Spasmolytic Effect of Efonidine Hydrochloride in Isolated Canine Coronary Artery: Comparison with the Effects of Nifedipine and Nisoldipine

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Spasmolytic effects of efonidine hydrochloride (efonidine) on high K+-, U46619- and 3,4-diaminopyridine (3,4-DAP)-induced contractions were evaluated in isolated canine coronary artery, and were compared with the effects of nifedipine and nisoldipine. Efonidine (0.3—30 nm), nifedipine (1—300 nm) and nisoldipine (0.1—100 nm) each relaxed the contractions induced by high K+ and U46619. However, relaxation produced by efonidine was slower than that produced by nifedipine or nisoldipine. The rank order of potency of these drugs for U46619-induced contraction was efonidine > nisoldipine > nifedipine, whereas in high K+-induced contraction, it was nisoldipine > efonidine > nifedipine. Thus, the relaxing effect of efonidine on U46619-induced contraction appeared to be more potent than its effect on high K+-induced contractions, when compared with the effects of nifedipine and nisoldipine. These three drugs also suppressed 3,4-DAP-induced rhythmic contractions. However, a marked time-dependent increase in potency was only observed for efonidine, and was similar to its time-dependent effect on high K+- and U46619-induced contractions. Efonidine did not change the contraction cycle length whilst suppressing the peak contractions. On the other hand, lower concentration of nifedipine at 3 nm and nisoldipine at 1 nm significantly shortened the cycle length. These results suggest that efonidine may be an effective agent for the treatment of angina pectoris. The high potency of efonidine for U46619-induced contractions will provide some advantages in the clinical use of this compound on thromboxane A2-mediated coronary vasoreactivity.

Key words: efonidine hydrochloride; calcium ion antagonist; high potassium; U46619; 3,4-diaminopyridine; coronary artery

Several calcium (Ca2+) channel blockers, particularly 1,4-dihydropyridine derivatives, have been shown to relax coronary arteries as well as peripheral resistance vessels in vitro and in vivo, and have subsequently been used in the treatment of angina pectoris and hypertension. Some of the first 1,4-dihydropyridine related compounds developed, such as nifedipine, have been known to dilate the vessels rapidly, causing reflex tachycardia which induces an increase in oxygen demand. This characteristic causes side effects and is undesirable in a therapeutic agent. Consequently, a large number of 1,4-dihydropyridines with slow onset actions have been developed to avoid the problem of reflex tachycardia.

Efonidine hydrochloride (efonidine), (±)-2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4(3-nitrophenyl)-3-pyridinecarboxylate hydrochloride ethanol, is a 1,4-dihydropyridine derivative with a voltage-dependent Ca2+ channel blocking action that shows slow-onset and long-lasting properties. Efonidine is reported to show potent antihypertensive effects in experimental-hypertension animal models, and is already used in the treatment of hypertension. The aim of the present study was to assess its spasmytic effect on isolated canine coronary artery, and to differentiate the characteristics of efonidine from those of nifedipine and nisoldipine.

We used U46619, a stable analog of thromboxane A2 (TXA2), 3,4-diaminopyridine (3,4-DAP), a potassium channel blocker, and 80 mM K+ solution (high K+) as spasmogenetic agents.

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MATERIALS AND METHODS

Preparation Mongrel and beagle dogs weighing 9—15 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and killed by bleeding from the femoral artery. The heart was rapidly removed from each animal and immediately immersed in ice-cold Krebs-Henseleit solution (KHS) of the following composition (in mm): NaCl 118, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, CaCl2 2.5 and glucose 11. The circumflex and anterior descending branch of the left coronary arteries were carefully isolated and cleaned of adhering fat and connective tissue. The arteries were cut into 2 mm lengths and suspended in an organ bath containing 10 mL KHS under a resting tension of 1 g. The solution was continuously aerated with 95% O2, 5% CO2 and kept at 37°C (pH 7.4). Isometric tension was measured by means of a force-displacement transducer (TB-651T, Nihon Kohden, Tokyo, Japan) connected to a carrier amplifier (EF-601G, Nihon Kohden, Tokyo, Japan) and was recorded on a pen-recorder (Recti-Horiz-8K, NEC San-Ei, Tokyo, Japan).

