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Original article

Effect of Amlodipine in Comparison to Nifedipine on Vascular Perfusion Pressure of Isolated Rat Kidney

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Abstract

Objective(s)
This study aimed to investigate and to compare the effects of nifedipine and amlodipine, dihydropyridine (DHP) calcium channel blockers (CCBs) on perfusion pressure of isolated perfused rat kidney.

Materials and Methods
Following the establishment of renal perfusion with a constant baseline pressure of 85-95 mmHg, the renal vasculature was constricted by phenylephrine (PE) injection. Changes in the baseline perfusion pressure were recorded. Then nifedipine and amlodipine prepared in perfusion medium was fed to the kidney for 30 min. Finally alterations in the baseline pressure arising from PE administrations in the presence of CCBs were recorded and data analyses were done.

Results
PE-induced increases in perfusion pressure attenuated significantly in the presence of 5 and 10 μM of nifedipine and 1, 5, and 10 μM of amlodipine. Increases in perfusion pressure arising from PE (100 and 200 μM) in the presence of amlodipine (1, 5, and 10 μM) was significantly less than that in the presence of nifedipine (1, 5, and 10 μM). Calculated EC_{50} value of amlodipine for inhibition was significantly lower than that of nifedipine. Based on the EC_{50} values, the potency of amlodipine in inhibiting PE-induced responses is significantly higher compared to nifedipine.

Conclusion
The potency of amlodipine in inhibiting PE-induced increments in renal perfusion pressure is significantly higher compared to nifedipine.

Keywords: Amlodipine, Isolated rat kidney, Nifedipine

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Introduction

Calcium-channel blockers (CCBs) have a significant role in the treatment of several cardiovascular and non-cardiovascular disorders (1, 2). These compounds are of special significant in the therapy of hypertension, angina pectoris and other cardiovascular disorders (1). These drugs also preserve or improve renal function in patients with essential hypertensive renal disease or diabetic renal disease. Studies in animal models of hypertension and in hypertensive humans have demonstrated reduction in renal vascular resistance, and preservation or enhancement of renal plasma flow (RPF), suggesting a relative renal selective action of these agents (3, 4).

Among the classes of CCBs, dihydropyridine derivatives are widely used because of their potent vasodilating activity and weak cardiodepressant action (5). Dihydropyridines (DHPs) reduced Ca^{2+} entry via L-type voltage gated Ca^{2+} channels (LVGCs) in vascular smooth muscle cells. LVGCs mediate the depolarization-induced Ca^{2+} entry and consequent vasoconstriction. Therefore DHPs markedly reduce myogenic tone which plays an important role in regulating blood flow and blood pressure (6).

The prototype of DHPs, nifedipine, is clinically effective but has a number of undesirable pharmacokinetic and pharmacodynamic properties, which include a rapid onset of vasodilating action, a short half life and side effects such as reflex tachycardia, flushing, headache and dizziness (7, 8). Several newer 1,4-DHP analogues, which provide a reduction in adverse effects and exhibit more stable pharmacokinetics, have been developed and include amlodipine, lacidipine, nicardipine, nitrendipine (9).

Amlodipine (2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethixycarbonyl-5-2-methoxycarbonyl-6-methyl-1,4 dihydropyridine) is a dihydropyridine derivative which is structurally related to other DHPs such as nifedipine but its chemical properties differ from typical DHPs (10).

In vivo, the cardiovascular effects of amlodipine are largely due to vasodilation. This action of amlodipine is relatively slow in onset and of long duration (11). Similar vasorelaxant properties have been observed in vitro (10). Amlodipine has been shown to block L-type Ca^{2+} channels selectively (12).

Previous researches have shown that under angiotensin II or norepinephrine-induced vasoconstrictor tone, both DHP classes (eg, nifedipine, nisoldipine, and amlodipine) and benzodiazepine class (eg, diltiazem) CCBs caused greater increases in glomerular filtration rate (GFR) than those in RPF. These observations indicated that these CCBs act predominantly on renal pre-glomerular vessels (13-16).

In this study, effects of nifedipine and amlodipine on renal perfusion pressure in the isolated perfused rat kidney were investigated and compared with each other. Using this model it is possible to evaluate accurately the renal vasoconstriction modulation by CCBs elicited by different types of vasoconstrictor agents.

