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Effect of Calcium-Channel Blockade on the Aldosterone Response to Sodium Depletion and Potassium Loading in Man

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Angiotensin II (Ang II) and potassium (K^+) increase aldosterone (Aldo) production in vitro via Ca^{2+}-dependent mechanisms. To determine the effects of Ca^{2+} antagonism in vivo, we examined the influence of nifedipine on the Aldo response to Na^+ depletion and K^+ loading in 11 healthy subjects. On the fifth day of a low-Na^+/high-K^+ diet (10 mmol Na^+/100 mmol K^+) the subjects were randomly given either nifedipine 30 mg po or placebo, and on the sixth day they received the alternative drug. KCl in 5% glucose was infused on days 5 and 6 from 10:00 to 12:00 AM (0.6 mmol/kg over 2 hours). Dexamethasone was given to suppress adrenal corticotrophic hormone. Plasma renin activity (PRA) and plasma Aldo were determined every 20 minutes. Nifedipine induced a rise in heart rate at 60 minutes but did not change blood pressure. During KCl/glucose infusions, plasma glucose increased significantly, but plasma K^+ remained stable. PRA, but not baseline plasma Aldo, was stimulated by nifedipine. KCl provoked a significant and similar Aldo rise ($P < .01$) under placebo and nifedipine. Baseline Aldo/PRA ratio was reduced under nifedipine when compared to placebo ($P < .01$), whereas during KCl infusions this ratio was similarly elevated under placebo and nifedipine. We conclude that acute inhibition of slow Ca^{2+} channels does not interfere with K^+-induced Aldo secretion in man, suggesting that adaptive mechanisms operate in vivo. Am J Hypertens 1988;1:245–248

KEY WORDS: Aldosterone response, calcium-channel blockade, nifedipine, potassium loading, sodium depletion.

Potassium (K^+) and angiotensin II (Ang II) stimulate aldosterone (Aldo) production via calcium (Ca^{2+})-dependent mechanisms. Earlier studies using isolated adrenal glomerulosa cells have shown that inhibition of Ca^{2+} uptake blocked the steroidogenic response to both K^+ and Ang II.[1–3] Recent work from this laboratory[4] indicates that both Ang II and K^+ increase intracellular Ca^{2+} ($[Ca^{2+}]_i$), but they do so by different mechanisms: Whereas Ca^{2+} antagonists can totally block the K^+-induced $[Ca^{2+}]_i$ rise resulting from the opening of voltage-dependent Ca^{2+} channels and the subsequent Aldo production, they do not suppress the receptor-mediated $[Ca^{2+}]_i$ rise induced by Ang II, though they reduce the steroidogenesis slightly.

An inhibition of Aldo response to Ang II by acute administration of Ca^{2+}-antagonists has been observed in human studies,[5–7] but the effect of calcium antagonism on K^+-induced Aldo stimulation has not yet been examined. The present study was therefore designed to compare the influence of nifedipine on the Aldo response to Na^+ depletion and K^+ loading in normal man. The experimental protocol we used raised a further working hypothesis regarding the mechanism by which K^+-induced Aldo stimulation may involve insulin action. Our results suggest that intracellular K^+ may also influence Aldo secretion via a Ca^{2+}-independent mechanism.

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METHODS

Eleven healthy male subjects aged 23 to 27 years volunteered to participate in the study. They gave their informed consent to the experimental protocol, which has been approved by the Ethical Committee of the Department of Medicine.

The subjects were kept for 6 days on a strict low-Na^+ (15 mmol) and high-K^+ (100 mmol) daily intake. On day 5 the subjects were randomly given either nifedipine (20 mg at 7:00 AM and 10 mg at 10:00 AM po) or a placebo. On day 6 they received the alternative drug. Dexamethasone (1 mg) was taken at midnight on days 4 and 5 to suppress adrenal corticotrophic hormone (ACTH). On the fifth and the sixth days two similar KCl-infusion tests were performed from 10:00 AM to 12:00 noon. After fasting overnight, the subjects remained supine from 7:00 AM to 12:00 noon. KCl diluted in 5% glucose (50 mmol KCl/L) was infused over 2 hours (0.6 mmol/kg) through a large humeral vein.

