Iranian Journal of Basic Medical Sciences
Vol. 11, No. 3, Autumn 2008, 159-165
Received: May 15, 2008; Accepted: Oct 20, 2008

IJBMS

Original article

Effects of 4-(2-Alkylthio-1-benzyl-5-imidazolyl)-Dihydropyridines on the Isolated Rat Colon and Right Atrium Contractility

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Abstract

Objectives

In order to provide a pharmacological profile for some newly synthesized dihydropyridines, we investigated their effects on the isolated rat colon segments and the isolated rat atrium contractility. The tested compounds include alkyl ester analogues of nifedipine, in which the ortho-nitrophenyl group at position 4 is replaced by 2-alkylthio-1-benzyl-5-imidazolyl substituent, and nifedipine as a positive control substance.

Materials and Methods

Isolated rat colon and atrial tissues were prepared. Rat colon was contracted with 80 mM KCl, and maximum response was recorded (100%). After washing tissue with Krebs solution it was preincubated with different concentrations of test compounds and again KCl was added and percent change in contraction was calculated. Spontaneous contractions and its frequency for colon and atrium before and after addition of test compounds were also recorded and percent change was calculated. Nifedipine (10^{-8} -10^{-5} M) was used as positive control at all experiments.

Results

The compounds showed similar effects to that of nifedipine on the isolated rat colon. The potency of these analogues with concentration range 10^{-5} to 10^{-4} M was compared to potency of nifedipine which was effective at 10^{-8} to 10^{-5} M (P<0.01). However, unlike nifedipine, the test compounds exerted significant positive inotropic effect on the isolated rat atrium ($P < 0.01$). Our observations suggest that these analogues of nifedipine selectively enhance contractility of heart muscle while causing relaxation of intestinal smooth muscle.

Conclusion

These compounds may serve as valuable probes to develop novel dihydropyridines with dual smooth muscle relaxant effect and positive inotropic action.

Keywords: Colon, Dihydropyridines, Heart Atria, Myocardial Contraction, Rat

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Introduction

1, 4-Dihydropyridine calcium channel antagonists are an important class of drugs which induce relaxation of vascular smooth muscle, preferentially in arteries, and display a negative inotropic effect on isolated cardiac muscle (1). Some of these drugs have selectivity for the vessels and other type of smooth muscles. Changes in the substitution pattern at the C3, C4, C5 positions of nifedipine (Figure 1) alter activity and tissue selectivity (2-8). Newer dihydropyridines with vascular selectivity such as felodipine (2) which exhibit a minimal negative inotropic effect are useful for the treatment of hypertension and spasmodic disorders (9-11).

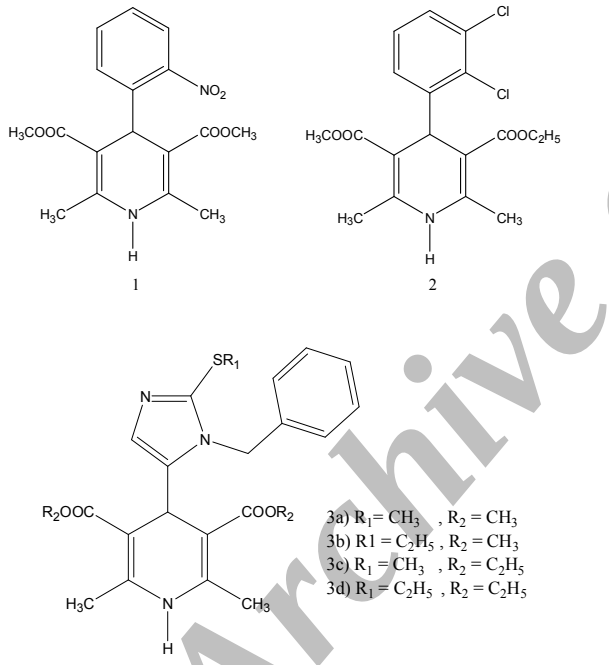

Figure 1. Structure of nifedipine (1), felodipine (2), analogues 3a-d

It has been documented that a C4 imidazole substituent led to active compounds as calcium channel antagonist (12-18).