Materials and Methods

Male wistar rats (300±10 g) having free access to food and tap water were anaesthetized with intraperitoneal injection of urethane (1.5 g/kg). After opening of the abdominal cavity by a ventricular incision, heparin was injected into the vena cava (500 U/kg) and renal artery was cannulated using a 20 G hypodermic needle with a polished tip via the superior mesenteric artery without disruption of flow. The ligatures around the cannula were tied and the kidney was removed and placed in a thermostated glass chamber contained perfusion medium at 37 °C. The perfusion medium consisted of Krebs solution with the following composition: NaCl (118 mM), KCl (4.8 mM), MgSO$_4$, 7H2O (1.2 mM), CaCl$_2$ (2.5 mM), KH$_2$PO$_4$ (1.2 mM), NaHCO$_3$ (25 mM) and Glucose (10 mM) and equilibrated with 95℅ O$_2$ and 5℅ CO$_2$ (5).

Perfusion was started *in situ* with a constant flow at 85-95 mmHg. Perfusion medium was fed to the kidney by means of a peristaltic pump (LKB, Varioperpex II) through PTFE tubings (Pharmacia Biotech, 18-8207-01).

The renal artery pressure was monitored through a pressure transducer (Beckman, 4-327)

situated parallel to perfusion cannula and was recorded on a Beckman polygraph (R-612).

The drugs were injected by using a load-inject valve placed in the perfusion circuit, just before the kidney. The valve provides two flow paths. In the load position, the valve connects the pump directly to the kidney. Using a syringe, the sample drug is injected into a loop with a small defined volume. In the inject position, the sample in the loop is directly inserted into the flow path of the perfusate and does not alter the baseline pressure (17).

In the method we used, following the establishment of isolated kidney perfusion and 30 min equilibration, first the renal vasculature was constricted by injection of 0.5, 2, 5, 10, 50, 100, and 200 μM phenylephrine (α-agonist) to the perfusion line via load-inject pump. Changes in baseline pressure due to phenylephrine (PE) injection were recorded on a physiograph trace. Then DHPs calcium channel blockers nifedipine, and amlodipine (1, 5, and 10 μM) prepared in perfusion medium was fed to the kidney by a peristaltic pump for 30 min. Finally alterations in perfusion pressure from baseline due to PE administration via load-inject pump in the presence of CCBs were recorded. Of note, PE concentrations have been selected based on concentration response-curve and in higher concentrations of PE (>200 μM) the curve took a plateu shape.

The DHPs were dissolved in dimethylsulfoxide (DMSO). Subsequent dilutions were made in krebs solution.

Maximum increase in baseline perfusion pressure arising from PE injection in the absence of CCBs was considered as 100% of response. PE-evoked increments in renal perfusion pressure in the absence or presence of CCBs were expressed as percent increase in perfusion pressure. Data (percent increments in perfusion pressure) were analysed by ANOVA using Nested design in MINITAB software.

EC_{50} was used to measure the potency of these compounds by quantifying the inhibition of perfusion pressure arising from PE as the primary response as done by other authors (18). The observed increases in the perfusion pressure in the presence of increasing concentrations of DHPs were transformed into percent inhibition of perfusion pressure responses. These data also were analyzed as mentioned before. The EC_{50} values of CCBs were calculated by fitting the data points into logistic model curve with r values of not less than 0.94 using the Curve Expert 1.3 software and were analysed using Student's *t*-test. All data were expressed as means±SEM. A *P*-value< 0.05 was considered to be significant.

The animal experiments were in accordance with international guidelines and approved by the ethical committee of the Iranian University of Medical Sciences, Teheran, Iran.

Results

Our results indicated that the increases in perfusion pressure arising from PE administration attenuated significantly in the presence of 5, and 10 μM of nifedipine (Figure 1).