Blood was drawn every 20 minutes during the KCl infusion for determination of plasma Na^+ and K^+, plasma renin activity (PRA) and Aldo. Plasma glucose and plasma cortisol were measured at 60 and 120 minutes. Plasma insulin was also determined in five subjects. Blood pressure and heart rate were checked regularly during the tests. Urinary Na^+ and K^+ were measured in 24-hour urine on the fifth day of the diet and in a urine spot on the sixth day.

Na^+ and K^+ were determined by flame photometry and plasma glucose by the glucose-oxydase method. PRA, plasma cortisol and insulin, and urinary Aldo were determined by radioimmunoassay (RIA). Plasma aldosterone was measured by RIA using a commercial kit (Coat-A-Count-Diagnostic Products Corp). The limit of detection was 1.5 ng/100 mL, and the intra-assay coefficient of variation was 5.1%.

Statistical analysis was performed by paired *t* test, the subjects being their own controls. Results are expressed as the mean ± SEM.

RESULTS

On the fifth day of low-Na^+/high-K^+ diet, 24-hour urinary excretion was 19.8 ± 2.1 mmol for Na^+ and 109.9 ± 9.0 mmol for K^+. Urinary excretion of Aldo was 42.0 ± 2.7 μg/24 hours. In the sixth day urine spot Na^+ was 12.0 ± 2.2 mmol/L and K^+, 77.0 ± 2.7 mmol/L. Mean body weight was 68.7 ± 3.3 kg on the fifth day and 68.5 ± 3.2 kg on the sixth day (difference not significant [NS]).

Before KCl infusion blood pressure was 114 ± 2/69 ± 2 mm Hg on placebo and 115 ± 2/69 ± 1 mm Hg on nifedipine (NS). Heart rate was 61 ± 2 and 66 ± 3 beats/minute, respectively (NS). At the end of the KCl infusion, blood pressure was 113 ± 2/71 ± 2 mm Hg on placebo and 115 ± 2/69 ± 2 mm Hg on nifedipine (NS), and heart rate was 60 ± 2 and 65 ± 3 beats/minute, respectively (NS). However at 60 minutes, heart rate was significantly higher on nifedipine (75 ± 4) than on placebo (60 ± 2, $P < .01$).

The mean amount of KCl infused over 2 hours (0.6 mmol/kg) was 41.1 ± 1.7 mmol. Despite this large load, plasma K^+ did not change significantly during the 2-hour infusion in either test (Figure 1). Plasma Na^+ remained constant. The infused volume was 831 ± 42 mL over 2 hours, providing a glucose load of 41.6 ± 2.1 g. On both placebo and nifedipine days, plasma glucose and plasma insulin increased significantly to a similar extent during KCl-5% glucose infusion with a peak at 60 minutes.

As shown in Figure 1, baseline PRA increased by Na^+ depletion was further stimulated by nifedipine treatment, whereas plasma Aldo levels were similar on placebo and nifedipine days. Consequently the Aldo/PRA ratio was significantly higher on placebo (5.6 ± 0.6 at time 0) than on nifedipine (3.9 ± 0.5, $P < .01$). During KCl infusion, despite the absence of change in plasma K^+ concentration, there was a marked and highly significant rise in plasma Aldo, reaching a peak at 40 minutes on placebo ($P < .01$ *vs* baseline) and at 60 minutes on

