Original Article

Blocking T-Type Ca^{2+} Channels with Efonidipine Decreased Plasma Aldosterone Concentration in Healthy Volunteers

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Efonidipine can block both L- and T- type Ca^{2+} channels. In a previous *in vitro* study, we clarified that efonidipine dramatically suppresses aldosterone secretion from human adrenocortical tumor cells during angiotensin II (Ang II)- and K^+ -stimulation, whereas nifedipine, a dominant L-type Ca^{2+} channel antagonist, does not. This study was conducted to assess the *in vivo* effects of efonidipine and nilvadipine on the plasma aldosterone concentration. Placebo, 40 mg of efonidipine, or 2 mg of nilvadipine was administered to five healthy male volunteers. Hemodynamic parameters (pulse rate [PR] and blood pressure [BP]), plasma concentrations of neurohormonal factors (plasma renin activity, Ang II, aldosterone, and adrenocorticotrophic hormone [ACTH]), and serum concentrations of Na^+ and K^+ were measured before and 6 h after administration of the agents. All three agents had little effect on PR and BP. Efonidipine and nilvadipine significantly increased plasma renin activity and Ang II. Both had little effect on ACTH, Na^+ , and K^+ . The plasma aldosterone concentration was significantly decreased after efonidipine treatment (88.3 ± 21.3 to 81.6 ± 24.9 pg/ml, $p=0.0407$), whereas it was significantly increased after nilvadipine treatment (66.5 ± 12.2 to 82.17 ± 16.6 pg/ml, $p=0.0049$). Placebo had little effect on neurohormonal factors. Efonidipine decreased plasma aldosterone concentration despite the increase in plasma renin activity and Ang II, suggesting that T-type Ca^{2+} channels may also play an essential role in the secretion of aldosterone in healthy human volunteers. (*Hypertens Res* 2006; 29: 493–497)

Key Words: efonidipine, aldosterone, T-type Ca^{2+} channel

Introduction

Recent molecular biology research has classified Ca^{2+} channels into five subtypes—L (long-lasting), T (transient), N (neuronal), P/Q (Purkinje), and R (residual drug-resistant)—according to their localization and function (1, 2). The L-type Ca^{2+} channels are distributed in the cardiac muscle, vascular smooth muscle, and skeletal muscle and are involved in their contraction and relaxation. The N-type Ca^{2+} channels are involved in cardiovascular sympathetic nerve activation, and

cilnidipine, an antagonist of both L- and T- type Ca^{2+} channels, was reported to suppress the occurrence of ischemia/reperfusion arrhythmia (3). The T-type Ca^{2+} channels, which were originally called low-voltage-activated channels, are known to be distributed in the sinoatrial node (4) and are involved in pacemaker activity (5). Furthermore, the T-type Ca^{2+} channels regulate vascular tone and hormone secretion (6, 7). Efonidipine antagonizes both T- and L-type Ca^{2+} channels with similar affinity and has little effect on the N-, P/Q-, and R-type Ca^{2+} channels. On the other hand, nifedipine and nilvadipine antagonize only the L-type channels and have lit-

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Table 1. Hemodynamic Parameters before and 6 h after Administration of Agents

Agent/hemodynamic parameters	Before	6 h after administration	<i>p</i>
Placebo			
Pulse rate (bpm)	60±8	60±5	n.s.
SBP (mmHg)	116±6	115±5	n.s.
DBP (mmHg)	69±8	66±11	n.s.
Efonidipine			
Pulse rate (bpm)	63±4	69±4	n.s.
SBP (mmHg)	118±13	115±8	n.s.
DBP (mmHg)	68±11	68±9	n.s.
Nilvadipine			
Pulse rate (bpm)	62±10	65±7	n.s.
SBP (mmHg)	114±5	114±6	n.s.
DBP (mmHg)	69±7	68±6	n.s.

SBP, systolic blood pressure; DBP, diastolic blood pressure; n.s., not significant. Mean±SD.

Table 2. ACTH and Electrolyte before and 6 h after Administration of Agents

Agent/ACTH and electrolyte	Before	6 h after administration	<i>p</i>
Placebo			
ACTH (pg/ml)	35.7±10	27.4±14	n.s.
Na ⁺ (mEq/l)	143.6±1.8	143.2±1.9	n.s.
K ⁺ (mEq/l)	3.96±0.2	4.10±0.3	n.s.
Efonidipine			
ACTH (pg/ml)	27.6±11	24.9±16	n.s.
Na ⁺ (mEq/l)	142.0±1.5	140.8±1.2	n.s.
K ⁺ (mEq/l)	4.15±0.3	3.97±0.2	n.s.
Nilvadipine			
ACTH (pg/ml)	23.9±8	23.4±4	n.s.
Na ⁺ (mEq/l)	142.0±2.1	142.3±2.7	n.s.
K ⁺ (mEq/l)	4.03±0.3	3.98±0.3	n.s.