Previously, we reported synthesis of 1, 4-dihydro-2, 6-dimethyl-4-(2-alkylthio-1-benzyl-5-imidazolyl)-3, 5-pyridine dicarboxylates 3a-d (17). As search was going on for finding more selective compounds with inotropic and spasmolytic effects, it became of interest to evaluate the effects of four analogs of nifedipine 3a-d synthesized in our laboratory on the isolated rat colon and the isolated rat atrium contractility.

Materials and Methods

Animals

Male Wistar rats (200–250 g) were obtained from a random bred colony in the animal house of Mashhad University of Medical Sciences. Animals were housed in colony room 12/12 hr light/dark cycle at 21±2 °C and had free access to water and food. Handling and care of animals met the guidelines of the Ethics Committee for the use of experimental animals for research at Mashhad University of Medical Sciences. All efforts were made to minimize the number and suffering of animals used in this study.

The isolated rat colon preparation

Animals were killed by cervical dislocation. The abdominal cavity was opened by a mid-line incision through the linea alba and the ascending colon (immediately adjacent to the caecum) was rapidly removed and immersed in Krebs solution. A segment of colon (2 cm) was removed and a thread was attached to each end of the preparation. Tissue was mounted under l g tension in 10 ml tissue bath containing Krebs solution ready for isometric recording (using an isometric transducer connected to an Oscillograph 400 MD/ 2). The composition of Krebs solution was as follow (mM): NaCl 118.4, KCl 4.7, $MgSO_4$ H_2O 1.2, KH_2PO_4, $2H_2O$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, and glucose 11.1 in distilled water. This solution was maintained at 37 ^oC, bubbled with 5% CO_2 and 95% O_2. The preparation was allowed to equilibrate for 30 min before commencing the experiments. The isolated segment of rat colon was contracted with 80 mM KCl, and maximum response was recorded. Then the tissue was washed with Krebs solution thoroughly and after reaching an steady state, was preincubated separately for 5 min with different concentrations (10^{-5} -10^{-4} M) of test compounds and again KCl was added with the same final concentration and maximum responses were

recorded and any change was calculated as a percentage of the control value. Amplitude and frequency of spontaneous contractions of rat colon before and after addition of test compounds were also recorded. Nifedipine (10^{-8} -10^{-5} M) was used as positive control.

The isolated rat right atria

Spontaneously beating right atria were dissected free and pierced by two threads, one of which was hooked through a static platinum loop embedded in an organ bath, the other was hooked through a thread loop terminating at an isometric force transducer. The isometric force transducer was fixed to a laterally constrained stage, which was raised or lowered vertically. Tissues were suspended in 10 ml organ bath containing Krebs solution. This solution was maintained at 37 °C, bubbled with 5% CO_2 and 95% O_2. An optimal tension of 1 g was applied, and preparations were allowed to equilibrate for 60 min at this tension prior to starting experiments. During the equilibration period, the bath solution was replaced every 15 min. Changes in muscle tension were recorded using an isometric transducer and recorded by an Oscillograph 400 MD/ 2. Spontaneous atrium contractions before and after addition of test compounds were recorded and changes were calculated as percentage of control (base-line) values. Nifedipine (10^{-8}-10^{-5} M) was used as positive control.

Synthesis of Drugs

The synthesis of the 1, 4-dihydropyridine derivatives a–d was achieved following the steps reported previously (17). Sodium chloride, potassium chloride, magnesium sulphate, sodium hydrogen carbonate, potassium dihydrogen orthophosphate, D-glucose and calcium chloride were obtained from Merck Laboratories. All compounds 3a-d except nifedipine (dissolved in ethanol) were dissolved in DMSO and then diluted with normal saline, to final concentration of 0.01% V/V. The responses of isolated tissues to different compounds 3a-d were not significantly affected by this concentration of DMSO or ethanol. Nifedipine was provided by Daru-Pakhsh Co., Tehran, Iran as a gift.