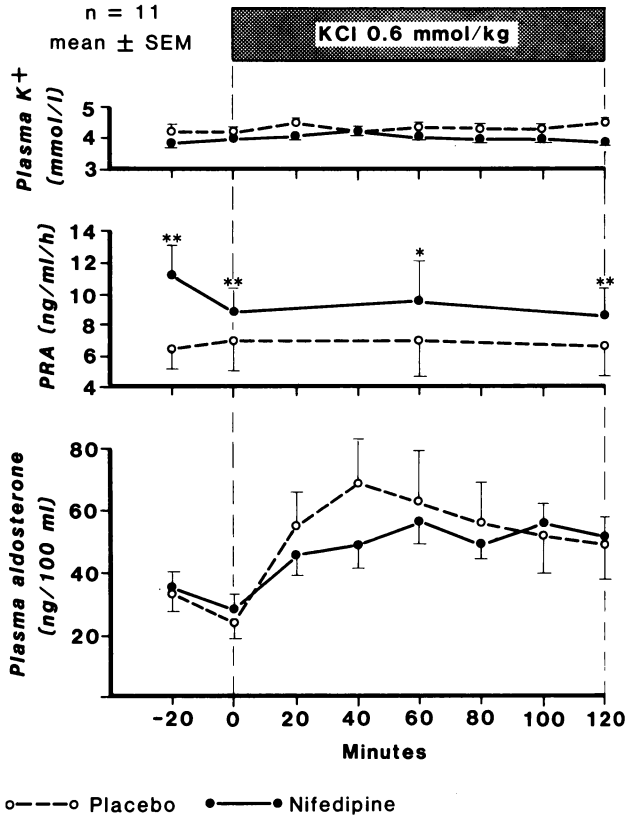

FIGURE 1. *Effect of a 2-hour KCl IV infusion (0.6 mmol/kg in 5% glucose) on plasma potassium concentration, plasma renin activity and plasma aldosterone levels, in 11 healthy subjects during sodium restriction. See text for statistics.*

nifedipine ($P < .001$ *vs* baseline); peak values were not significantly different on placebo day (41.2 ± 10.0 ng/dL) and on nifedipine day (34.8 ± 3.8 ng/dL). The Aldo/PRA ratio during KCl infusion was not significantly different on placebo and on nifedipine. Plasma cortisol levels remained suppressed during the tests (1.7 ± 0.4 μg/dL on placebo and 1.3 ± 0.3 on nifedipine/NS).

DISCUSSION

In vitro studies using adrenal glomerulosa cells indicate that although Ang II and K^+ both stimulate Aldo secretion by increasing $[Ca^{2+}]_i$, only the K^+-mediated stimulation, acting through voltage-dependent Ca^{2+} channels, is specifically blocked by Ca^{2+} antagonists.[4] Slow Ca^{2+}-channel blockers can also decrease Ang II–induced Aldo production, but their effect is less marked than on KCl-induced steroidogenesis. This lesser effect is a consequence of a lower basal intracellular Ca^{2+} concentration resulting from depletion of $[Ca^{2+}]_i$ stores by these drugs and preventing attainment of the critical threshold for triggering steroidogenesis. From these in vitro findings, it might therefore be inferred that acute inhibition of Ca^{2+} channels would interfere with K^+-induced Aldo stimulation. In a sheep whose adrenal gland was transplanted to its neck, the local infusion of Ca^{2+} antagonists reversed the Aldo response to K^+ but did not affect Aldo secretion induced by Na^+ depletion.[8,9]

