Vasodilator Effects of *Bis*-Dihydropyridines Structurally Related to Nifedipine

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Abstract: Calcium channel blockers are widely used in therapy for hypertension and angina pectoris, and among these blockers some 1,4-dihydropyridines (e.g. amlodipine, nitrendipine and nifedipine) have had widespread clinical use. In this work we investigated the vascular effects of four *bis*-1,4-dihydropyridines (*bis*-DHPs: 01-04), structurally related to nifedipine, in which a second 1,4-dihydropyridinic moiety was incorporated in the corresponding arylic moiety in *para* and *meta* position. Of these four *bis*-DHPs, the *meta* regioisomers (*bis*-DHP-03 and *bis*-DHP-04; 0.01-3.16 mg kg^{-1}) and nifedipine induced a greater decrease on diastolic and systolic blood pressure than the *para* isomers (*bis*-DHP-01 and *bis*-DHP-02), as shown in two experimental models: normotensive and spontaneously hypertensive rats. Complementarily, *bis*-DHPs action was examined in intact and endothelium-denuded rat aorta, depolarized by KCl [80 mM] in one group and stimulated by noradrenaline ($1x10^{-7}$ M) in another and the corresponding IC_{50} values were obtained ($1.5x10^{-6}$-$2.4x10^{-7}$M). Later, the relaxing action of *bis*-DHP-03,04 and nifedipine on the contraction evoked by Ca^{2+} in K^{+}-depolarized rat aorta was analyzed and the corresponding EC_{50} values for the *meta* isomers and nifedipine were obtained. The results showed a concentration dependent vasodilating activity in both KCl precontracted and noradrenaline stimulated aorta rings. The apparent order of potency with and without endothelium in both experimental models was nifedipine>*bis*-DHP-04>*bis*-DHP-03. The cumulative concentration-effect curves for Ca^{2+} in the presence of the *bis*-DHPs tested show the same potency order.

Unlike nifedipine, the tested compounds are not photosensitive, which makes them more attractive in therapy for hypertension related diseases.

Key Words: Vascular smooth muscle, dihydropyridines, vasodilator, nifedipine, calcium channel blockers, spontaneously hypertensive rats.

INTRODUCTION

Primary hypertension is the most prevalent cardiovascular disorder [1], thus creating the need for effective treatment. In this sense, 1,4-dihydropyridines (1,4-DHPs), also known as Hantzsch esters, a class of calcium channel blocker agents that include the first generation nifedipine [2], have been used for many years in the treatment of hypertension, *angina pectoris*, and in other cardiovascular pathologies [3]. Their mechanism of action is based on the inhibition of the smooth muscle L-type Ca^{2+} channels, thereby decreasing intracellular Ca^{2+} concentration and inducing smooth muscle relaxation [4]. Among this class of cardiovascular agents, some other dihydropyridines [5,6] (e.g. amlodipine and nitrendipine) have also had widespread clinical use and have served as important tools for the study of Ca^{2+} channel structure and function [7,8]. It is worth noting that their effects and potency depend on modifications of substituents in the dihydropyridinic moiety. Despite many studies on the structure-function relationships of this type of calcium channel modulators, there is still debate on the exact stereochemical/conformational requirements for activity [9]. The stereochemical relationship between the aryl group and the dihydropyridine ring was found to be one of the factors having a pronounced effect on the biological activity [10]. Recently, it has been reported that some *bis*-compounds exhibit diverse biological activities and have a higher potency than the *mono*-compounds [11,12]. For example, Azab *et. al* have demonstrated that *bis*-thiazolodihydropyridine derivatives show moderate to high antimicrobial activity [11].

In view of the above facts and in continuation of our work on the chemistry of 1,4-dihydropyridines, we report the synthesis of some novel types of conformationally highly flexible *bis*-dihydropyridines, which are of interest in rela-

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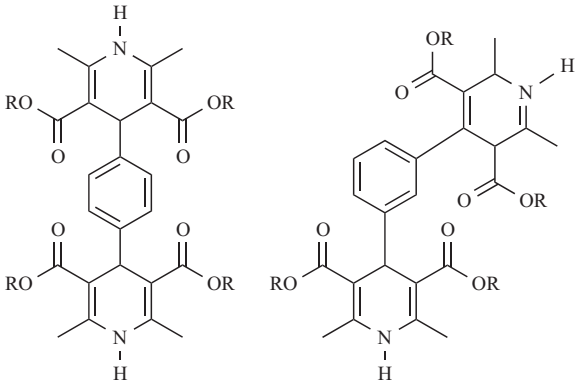
tion to the production of molecules for pharmacological purposes.