Statistical analysis

The data are expressed as mean values±SEM from five experiments and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer to evaluate the significance of differences. The number of experiments (repetitions= n) refers to the number of preparations isolated from different rats. Thus, for example, n= 5 means that those particular set of experiments were performed on tissues isolated from 5 rats.

Results

The effects of 3a-d on the isolated rat colon strips

The isolated rat colon strips exhibited spontaneous contractions with a frequency of 9±0.5 contractions per min. Addition of compound 3d at 4×10^{-5} M reduced the frequency of spontaneous contractions by 70±0.6 % of control (P<0.001, Figure 2).

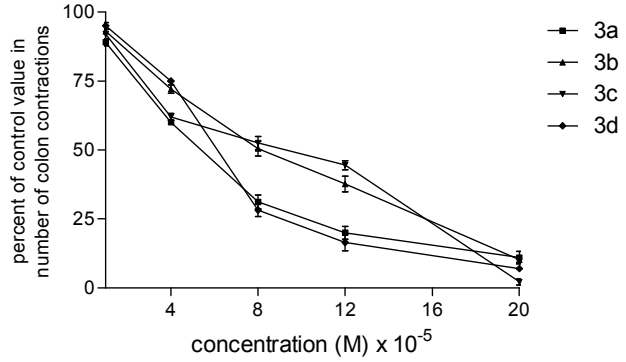

Figure 2. Percent of control value in number of spontaneous rat colon contractions against different concentrations of test compounds 3a-d, data were shown as mean ± SEM. Nifedipine was used at concentrations (M) 5×10^{-8}, 5×10^{-7}, 5×10^{-6} and 5×10^{-5} (data not shown).

Nifedipine at 2.5×10^{-7} M decreased the frequency of spontaneous contractions of the colon by 36±1.3% of control (P< 0.001). Addition of all 3a-d reduced the amplitude of spontaneous contractions in a concentration-dependent manner. For instance, compound 3d at 4×10^{-5} M decreased the amplitude of spontaneous contractions by 75±0.9% of control (P<0.001, Figure 3).

However, this reduction was reversible (recovered after washing with Krebs solution for 20 min). Among the compounds tested, 3d had the strongest inhibitory effect on colon spontaneous contraction (Figure 3). Nifedipine at 2.5×10^{-7} M decreased amplitude of spontaneous contractions of the rat colon by 25±0.64 % of control (P<0.001). Pre-incubation of the isolated colon strips with different concentrations of compounds 3a-d caused a reduction in contractions induced by 80 mM KCl. Preincubation with compound 3d at 4 x 10^{-5} M reduced KCl-induced contraction by 78±0.75% of control (P<0.001, Figure 4). Pre-incubation with 3d had the strongest inhibition on the KCl-induced contractions (P<0.001, Figure 4). Nifedipine at 10^{-7} M decreased KCl-induced contractions of colon by 50±1.2 % of control (P<0.001).

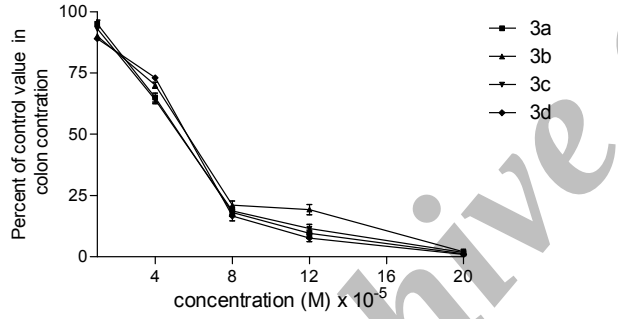

Figure 3. Percent of control value in spontaneous rat colon contractions against different concentrations of test compound 3a-d, data were shown as mean±SEM. Nifedipine was used at concentrations (M) 5×10^{-8}, 5×10^{-7}, 5×10^{-6} and 5×10^{-5} (data not shown).

