricosuric effect in rats is not due to a tubular action. However, in order to determine whether or not the same is true of other dihydropyridines in rats and, further, to determine whether or not the uricosuric action of calcium channel blockers in rats is the same as in humans, more extensive studies are required since ferodipine and manidipine have been reported to increase the fractional excretion of urate in healthy normotensive volunteers (7) and in hypertensive patients (5), respectively.

In the present study, RBF was increased by all the tested drugs. Our results are in agreement with those in our own and other reports: diltiazem (9) and nifedipine (15) increased RBF in rats after i.v. infusion, and intrarenal infusion of nicardipine increased RBF in dogs (12–14). Surgical correction of renal hemodynamics, namely, simple correction of reduced RBF leads to reduction in the serum concentration of uric acid (16). Thus, increases in RBF caused by the calcium channel blockers, including diltiazem and nicardipine, might play a role in the modulation of the renal handling of urate.

In addition to RBF, receptors for angiotensin II (17), the adrenergic system (17) and receptors for dopamine (18) are considered to be related to the urate transport system in renal proximal tubules. Many calcium channel blockers inhibit angiotensin II-induced renal vasconstriction (19). Moreover, it was reported recently that calcium channel blockers, including diltiazem and nicardipine, interact with the dopamine receptor system in renal tubules to induce natriuresis (20, 21). In view of the indirect coupling of the transport of sodium and urate in the proximal tubules (22), we can speculate that the mechanisms of the uricosuric actions of calcium channel blockers might be partially explained by their inhibitory effects on receptors for angiotensin II and/or interactions with receptors for dopamine. However, the difference between the uricosuric effects of diltiazem and nicardipine is harder to explain. Diltiazem and nicardipine are well-known to be structurally and pharmacologically heterogeneous, exibiting clear tissue-selectivity. Therefore, it seems possible that only diltiazem might act somewhere in the metabolism of urate, but not in the excretory system, or this drug might have some unidentified direct action on the urate transport system in renal tubules.

In conclusion, the present data confirm our previous report in which we showed that diltiazem induced uricosuria by a tubular action and, additionally, by alterations in renal hemodynamics. The results also suggest that nicardipine, but not nifedipine, has a uricosuric action in rats, probably via alterations in renal hemodynamics that include increases in GFR and RBF.

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