Thus, recently a new method was developed in order to produce a set of DHPs with short reaction times and good yields under the green chemistry protocol [13-15]. It is worth mentioning that while chemistry provides immense benefits to society, it can be quite hazardous; in this sense a new protocol, to make chemistry environmentally benign is known as Green Chemistry [16].

In this context, a set of four *bis*-DHPs (01-04) Fig. (**1**) was synthesized, in which a second 1,4-dihydropyridinic



bis-DHP-01 R = Me *bis*-DHP-03 R = Me
bis-DHP-02 R = Et *bis*-DHP-04 R = Et

Fig. (1). Structure of the studied molecules.

moiety was incorporated instead of the corresponding nitro group of nifedipine. The design of this type of analogues was based on the following considerations: a) two dihydropyridines were bonded to the phenyl moiety, which favors a larger number of effective collisions with the dihydropyridine receptor (thus a greater probability of bonding between the compounds and the receptor site), b) more importantly, the phenyl moiety does not have any substituents, allowing for free rotation through the sp^2 and sp^3 carbons, which in turn permits the moieties to have a faster interchange than is the case for nifedipine, and does not permit conformational isomerism (atropisomerism) and c) recognition was confirmed by the docking simulations that we frequently use for measuring binding affinities [17]; these results will be published later. The aim of this work was to test these molecules *in vivo* for their expected hypotensive effect on normotensive and spontaneously hypertensive rats, and *in vitro* for their vasodilating action (compared to nifedipine) on noradrenaline- and K^+-depolarization-evoked contractions, as well as the cumulative Ca^{2+} concentration-response curves.

MATERIALS AND METHODS

Animals

Male Wistar normotensive and male spontaneously hypertensive rats (SHR) of 250-300 g were used. The animals were kept at constant temperature (22 ± 2 °C) and humidity (50%), with food and water freely available in their home cages. All procedures were conducted in accordance with the guidelines for *Use and Care of Laboratory Animals* (NOM-062-ZOO-1999, Ministry of Agriculture, México),and the protocol was approved by the institutional committee for animal use.

Anaesthetized Rat Protocol

The effect of *bis*-DHPs (01-04) on diastolic and systolic blood pressure and heart rate in normotensive and spontaneously hypertensive rats was determined. The experiments were carried out with a total of 48 Wistar and 12 SHR rats, under anesthesia with urethane (1750 mg kg^{-1}, i.p.). Rats were cannulated and artificially ventilated (ideal Palmer pump at 56 strokes min^{-1}, with a stroke volume of 5 mL kg^{-1}) [18], adjusting the arterial pH within normal limits. After bilateral vagotomy, catheters were inserted *via* a femoral vein for administration of *bis*-DHPs, while the carotid artery was connected to a TSD105 pressure transducer coupled to a MP 100 data acquisition system (Biopac Instruments, Santa Barbara, CA, USA) for the measurement of arterial blood pressure and heart rate. After drug administration, the cannula was flushed with 0.25 mL of physiological saline solution and the body temperature of the animal was maintained at 37ºC.

Once the animals were in a stable haemodynamic condition for at least 45 min, baseline values of blood pressure and heart rate were established. After collection of these data, the normotensive animals were divided into five groups (n = 8 each). Blood pressure and heart rate dose-response curves were determined for *bis*-DHP 01 (1.0, 1.53, 2.37, 3.65 mg kg^{-1}, i.v.), *bis*-DHP-02 (1.0, 3.16, 10, 31.6 mg kg^{-1}, i.v.), *bis*-DHP-03 and 04 (0.1. 0.177, 0.316, 0.563, 1.0 mg kg^{-1}, i.v.), nifedipine (0.1, 0.316,1.0, 3.16 mg kg^{-1}, i.v.) as well as for the vehicle. Then, SHR rats were divided into two treatment groups (n = 4 each) and the effects of *bis*-DHP-03 and 04 (0.01. 0.0177, 0.0316, 0.0563, 0.1 mg kg^{-1}, i.v.), were determined. Drugs doses were selected on the basis of results obtained from preliminary experiments. Each dose of *bis*-DHPs was sequentially administered once the effect produced by the preceding dose returned to baseline values (after 10-15 min).