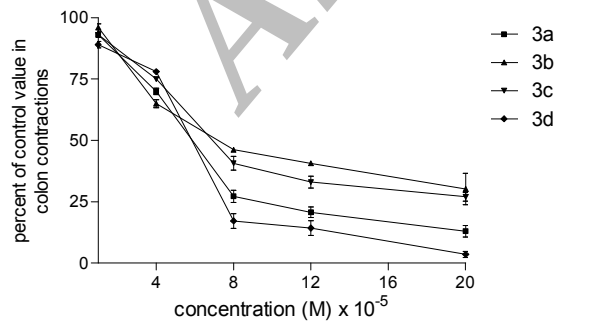

Figure 4. Percent of control value in contraction of rat colon with KCl (80 mM) when pre-incubated with different concentrations of test compounds 3a-d, data were shown as mean ± SEM., Nifedipine was used at concentrations (M) 5×10^{-8}, 5×10^{-7}, 5×10^{-6} and $5x10^{-5}$ (data not shown).

Effects of 3a-d on the isolated rat right atria

In the isolated rat atria, the spontaneous beating was 235±2 (beats per min). All compounds 3a-d reduced the number of atrial beating. For example compound 3d at 6×10^{-5} M reduced number of atrial beating by 46.5±1.9% of control ($P\leq$0.001, Figure 5). Compound 3d caused the highest decrease in atrial beating (P <0.001, Figure 5). Nifedipine at 2.5×10^{-7} M decreased frequency of contractions of rat isolated atrium by 35 ± 1.4 % of control (P<0.001). Addition of different compounds 3a-d to the organ bath caused a significant increase in atrial contractility. Compound 3d at 6×10^{-5} M increased atrial contractility by 152±4.2 % of control (P< 0.001, Figure 6). Compound 3d caused the highest increase in atrial contractility (P< 0.001, Figure 6). Nifedipine in contrast at 2.5×10^{-7}M decreased contraction of atrium by 34±3.2 % of control ($P\leq$ 0.001).

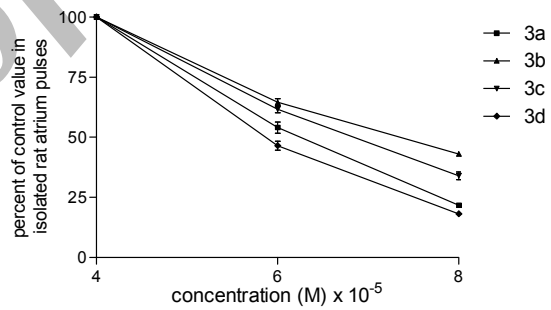

Figure 5. Percent of control in isolated rat atrium pulses against different concentrations of test compounds 3a-d, data were shown as mean ± SEM. Nifedipine was used at concentrations (M) 5×10^{-8}, 2.5×10^{-7} and 5×10^{-7} (data not shown).

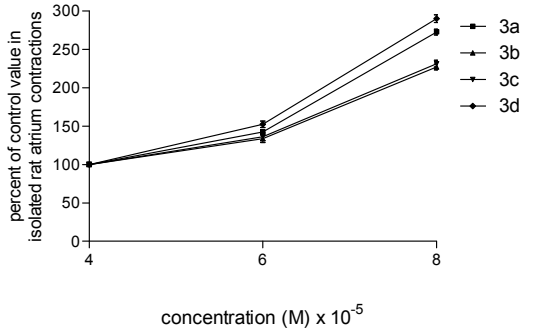

Figure 6. Percent of control value in isolated rat atrium contractions against different concentrations of test compounds 3a-d, data were shown as mean±SEM. Nifedipine at concentrations (M) 5×10^{-9}, 5×10^{-8}, 2.5×10^{-7} and 5×10^{-7}, decreased contractions (data not shown).