ACTH, adrenocorticotrophic hormone; n.s., not significant. Mean±SD.

the effect on the other types of Ca²⁺ channels (8, 9). Hence, we compared efonidipine, nifedipine and nilvadipine with regard to their characteristic action of T-type Ca²⁺ channel antagonism.

Aldosterone plays an important role in the pathophysiology of hypertension, cardiac hypertrophy, and heart failure. Targeting aldosterone synthesis and/or secretion represents a potentially useful approach for the prevention of cardiovascular disease (10). Aldosterone is synthesized in the zona glomerulosa of the adrenal gland, where it is secreted in a Ca²⁺-dependent manner in response to stimuli such as angiotensin II (Ang II), adrenocorticotrophic hormone (ACTH), and increased extracellular K⁺ (11). Bovine and human glomerulosa cells express L-type and T-type voltage-dependent Ca²⁺ channels (12); however, recent studies suggest that T-type Ca²⁺ channel activity is more closely related to aldosterone production than L-type channel activity (13, 14).

In a previous *in vitro* study, we have shown that efonidipine dramatically suppresses aldosterone secretion from human

adrenocortical tumor cells (H295R) during Ang II stimulation, whereas nifedipine does not. Further, we confirmed that this action of efonidipine is attributed to the blockade of T-type Ca²⁺ channels (15). However, there have been few clinical reports on the role of Ca²⁺ channel antagonists in reducing plasma aldosterone concentration. The present study was conducted to assess the *in vivo* effects of efonidipine and nilvadipine on plasma aldosterone concentration.

Methods

Study Design

The present study was approved by the Ethical Committee (No. 311-6) of Nara Medical University. All subjects provided written informed consent to participate in the study.

Five healthy male volunteers (38±7 years) were recruited for the study. None of the volunteers had a history of smoking, and no evidence of previous or current cardiovascular,

respiratory, renal, or hepatic disease was obtained from their medical history. None of the volunteers had received medication for at least 1 month before the beginning of the study.

This study was a placebo-controlled crossover study with three periods of treatment that were separated by a washout period of at least 7 days. In each subject, three agents were administered in the following order: a placebo sugar tablet, efonidipine (40 mg), and nilvadipine (2 mg). The dosages used in this study were determined as those generally prescribed for patients with hypertension and have been reported to show few side-effects in healthy volunteers (16, 17). The maximum blood pressure (BP) reduction was attained approximately 6 h after administration of either drug to healthy volunteers. During each period, the drug was administered as a single oral dose at 8:00 AM, and an analysis of its effect was performed before medication (basal state) and 6 h after its administration (posttreatment state). The volunteers were hospitalized from 6:00 AM to 6:00 PM, and were maintained in the supine position in a quiet room. They had fasted from 8:00 PM on the day before the beginning of the study; on the day of the study itself, they did not have breakfast or lunch, and were asked to consume as little water as possible. They were also requested to refrain from consuming alcohol and foods/drinks containing caffeine on the day before the beginning of this study and continuing until the end of the study. We took special care to keep the sampling conditions quiet and stable.

Hemodynamic Analysis

We used an automated sphygmomanometer (HEM-780; Omron Corp, Matsusaka, Japan) to record the pulse rate (PR) and BP. All measurements were obtained with the volunteers in the supine position, after they had evacuated their bladders, in a quiet room.

Neurohormonal Assays

Blood samples were taken in the supine position, immediately after each hemodynamic evaluation time. Plasma renin activity and the Ang II, aldosterone, and ACTH concentrations were measured by radioimmunoassay (RIA) at a contract laboratory (SRL, Inc., Tokyo, Japan). The effect of aldosterone on electrolytes was assessed by routine measurement of electrolytes.

Statistical Analysis

Based on a SD of 5% for plasma aldosterone concentration, the sample size necessary to detect a 10% change in a normal plasma aldosterone concentration was calculated. To decrease the variability among subjects in the control group and in the treatment group, a crossover design was chosen. It was found that a minimum of 6 patients would be required for

Fig. 1. Plasma renin activity before medication and 6 h after administration of agents.

each group to demonstrate changes of this magnitude. Data are expressed as the mean $\pm$ SD. One-way analysis of variance (ANOVA) was performed to check for any differences at the basal state between the treatment sequences. A paired *t*-test was performed to check for any differences between the basal and posttreatment state. A value of $p < 0.05$ was considered to indicate statistical significance.