To assess the effect of K^+ on Aldo secretion, KCl was infused acutely after stimulation of the renin–angiotensin system as in previous studies.[10,11] It is known that in normal subjects a clear Aldo response to K^+ occurs only in the presence of high concentrations of Ang II.[10] This fact emphasizes the important interdependence of Ang II and K^+.[12] KCl was diluted in glucose to prevent a local venous toxic reaction. Although plasma K^+ did not rise during the KCl-glucose infusion, there is strong evidence that Aldo stimulation was directly induced by K^+, as previously reported.[10] Glucose ingestion acutely lowers plasma concentrations of K^+ and Aldo,[11] whereas plasma Aldo levels rise during KCl–glucose infusion, despite an unchanged K^+ plasma concentration,[10] as confirmed in the present study. Whether K^+ enters the cell in order to stimulate steroidogenesis is still conjectural, as opposite conclusions have been drawn from in vitro or in vivo studies using direct or indirect estimation of intracellular K^+ content.[10,11,13] Insulin stimulates K^+ cellular uptake by the liver and muscle tissues,[14] but it is not known whether tissues like the adrenal cortex are also involved. These findings suggest that either membrane depolarization occurs without any apparent change in K^+ extracellular concentration (which is unlikely, as high concentration is required in vitro to depolarize glomerulosa cells[4]), or that the signal may result from transmembrane K^+ fluxes leading to a higher intracellular K^+ concentration. If these processes are mediated by the Ca^{2+} fluxes demonstrated in vitro,[4] they would also be blocked in vivo by Ca^{2+} antagonists. This was not the case in this study because, contrary to our expectation, nifedipine was unable to prevent the K^+-induced rise in Aldo concentration, the peak value of which during KCl infusion being similar on placebo and nifedipine treatment. Similar results showing no interference by nifedipine with Aldo secretion have been reported recently in hypertensive patients given an acute KCl infusion.[15] Our findings indicate that the mechanism of K^+-induced Aldo secretion under these particular in vivo circumstances differs from that observed in vitro in isolated cells. In this study, as in an earlier one,[16] insulin response to hyperglycemia was not altered by nifedipine. The two KCl infusions were therefore comparable in terms of glucose load and insulin secretion. Insulin alone can influence plasma K^+ concentration, baseline plasma Aldo levels and its response to Ang II in various ways.[17]

In the present in vivo human study under low salt intake, nifedipine reduced the Aldo/PRA ratio but did not alter K^+-induced Aldo secretion. Renin secretion was clearly stimulated as a result of a combined effect of Na^+ depletion and acute inhibition of slow Ca^{2+} channels, and as previously reported,[18] it was not modified by KCl administration. The high basal plasma concentration of Aldo induced by Ang II stimulation was similar on nifedipine and placebo. Despite the known higher sensitivity of adrenal cells after Na^+ depletion,[19] no further increase in Aldo secretion could be obtained in response to the acute renin rise under nifedipine treatment. The finding of a lower Aldo/PRA ratio during nifedipine treatment, previously reported in hypertensive patients,[20] is compatible with the acute inhibitory effect of nifedipine on steroidogenesis during Ang II infusion in man.[5–7] Another unrelated Ca^{2+} antagonist, verapamil, did inhibit Aldo response to Ang II, but only when given chronically.[21] During sodium restriction in normal subjects nifedipine and diltiazem reduced the sensitivity of Aldo secretion to Ang II.[7]

As nifedipine has been shown to increase apparent liver blood flow in normal subjects,[22] an alteration of Aldo metabolic clearance rate is to be considered so that changes in Aldo plasma concentrations may not parallel changes in Aldo secretion. To exclude any contribution of another Ca^{2+}-independent adrenal trophic factor, ACTH was constantly suppressed by dexamethasone during the two test days. In fact Guthrie et al[21] have reported that verapamil does not alter the Aldo response to ACTH. Nifedipine, known to bind with high affinity and specificity to the voltage-dependent Ca^{2+} channel,[23] was administered shortly before KCl infusion. It may be inferred from pharmacokinetic data that the dose selected would result in plasma concentrations in the same micromolar range as the in vitro studies, although individual bioavailability may vary.[24] The PRA

rise obtained in every subject indicates that effective drug levels were achieved. Blood pressure was not influenced, as would be expected in normotensive subjects.[25]

In summary, acute blockade of slow Ca^{2+}-channels does not interfere with K^+-induced Aldo secretion but reduces the Aldo/PRA ratio. These findings suggest some inhibition of the Ang 2-induced Aldo secretion. The discrepancy with in vitro findings indicates that adaptive mechanisms operate in vivo allowing the maintenance of Aldo secretion in normal man. Finally, these results support the existence of a role for intracellular K^+ in stimulating Aldo secretion.

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