Discussion

The present study demonstrates that all four tested analogues of nifedipine exert discriminatory effects on the intestinal smooth muscle and heart muscle contractility. These compounds inhibit depolarization-induced contraction of the isolated rat colon preparations but not that of the isolated rat atrium. In fact, all of these drugs increased heart atrium contractility, an indication of the positive inotropic effect. Perhaps this was the most intriguing observation we had during our investigation. In the case of classic calcium channel blockers dihydropyridines, it is expected to have both negative inotropic and negative chronotropic effects. However, with regard to our test compounds, it appeared that these agents acted in a way similar to that of (-)-cis-diltiazem (19). It seems that our test compounds do not fall in classic L-type calcium channel blocker dihydropyrindines category. It has been shown that (-)-cis-diltiazem had a positive inotropic effect in the electrically stimulated left atrial preparation, and that it also exerted a negative chronotropic effect in the isolated, spontaneously beating rat right atrium at the same concentration-range as that producing the positive inotropic effect (19). Furthermore, calcium channel blockers have a weak positive inotropic action in lower concentrations and a negative inotropic action in higher concentrations. Low concentrations of (+)-cis-diltiazem increase the tension of canine papillary muscle, while high concentrations of the drug decrease it (19). The weak positive inotropic effect of (+)-cis-diltiazem is considered to be due to an action that is unrelated to calcium channel blockade. There are some other compounds such as fentanyl and remifentanil which cause direct negative chronotropic and positive inotropic effects in isolated rat heart (20). Thus, the positive inotropic effect of our test compounds may also be due to an action unrelated to calcium channel blockade. This could be due to an indirect effect through increased systolic ventricular pressure caused by vasodilatation. The mechanism of the positive inotropic effects of these compounds can be explained as follows. The coronary vasodilatation caused by calcium channel blockers results in an increased fiber tension of the myocardium and, an increase of the myocardial contractile strength.

Under basal conditions, all concentrations of 3a-d significantly reduced the amplitude of spontaneous contractions of the proximal portion of rat colon supporting the hypothesis that these drugs may interfere with calcium channel activity. Evaluation of novel compounds effects on potassium chloride induced contraction of the isolated rat colon, is a well established assay for screening calcium channel blocking activity (21). Although, the most likely mechanism of action of compounds 3a-d for decreasing contraction of the isolated rat colon preparations seems to be calcium channel blockade, we cannot exclude some other effects of these compounds on adrenergic pathway and nonadrenergic-noncholinergic system (NANC). Activation of either β-adrenoreceptors (22) or NANC (23) could have an inhibitory effect on colonic contraction and contractility. Also, further direct investigations on the isolated calcium channel currents recorded from colon smooth muscle cells employing patch clamp techniques are required for determining affinities of the channels for these drugs under different resting, open and inactivated states.

Our result on the isolated rat atrium showed that compounds 3a-d had a positive inotropic and negative chronotropic effects. Therefore, in condition such as heart failure these compounds 3a-d could have beneficial effects (24).

It has been shown that dihydropyridine derivatives are selective for voltage-operated calcium channels (25). There is evidence that supports the presence of different subunit structure of calcium channels in colon smooth muscle cell (26). Considering these differences among calcium channels in intestinal smooth muscle and cardiac muscle, the differential effects of the test compounds on contraction of the isolated rat colon and on the isolated rat atrium may be plausible.

Conclusion

The tested compounds have antispasmodic effect on rat colon smooth muscle and positive inotropic effect on rat isolated atrium. Further pharmacological and toxicological studies are required in order to provide a comprehensive profile of these compounds for their prospective use in drug therapy.

Acknowledgments

This work was supported by a grant from Research Council of Mashhad University of Medical Sciences.

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