Results

The hemodynamic parameters are shown in Table 1. No significant difference was observed with regard to the systolic BP, diastolic BP, and PR at the basal state between the treatment sequences. All three agents had little effect on BP and PR. The concentrations of ACTH and electrolytes are shown in Table 2. No significant difference was observed with regard to ACTH and electrolytes at the basal state between the treatment sequences (ACTH: $p=0.41$; Na^+ : $p=0.13$; K^+ : $p=0.43$). These three agents had little effect on the concentrations of ACTH and electrolytes. The plasma renin activities at the basal and posttreatment states are shown in Fig. 1. The plasma renin activity was significantly increased 6 h after the administration of both efonidipine (from 0.78 ± 0.47 to 1.85 ± 0.99 ng/ml/h, $p=0.015$) and nilvadipine (from 0.91 ± 0.54 to 1.41 ± 0.70 ng/ml/h, $p=0.0047$). The plasma Ang II concentrations are shown in Fig. 2. The plasma Ang II concentration was significantly increased after the treatment with both efonidipine (from 6.66 ± 3.14 to 10.3 ± 4.76 pg/ml, $p=0.023$) and nilvadipine (from 8.16 ± 3.60 to 11.5 ± 4.18 pg/ml, $p=0.042$). However, the plasma aldosterone concentration was significantly decreased after efonidipine treatment (from 88.3 ± 21.3 to 81.6 ± 24.9 pg/ml, $p=0.040$), but it was increased after nilvadipine treatment (from 66.5 ± 12.2 to 82.17 ± 16.6 pg/ml, $p=0.0049$) (Fig. 3). The placebo did not have any effect on the neurohormonal factors.

Fig. 2. Plasma angiotensin II levels before medication and 6 h after administration of agents.

Discussion

The present study was the first to demonstrate that efonidipine, an antagonist of both T- and L-type Ca^{2+} channels, was able to decrease the plasma aldosterone concentration despite an increase in plasma renin activity and Ang II concentrations in healthy volunteers, whereas nilvadipine, a dominant L-type Ca^{2+} channel antagonist, did not exert such inhibitory effects on the renin-angiotensin-aldosterone system.

These findings were consistent with our recent *in vitro* study, which showed that efonidipine suppresses Ang II- and K^{+} -induced aldosterone secretion from the human adrenocortical tumor cells (H295R), while nifedipine does not. These findings suggest that efonidipine suppresses aldosterone secretion through the blockade of T-type Ca^{2+} channels (15). The present study is also consistent with earlier studies indicating that two other T-type Ca^{2+} channel antagonists—mibefradil and tetrandrine—suppress aldosterone secretion from bovine adrenal glomerulosa cells (18, 19).

Recent clinical studies such as Randomized Aldactone Evaluation Study (RALES) (20) and Eplerenone Postacute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) (21) have shown that suppression of the biological action of aldosterone with a mineralocorticoid receptor (MR) antagonist reduces the incidence of cardiovascular events among patients with severe heart failure. However, MR antagonists trigger life-threatening hyperkalemia in quite a few patients (22). Patients with cardiovascular disease or renal insufficiency who are treated by combination therapy with an MR antagonist and an angiotensin converting enzyme inhibitor (ACE-I) or an Ang II receptor antagonist should be frequently checked for unexpected increases in serum K^{+} concentration. Efonidipine, which suppresses only the Ang II- and K^{+} -stimulated aldosterone secretion, is likely not to induce such hyperkalemia, suggesting that efonidipine may

Fig. 3. Plasma aldosterone levels before medication and 6 h after administration of agents.

suppress the action of aldosterone more safely than the MR antagonists.

Many clinical studies have proved that antihypertensive therapy is essential for the treatment of cardiovascular disease (23–25), and Ca^{2+} channel antagonists, including efonidipine, have the potential to lower the high blood pressure. However, most Ca^{2+} channel antagonists stimulate the renin-angiotensin-aldosterone system, and the mechanisms of this stimulation, which are probably multifactorial, are still not completely understood (26–28).

The precise correlation between plasma aldosterone concentration and cardiovascular disease is controversial (29), but with respect to blockade of the renin-angiotensin-aldosterone system, efonidipine, which antagonizes both the T- and L-type Ca^{2+} channels, is more useful than other dominant L-type Ca^{2+} channel antagonists in patients with hypertension.

The conclusions of this study may be limited by the sampling condition. That is, water and sodium intake were not restricted until the day before the experiments. Stricter control of the salt balance may have minimized the deviation of the results among the three groups of patients.

In conclusion, efonidipine can decrease the plasma aldosterone concentration, despite the increase in the plasma renin activity and Ang II concentrations, which indicates that T-type calcium channels may also play an essential role in the secretion of aldosterone in healthy human volunteers.

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