Experimental Protocol Preparations were equilibrated for at least 2 h prior to experiments. In the experiment using high K+ and U46619, the preparations were conditioned by exposure to high K+ KHS (80 mm, NaCl was replaced by an equimolar amount of KCl) or 100 mm U46619, after the equilibration period. When the sustained contraction had stabilized, each concentration of each test drug, was added to the bath. The resulting relaxation responses were observed for 3 h. At the end of the measurement period, 100 μM papaverine was added to determine the maximum relaxation of each preparation.
Relaxation responses were calculated as percentages of the maximum relaxation.  
In the experiment using Ca$^{2+}$-free KHS, the preparation was incubated in normal KHS for 5 h with a test drug.  
then, normal KHS was replaced with Ca$^{2+}$-free KHS containing the drug; 10 min later, U46619 (100 nm) was added.  
The peak tension of the transient contraction was calculated as a percentage of a high K$^+$ (50 mm)-induced contraction.

In the experiment using 3,4-DAP, 10 nm 3,4-DAP was added to induce rhythmic contractions.  
The test drug was added once a stable amplitude and cycle length were observed, and the inhibitory effect was observed for 3 h.  
To avoid decomposition of the drugs, all experiments were carried out under darkened conditions.

Drugs Used Efonidipine and nisoldipine were synthesized by Nissan Chemical Industries, Ltd. (Tokyo, Japan).  
Nifedipine, 3,4-DAP, U46619 and other chemicals were purchased from commercial sources.

Dihydropyridines were dissolved in 5% polyoxyethylene 40 hydrogenated castor oil (HCO-60, Japan Surfactant,  
Tokyo, Japan).  U46619 was dissolved in ethanol to a concentration of 1 mm as a stock solution.  
These solutions were diluted with distilled water.  Other drugs or chemicals were dissolved in distilled water.  
HCO-60 or ethanol at their final concentrations did not affect the tissue responses (data not shown).

Statistical Analysis IC$_{50}$ values were calculated as the concentrations of drugs required to produce 50%  
relaxation or suppression by linear regression analysis  
following log-logit transformation, then represented with  
95% confidential limits.  The other data are expressed as  
the mean ± S.E.M.  Statistical differences were calculated  
by one-way analysis of variance with repeated measures,  
followed by Dunnnett's test.  A probability less than 0.05  
was considered to be statistically significant.

RESULTS

Response to High K$^+$-Induced Contraction Figure 1  
shows the time course of relaxation induced by efonidipine,  
nifedipine and nisoldipine in a high K$^+$-contracted artery.  
Efonidipine (0.3—30 nm) produced a slowly developing  
relaxation in a concentration-dependent manner which  
required more than 3 h to reach equilbrium.  By contrast,  
nifedipine (1—30 nm) and nisoldipine (0.1—3 nm) rapidly  
relaxed the high K$^+$-contracted artery; the relaxation  
response reached equilibrium 30 to 60 min after addition  
of the drug.  IC$_{50}$ values obtained 1 or 3 h after addition  
of the drugs are presented in Table 1.  Based on these  
values, the potency of efonidipine 1 h after its addition  
was similar to that of nifedipine, and was 10 times lower  
than that of nisoldipine.  However, at 3 h after drug  
addition, the potency of efonidipine had increased and it  
was more potent than nifedipine.  The rank order of  
potency 1 h after the addition was nisoldipine > nifedipine  
> efonidipine.  Prolonging the incubation time altered the  
rank order of potency to nisoldipine > efonidipine > nifedipine.

Response to U46619-Induced Contraction Efonidipine  
(0.3—30 nm) relaxed U46619-induced contractions in a  
concentration-dependent manner.  Nifedipine (3—300 nm)  
and nisoldipine (1—100 nm) also relaxed U46619-induced contractions (Fig. 2).  In comparison with the relaxation  
of high K$^+$-induced contractions, relatively higher concentrations of the drugs were required to relax U46619- 
induced contractions (Table 1).  The potency of efonidipine  
was approximately 4 times higher than that of nifedipine,  
and 2 times lower than that of nisoldipine, 1 h after drug  
addition.  However, 3 h after drug addition the potency  
of efonidipine had increased and was nearly equal to that  
of nisoldipine, and was 10 times higher than that of  
nifedipine.  Prolonging the incubation time altered the rank  
order of potencies from nisoldipine > efonidipine > nife- 
dipine to efonidipine > nisoldipine > nifedipine.  Thus,  
the relaxation effect of efonidipine on U46619-induced  
contraction was greater than those of the other dihydro- 
pyridines tested.