CONTRACTION IN ISOLATED AORTA

K^+ Depolarization-Evoked Contraction

Animals were sacrificed and the thoracic aorta was excised and cleaned from surrounding connective tissue. The isolated arteries were cut into rings (≈4-5 mm in length) and the endothelium was removed by gently rubbing the intimal surface with a metal device [19] (n = 4 each). In brief, aortic rings were placed in tissue chambers filled with 10 mL Krebs-Henseleit solution, maintained at 37 ºC, pH 7.4 and bubbled with 95% O_2 containing 5% CO_2. Arterial rings were hooked to the bottom of the chamber and to a Grass FT03 force displacement transducer (Astro-Med, Inc. West Warwick, RI, USA), connected to a MP100 data-acquisition system (Biopac), to record the isometric tension developed by aortic rings. An optimal tension of 3.0 g was applied to rat aorta, then challenged with phenylephrine ($1x10^{-7}$ M) and washed every 30 min for 2 h. During the last stimulation with phenylephrine, arterial rings were exposed to carbachol ($1x10^{-6}$ M) to verify the functionality of endothelium. The

absence of endothelium was confirmed by the lack of a relaxation response to carbachol. Contraction was evoked by increasing the KCl (80 mM), then arteries were preincubated for ≈ 5 min in this solution to reach maximal contraction and reproducible cumulative concentration-response curves to *bis*-DHPs ($1x10^{-10}$ to $1x10^{-5}$ M).In some experiments endothelium was gently removed; the absence of endothelium was confirmed by the lack of a relaxation response to carbachol [5]. Solutions were prepared daily and the concentrations were calculated in accordance with the free base of the substances.

In another set of experiments the contraction was evoked by adding noradrenaline ($1x10^{-7}$ M) and then *bis*-DHP-03 and 04 ($1x10^{-10}$ to $1x10^{-5}$ M) were tested.

Calcium Concentration-Response Curves

The cumulative Ca^{2+} contraction-response relationship was investigated in a Ca^{2+}- free solution containing 80 mM KCl. Aortic rings were preincubated for 10 min and washed with this solution three times for 30 min. Then, cumulative Ca^{2+} concentration-response curves were obtained for each artery in presence of *bis*-DHP-03 ($1x10^{-7}$ to $1x10^{-5}$ M), *bis*-DHP-04 ($1x10^{-8}$ to $1x10^{-6}$ M) and nifedipine ($1x10^{-9}$ to $1x10^{-7}$ M). The Ca^{2+} concentrations tested were from $1x10^{-6}$ to $1x10^{-2}$ M and ion addition was made cumulative as soon as a plateau was obtained at the previous concentration. The responses are expressed as the percentage of maximal contraction evoked before the addition of the compounds and nifedipine.

Statistics

All data are presented as mean ± SEM. The difference between changes in diastolic and systolic blood pressure, and heart rate elicited by the different treatments and doses were compared by the use of the Student Newman-Keuls test, once a two way ANOVA for repeated measures had revealed that the samples represented different populations [20]. IC_{50} values were calculated using linear regression equations [21]. Differences were considered significant when the P value was ≤ 0.05 (two tailed). Statistical tests were run in Sigma Stat 2.03 (Jandel Corp. SPSS Inc. San Rafael, CA., U.S.A.).

Drugs

Urethane, nifedipine, phenylephrine, carbachol, and noradrenaline were obtained from Sigma Chemical Co. (St. Louis, MO, USA). For *in vivo* tests, the compounds were dissolved in polyethylene glycol 200 (0.01%) and physiological saline solution; for *in vitro* tests dimethyl sulfoxide was employed. The four *bis*-dihydropyridines were obtained according to a recent procedure [14].

RESULTS

Anaesthetized Rat Experiments

The results of the tested compounds *bis*-DHPs (01-04) are shown in Fig. (**2-6**). The baseline values of diastolic and systolic blood pressures, and heart rate in Wistar rats were: 81 ± 2 mmHg, 102 ± 3 mmHg, 345 ± 5 bpm and respectively, while in SHR rats these values were 142 ± 19 mmHg,