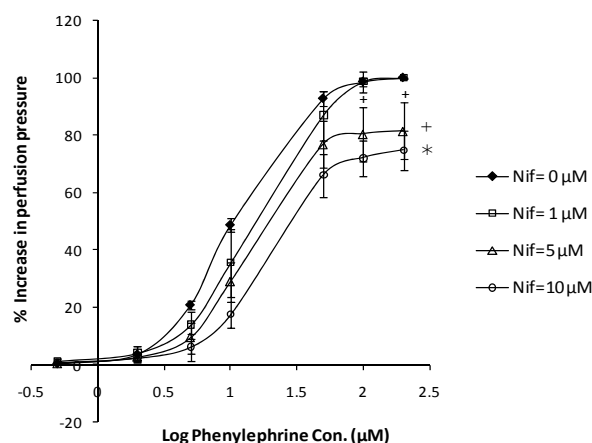Figure 1. The inhibitory effect of nifedipine on PE-induced increases in perfusion pressure. Values are means±SEM from 5 kidneys. Percent increases in perfusion pressure arising from PE decreased significantly in the presence of increasing concentrations of nifedipine (5 μM +*P*<0.001; 10 μM ∗*P*<0.0001, vs. control group). Nifedipine at 5 μM shows significant difference with nifedipine at 1 μM (+*P*<0.001, error bars). PE-evoked increments in renal perfusion pressure in the presence or absence of CCBs were expressed as percent increase in perfusion pressure. The signs across the curves show significant differences vs. control groups.

PE-induced increases in perfusion pressure attenuated significantly in the presence of 1, 5, and 10 μM amlodipine (Figure 2). Amlodipine at 1 μM and 5 μM showed significant differences with each other (*P*<0.001).
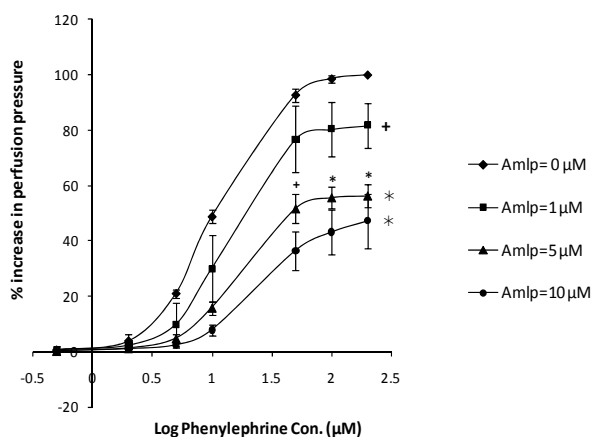
Figure 2. The inhibitory effect of amlodipine on PE-induced increases in perfusion pressure. Values are means±SEM from 5 kidneys. PE-induced increments in perfusion pressure attenuated significantly in the presence of increasing concentrations of amlodipine (+ *P*<0.001, *＊P*<0.0001, vs. control group). Amlodipine concentrations of 1 μM, and 5 μM differ significantly from each other (+*P*<0.001, *P*<0.0001, error bars). The signs across the curves show significant differences vs. control groups.

A sample trace representing the inhibitory effect of amlodipine on perfusion pressure rise induced by PE injection was shown in Figure 3.
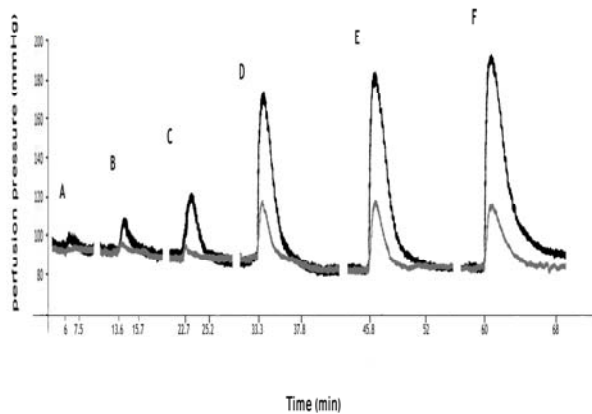

Figure 3. A sample trace representing the inhibitory effect of amlodipine (10 μM) on increases in perfusion pressure induced by increasing concentration of PE [2 μM (A), 5 μM (B), 10 μM (C), 50 μM (D), 100 μM (E), 200 μM (F)]. PE-induced peak pressure rise in the absence of amlp (■), PE-induced peak pressure rise in the presence of 10 μM amlp (□).