The U46619 (100 nm) induced contraction in Ca$^{2+}$-free  
KHS containing 1 mm EGTA was 21.8±3.0% of the  
maximal high K$^+$ (50 mm)-induced contraction in the  
presence of Ca$^{2+}$.  As shown in Fig. 3, pretreatment  
with efonidipine for 5 h did not affect U46619-induced  
contraction, nor did preincubation with the other

Fig. 1. Time Course for Relaxation Induced by Efonidipine, Nifedipine  
and Nisoldipine on High K$^+$ (80 mm)-Induced Contractions in Isolated  
Canine Coronary Arteries.  
Each point represents the mean ± S.E.M. of five to eight experiments.
dihydropyridines.

3,4-DAP-Induced Rhythmic Contraction Representative recordings of the effects of the three drugs on 3,4-DAP-induced rhythmic contractions are shown in Fig 4. The addition of 10 mM 3,4-DAP gradually produced these contractions. Addition of vehicle did not influence the tension or cycle length, and constant rhythmic contractions were observed for 3 h after the addition. Efondipine (3—300 nM) suppressed peak contractions in a concentration-dependent and time-dependent manner, but the suppression did not reach equilibrium level even 3 h after addition; the IC$_{50}$ value decreased from 103 nM 1 h after efondipine addition to 27.8 nM after 3 h. Nifedipine (1—100 nM) and nisoldipine (0.1—10 nM) also suppressed 3,4-DAP-induced contractions with IC$_{50}$ values of 3.79 nM and 0.81 nM, respectively; however, significant time-dependent increases in potency were not observed (Table 1). As shown in Fig. 5, efondipine did not change the contraction cycle length, even at high concentrations, whereas lower concentration of nifedipine and nisoldipine significantly shortened the cycle length.

DISCUSSION

The present results showed that efondipine relaxed high K$^+$-induced contractions of canine coronary artery in a concentration-dependent manner. The onset of action of efondipine was much slower than that of nifedipine and nisoldipine, in agreement with previous observations using isolated rabbit aorta.15 These effects are explained by the slow association and slow dissociation of efondipine with dihydropyridine binding sites.17 Thus, slow onset of action of this drug in dog coronary artery also seems to be

![Graph of Efondipine Relaxation](image)

![Graph of Nifedipine Relaxation](image)

![Graph of Nisoldipine Relaxation](image)

Fig. 2. Time Course for Relaxation Induced by Efondipine, Nifedipine and Nisoldipine on U46619 (100 nM)-Induced Contractions in Isolated Canine Coronary Arteries

Each point represents the mean±S.E.M. of four to eight experiments.

![Graph of Contractions](image)

Fig. 3. Effects of Efondipine, Nifedipine and Nisoldipine on U46619 (100 nM)-Induced Transient Contractions in Ca$^{2+}$-Free KHS (containing 1 mM EGTA)

Each column and vertical bar represent the mean±S.E.M. of four experiments.

Table 1. IC$_{50}$ Values of Efondipine, Nifedipine and Nisoldipine on High K$^+$-, U46619- and 3,4-Diaminopyridine-Induced Contractions in Isolated Canine Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>K$^+$</th>
<th>U46619</th>
<th>3,4-Diaminopyridine</th>
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<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>3 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Efondipine</td>
<td>5.4 (3.1—9.3)</td>
<td>1.6 (1.1—2.2)</td>
<td>19.5 (10.2—37.2)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>4.9 (3.8—6.5)</td>
<td>5.8 (3.4—9.8)</td>
<td>79.4 (39.8—158)</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>0.52 (0.41—0.69)</td>
<td>0.50 (0.40—0.63)</td>
<td>8.32 (5.13—13.8)</td>
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<td></td>
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<td>103 (57.4—183)</td>
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<td></td>
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<td>3.79 (2.53—5.69)</td>
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<td>0.81 (0.47—1.39)</td>
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Figures in parentheses represent 95% confidence limits.
attributable to its slow association and dissociation with dihydropyridine binding sites.