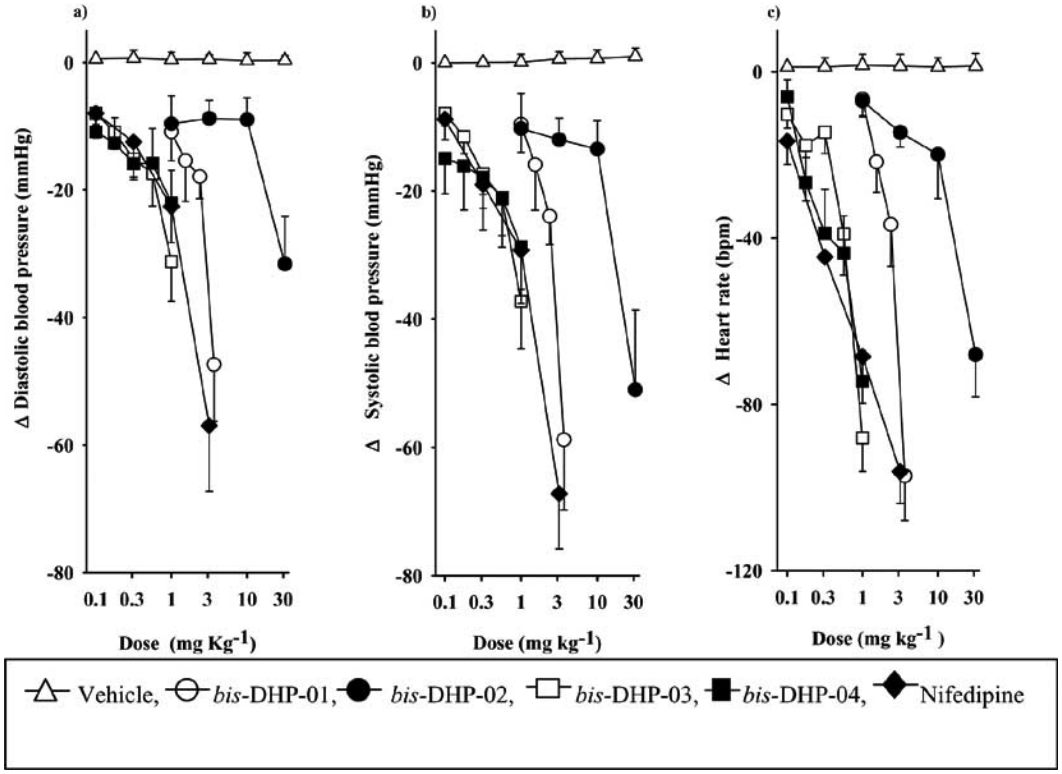


Fig. (2). a) Effect produced by *bis*-dihydropyridines on the diastolic blood pressure, **b**) Changes produced by *bis*-dihydropyridines on the systolic blood pressure **c**) Effect produced by *bis*-dihydropyridines on the heart rate. Each point represents the mean value, n = 8, and the bar indicates the SEM. The effect of nifedipine was compared with each studied molecule in normotensive male rats anaesthetized with urethane.