Our findings showed that PE-induced increments in perfusion pressure in the presence of amlodipine at all three administered doses, was significantly lower than that of nifedipine (1, 5 μM *P*<0.001; 10 μM *P*<0.0001; Figures 4- 6).
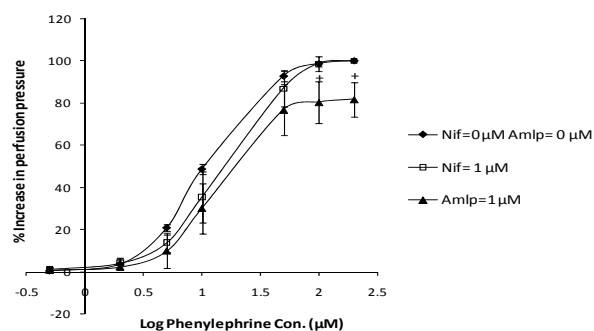

Figure 4. The inhibitory effects of amlodipine and nifedipine (1 μM) on PE-induced increases in perfusion pressure. Values are means±SEM from 5 kidneys. Amlodipine (1 μM) significantly decreased PE-induced increments in perfusion pressure (*P*<0.05). The difference between amlodipine (1 μM) and nifedipine (1 μM) was significant in this respect (+*P*<0.001).
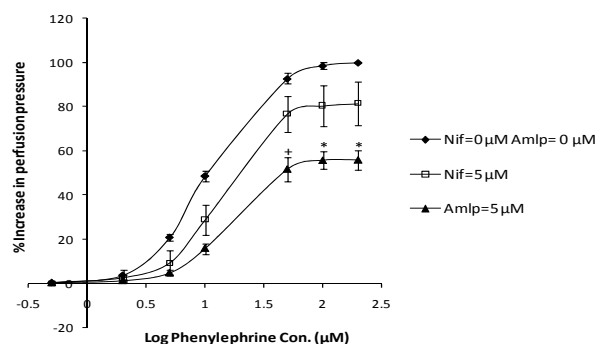

Figure. 5. The inhibitory effects of amlodipine and nifedipine (5 μM) on PE-induced increases in perfusion pressure. Values are means±SEM from 5 kidneys. nifedipine and amlodipine (5 μM) significantly decreased PE-evoked responses (Nif. *P*<0.001; amlp. *P*<0.0001). The difference between amlodipine (5 μM) and nifedipine (5 μM) was significant in this respect (+*P*<0.001, *P*<0.0001), reflecting that inhibitory effect of amlodipine (5 μM) on responses induced by PE (50, 100, 200 μM) was greater than that of nifedipine.

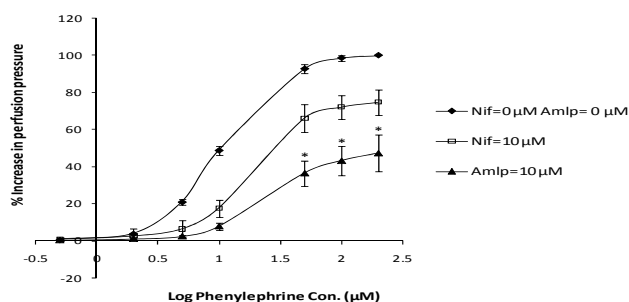Figure 6. The inhibitory effects of amlodipine and nfedipine (10 μM) on PE-induced increases in perfusion pressure. Values are means±SEM from 5 kidneys. Nifedipine and amlodipine (10 μM) significantly inhibited PE-induced responses (*P*<0.0001). The data analysis indicated that nifidipine (10 μM) and amlodipine (10 μM) were significantly different from each other (*P*<0.0001). Consequently inhibitory effect of amlodipine (10 μM) on responses induced by PE was greater than that of nifedipine (10 μM).

EC_{50} was used to measure the potency of these compounds by quantifying the inhibition of perfusion pressure arising from PE as the primary response as done by other authors (18). The concentration-response curve for DHP-induced inhibition (1-10 µM) exhibited a half maximal response (EC_{50}) of 2.17±0.73, and 4.4±0.32 for amlodipine and nifedipine, respectively (Figure 7). EC_{50} value of amlodipine for inhibition was significantly lower than that of nifedipine, indicating that amlodipine has more potency in inhibiting PE-induced responses as compared to nifedipine ($P<0.001$).
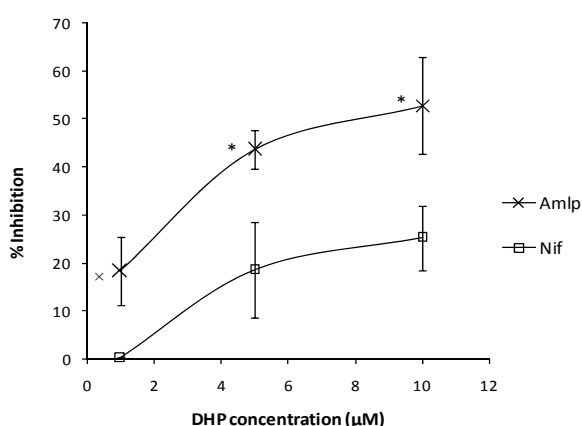

Figure 7. The concentration-response curve for DHP-induced inhibition. Values are means±SEM from 5 kidneys. Data analysis showed that inhibitory effects of increasing concentrations of amlodipine on PE-induced pressure responses (E_{max} of PE) were significantly greater than that of nifedipine (×$P<0.01$, *$P<0.0001$). Calculated EC_{50} of amlodipine for inhibition was significantly lower than that of nifedipine ($P<0.001$).