All the Ca\textsuperscript{2+} channel blockers tested in this study relaxed canine coronary arteries pre-contracted with U46619 in a concentration-dependent manner. Kamuru et al.\textsuperscript{9} reported that the contraction of isolated rabbit aorta induced by TXA\textsubscript{2} analog depends in part on extracellular Ca\textsuperscript{2+} influx through membrane voltage-dependent Ca\textsuperscript{2+} channels. Therefore, inhibitory effects of Ca\textsuperscript{2+} channel blockers seemed to be due to the effect of these compounds on voltage-dependent Ca\textsuperscript{2+} channels. However, the rank order of potency of the three drugs for the relaxation of U46619-induced contractions was different from that for the relaxation of high K\textsuperscript{+}-induced contractions. The potency of efonidipine in relaxing U46619-induced contractions was equipotent with nilosdipine, but it was less potent than nilosdipine in relaxing high K\textsuperscript{+}-induced contractions. These results suggest that in addition to its voltage-dependent Ca\textsuperscript{2+} channel blocking effect, efonidipine also relaxes U46619-induced contractions by another vasorelaxant mechanism(s).

To study the mechanism(s) of the inhibitory effect of efonidipine on U46619-induced contraction, we tested the drug’s abilities to inhibit the release of Ca\textsuperscript{2+} from intracellular storage sites and to bind TXA\textsubscript{2}/PGH\textsubscript{2} receptors, because Bay K 8644, a dihydropyridine derivative, has been reported to antagonize the TXA\textsubscript{2} receptor competitively.\textsuperscript{10} However, efonidipine did not show inhibition of contractions mediated by Ca\textsuperscript{2+} release from intracellular stores nor TXA\textsubscript{2} receptor antagonistic action (data not shown). Thus, these mechanisms do not appear to be responsible for the selectivity of efonidipine relaxation of TXA\textsubscript{2}-mediated contractions.

Uchida\textsuperscript{11} reported that exposure of isolated canine coronary artery to 3,4-DAP causes rhythmic contractions similar to those observed in human coronary arteries. Although the inhibitory effect of efonidipine on high K\textsuperscript{+} contractions was more potent than that of nilosdipine when the incubation time was prolonged, it was still less potent than nilosdipine under the 3,4-DAP exposure despite prolongation of the incubation time. Thus, the potency of efonidipine was not the same as for high K\textsuperscript{+} contractions. We only observed for 3 h after addition of drugs in the present study, and by that time the suppression response of efonidipine had not reached equilibrium level (Fig. 4). Therefore, it is likely that efonidipine may show comparable potency with that in high K\textsuperscript{+} contractions if incubation time is prolonged for 6 h, for instance. 3,4-DAP depolarizes the cell membrane rhythmically by decreasing K\textsuperscript{+} conductance, which increases Ca\textsuperscript{2+} influx into the cell through voltage-dependent Ca\textsuperscript{2+} channels, which in turn triggers the rhythmic contractions.\textsuperscript{11} The duration of depolarization induced by 3,4-DAP was thought to be shorter than a sustained cell membrane depolarization under high K\textsuperscript{+} conditions.\textsuperscript{12} The duration of open and inactivated states of Ca\textsuperscript{2+} channels were also thought to
Efonidipine showed characteristic action compared with nifedipine and nisoldipine. It did not shorten contraction cycle length while inhibiting the contractions, whereas nifedipine and nisoldipine did. It has been reported that Ca\(^{2+}\) channel blockers, not only nifedipine but also diltiazem and verapamil, cause increases in contraction frequency whilst strongly inhibiting the contractions.\(^{13}\) Although the underlying mechanisms remain unknown, this characteristic effect of efonidipine would be beneficial in treatment of angina pectoris.

In conclusion, the present study demonstrates that efonidipine has a vasorelaxant potency similar to those of other dihydropyridines, and that it has characteristic effects on U46619- and 3,4-DAP-induced contractions of canine coronary artery. Thus, we believe efonidipine would be an effective agent in the treatment of angina pectoris. A high potency for U46619-induced contractions may provide some advantages in the clinical use of this compound on TXA\(_2\)-mediated coronary vasoconstriction.

REFERENCES