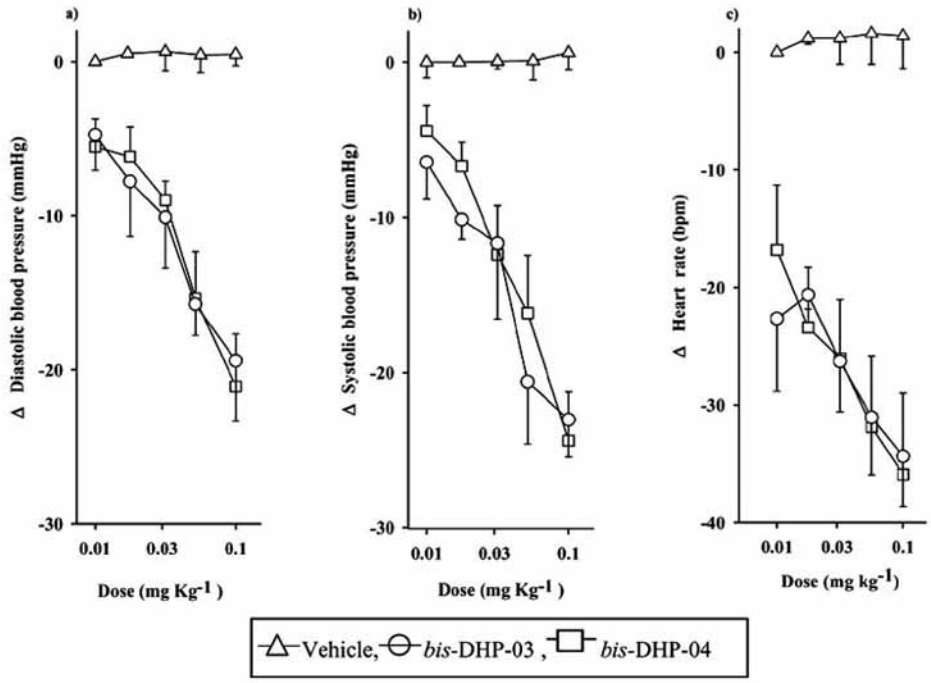


Fig. (3). a) Changes produced by *bis*-dihydropyridines on the diastolic blood pressure in hypertensive male rats; **b**) Effect produced by *bis*-dihydropyridines on the systolic blood pressure in hypertensive male rats; **c**) Effect produced by *bis*-dihydropyridines on the heart rate for hypertensive male rats. Each point represents the mean value, n = 4, and the bar indicates the SEM. The effect of nifedipine was compared with each studied molecule in normotensive male rats anaesthetized with urethane.

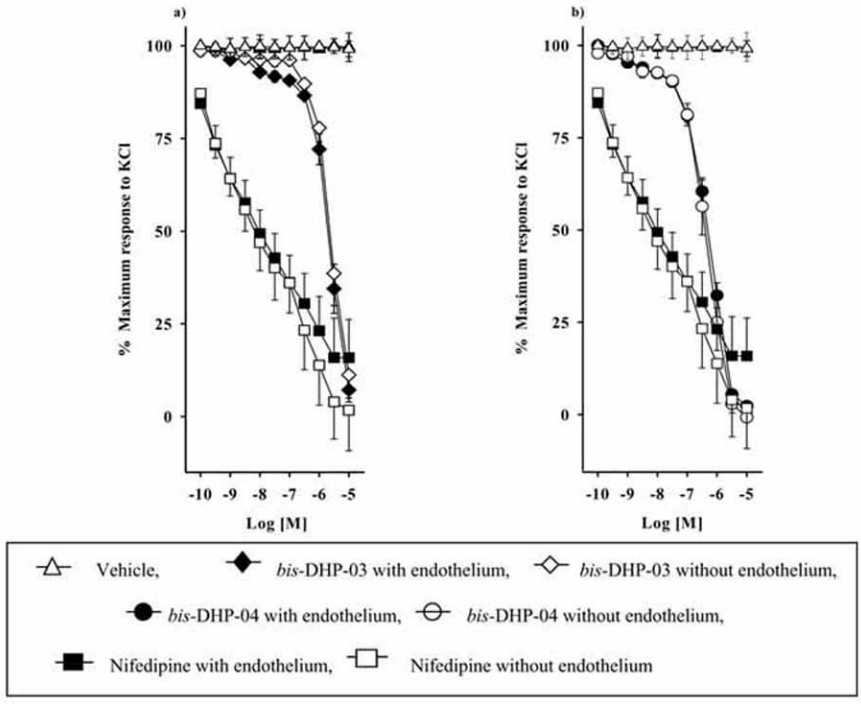


Fig. (4). a) Percentage of maximum response of aortic rings treated with KCl to the *bis*-dihydropyridines; the effect of nifedipine was compared with *bis*-DHP-03. **b**) Percentage of maximum response of aortic rings treated with KCl to the *bis*-dihydropyridines. The effect of nifedipine was compared with *bis*-DHP-04. Each point represents the mean value, n = 4, and the bar indicates the SEM.