Discussion

Calcium channel blockers (CCB) are clinically useful vasodilators, used widely in the treatment of hypertension. These agents are reported to preserve or even increase renal blood flow in the face of reduction in systemic blood pressure (4). Previous studies have shown that amlodipine a CCB of DHP chemical class, inhibited calcium currents in single vascular smooth muscle cells isolated from rabbit ear artery (10). It was found that amlodipine selectively blocks L-type Ca^{2+} channels (12, 19).

Present study indicated that amlodipine, a dihydropiridine derivative inhibited PE-induced increments in perfusion pressure

in isolated perfused rat kidney. Nifedipine also reduced responses to PE. DHPs reduced Ca^{2+} entry via L-type voltage gated Ca^{2+} channels (6). Amlodipine is shown to selectively block L-type Ca^{2+} channels (12).

A recent study implies that Ca^{2+} entry via $Ca_v1.2$ LVGCs is obligatory for myogenic tone and nifedipine inhibits myogenic tone exclusively by blocking LVGCs and not other Ca^{2+} entry channels. It also implies that the DHP receptors on $Ca_v1.2$ channels are necessary and sufficient for nifedipine block of VGCs in mesenteric small arteries (6).

Our findings showed that the inhibitory effect of amlodipine on PE-induced increases in perfusion pressure was greater than that of nifedipine. Based on the EC_{50} values, amlodipine is more potent in inhibiting response to PE compared to nifedipine. Amlodipine has more vasoselectivity property compared to nifedipine (20). Tissue selectivity improves pharmacokinetic parameters and gives some additional properties to the molecule (21).

It has been reported that dihydropiridine-class (nifedipine and amlodipine) caused predominant vasodilation of the afferent arteriole in isolated perfused hydronephrotic kidney (22). Since the traditional calcium antagonists (e.g. nifedipine) act on L-type voltage dependent calcium channel and these channels prevail predominantly at the afferent arteriole (23, 24). The effects on the efferent arteriole by these calcium antagonists are most likely attributed to additional actions of these antagonists, but not due to the class affects of these agents (4).

Amlodipine is a longer-acting CCB, with a longer *in vivo* elimination half-life, than nifedipine (25). Although it is a dihydropyridine, the action of amlodipine may also involve binding to non- dihydropyridine sites on the voltage-operated calcium channel. Ligand binding studies indicate that amlodipine interacts not only with a dihydropyridine binding site but also with a site which binds to diltiazem (26) and another that binds to phenylalkylamines such as (-)-D888 (27).

Amlodipine is demonstrated to posses an inhibitory action on both L-type and N-type

Ca channels (28). It is also reported to cause a substantial dilation of efferent as well as afferent arterioles in the *in vivo* hydronephrotic kidney (29).

However amlodipine vasodilatory properties have been ascribed to it's inhibitory action on voltage-gated calcium channels in vascular smooth muscle. Some studies have suggested that amlodipine has an additional vasodilatory effect through stimulation of nitric oxide (NO) release from vascular endothelium. It was found that amlodipine unlike nifedipine increases nitrite production from healthy canine coronary microvesseles *in vitro*, expressing an increase in NO biosynthesis (30). A recent study has demonstrated that the vasorelaxant effects of amlodipine unlike nifedipine are partly dependent on NO generation in rabbit femora artery *in vivo*. This effect of amlodipine occurs through β_2 receptor activation and may be related to an increase in local bradykinin (25). These findings indicate that this compound acts as two enantiomers, one with the ability to block L-type Ca channels whilst the other has NO releasing property (31).

Conclusion
The present study demonstrates that amlodipine is more potent in inhibiting PE-induced increments in renal perfusion pressure compared to nifedipine. In other words, amlodipine has more ability to decrease renal microvascular resistance compared to nifedipine.

Acknowledgment

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