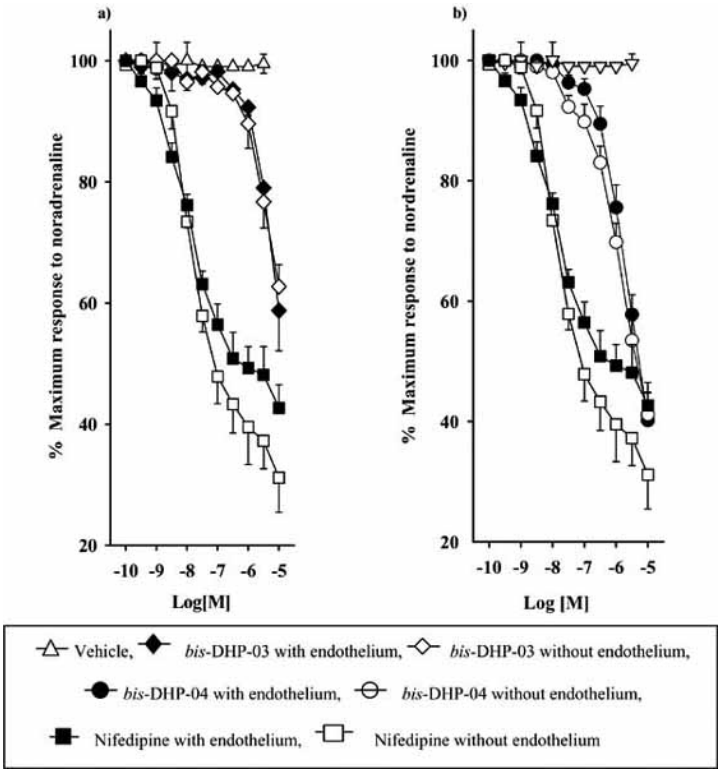


Fig. (5). a) Effect of *bis*-DHP-03 and nifedipine on noradrenaline-induced contractions of aorta rings. b) Effect of *bis*-DHP-04 and nifedipine on noradrenaline-induced contractions of aorta rings. Each graph shows concentration effect curves obtained in the absence and presence of endothelium. Each curve is the mean of four experiments and the bar indicates the SEM. The preparations were preincunbated for 60 min in the presence of noradrenaline.

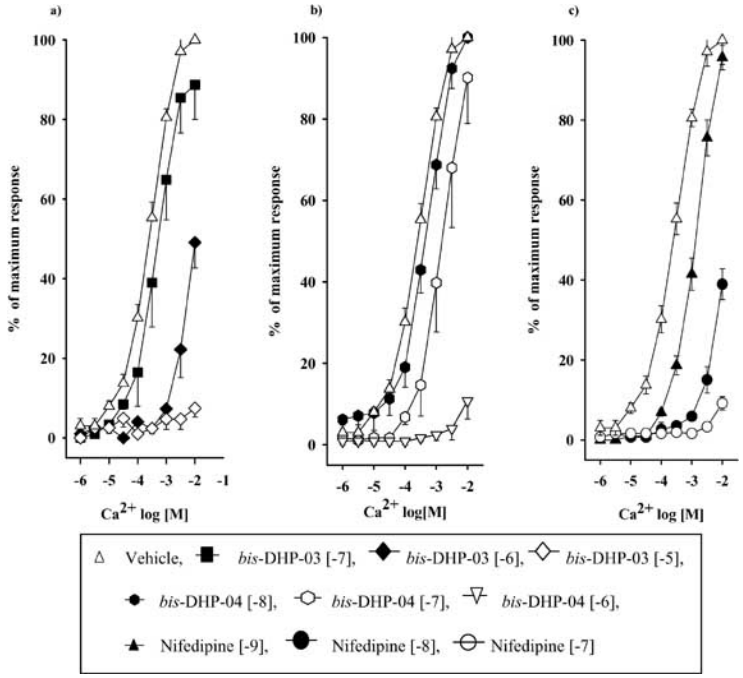


Fig. (6). a) Effect of *bis*-DHP-03 on contractions evoked by Ca^{2+} in K^{+}-depolarized aorta rings. b) Effect of *bis*-DHP-04 on contractions evoked by Ca^{2+} in K^{+}-depolarized aorta rings. c) Effect of nifedipine on contractions evoked by Ca^{2+} in K^{+}-depolarized aorta ring, cumulative Ca^{2+} concentration-effect curves were obtained before and after addition of the compounds at the concentrations indicated. Arterial preparations were preincubated in Ca^{2+}-free physiological solution depolarized in Ca^{2+}. Each curve is the mean of four experiments and the bar indicates the SEM.

205 ± 17 mmHg and 368 ± 6 bpm, respectively. The i.v. bolus injections were: *bis*-DHP-01 (1-3.16 mg kg^{-1}), *bis*-DHP-02 (1-31.6 mg kg^{-1}), nifedipine (0.1-3.16 mg kg^{-1}), *bis*-DHP-03 and *bis*-DHP-04 (0.1-1.0 mg kg^{-1}). Fig. (**2a-c**) shows the delta values on diastolic blood pressure, systolic blood pressure and heart rate for normotensive rats with the studied *bis*-DHPs, nifedipine and the vehicle. As observed, the rank order of the apparent vasodilator potency was: *bis*-DHP-03 ≅ *bis*-DHP-04 ≅ nifedipine >*bis*-DHP-01 > *bis*-DHP-02 for diastolic and systolic blood pressure Fig. (**2a-b**), whereas the rank order of the bradycardic effect was *bis*-DHP-04 ≅ *bis*-DHP-03 ≅ nifedipine >*bis*-DHP-02 > *bis*-DHP-02 Fig. (**2c**). These results evidenced that both *bis*-DHP-03 and 04, in which the second pyridinic unit was incorporated in *meta* position, induced a similar vasodilator effect as nifedipine and greater than that of the *para* regioisomers (*bis*-DHP-01 and 02).

On the other hand, Fig. (**3a-c**) shows the delta values of diastolic and systolic blood pressure and heart rate for SHR rats, where doses were ten-fold lower for *bis*-DHP-03 and 04 compared with normotensive rats. In addition, no significant differences were observed between *bis*-DHP-03 and 04 on diastolic and systolic blood pressure and heart rate, although there were significant differences between SHR and normotensive rats in respect to heart rate Fig. (**2a-3c**).

Contraction In Isolated Aorta

Concentration-response curves on aortic rings precontracted with KCl showed that the effect of the IC_{50} of bis-DHP-04 was approximately 7 times and bis-DHP-03 37 times higher than nifedipine ineliciting dilatation in aortic rings of normotensive rats Fig. (**4a-b**), (Table **1**), while *bis*-DHP-01 and *bis*-DHP-02 were inactive (data not shown). In addition, no significant differences were obtained between responses of aortic rings with and without endothelium for *bis*-DHP-03, 04 and nifedipine. The action of the latter *bis*-DHPs and nifedipine were examined on the noradrenaline evoked contraction. All three compounds produced a concentration-dependent inhibition of noradrenaline-induced contractions Fig (**5a-b**). The IC_{50} of *bis*-DHP-03, 04 and nifedipine in aorta with endothelium was $1.9x10^{-5}$ M, $5.4x10^{-6}$ M and $2.1x10^{-7}$ M while without endothelium such values were $2.6x10^{-5}$ M, $4.9x10^{-6}$ M and $1.1x10^{-6}$ M, respectively. The IC_{50} values for noradrenaline-induced contractions and K^+-evoked contractions were significantly different ($P < 0.001$). As observed, the order of apparent vasodilator potency was nifedipine > *bis*-DHP-04 > *bis*-DHP-03 (Table **2**).

Table 1. Antagonistic Activity of 1,4-Dihydropyridine Derivatives on Contraction Induced by KCl [80mM] on Aorta ± SEM

Compound	IC_{50} with endothelium [M]	IC_{50} without endothelium
Bis-DHP-03, *m*-Me	$1.5x10^{-6}$	$2.5x10^{-6}$
Bis-DHP-04, *m*-Et	$2.9x10^{-7}$	$2.3x10^{-7}$
Nifedipine	$4.0x10^{-8}$	$2.5x10^{-8}$

Table 2. Antagonistic Activity of 1,4-Dihydropyridine Derivatives on Contraction Induced by Noradrenaline [$1x10^{-5}$ M] on Rat Aorta with and Without Endothelium ± SEM

Compound	IC_{50} with endothelium [M]	IC_{50} without endothelium [M]
Bis-DHP-03, *m*-Me	$1.9x10^{-5}$	$2.6x10^{-5}$
Bis-DHP-04, *m*-Et	$5.4x10^{-6}$	$4.9x10^{-6}$
Nifedipine	$2.1x10^{-7}$	$1.1x10^{-6}$

Calcium Experiments

Fig. (**6a-c**) shows the effect of *bis*-DHP-03, 04 and nifedipine on Ca^{2+}-evoked contractions. In aorta arteries depolarized by KCl and incubated in a Ca^{2+}-free medium, the addition of Ca^{2+} evoked an increase in tension that was concentration-dependent. In the presence of the compounds and nifedipine, concentration-effect curves were shifted to the right and their maximum was progressively depressed. The order of apparent vasodilator potency was nifedipine > *bis*-DHP-04 > *bis*-DHP-03 (Table **3**). These compounds showed a concentration-dependence in blocking the KCl-induced contraction, suggesting that antagonism was noncompetitive at higher concentrations.

Table 3. Antagonistic Activity of 1,4-Dihydropyridine Derivatives on Contraction Induced by Ca^{2+} in K^+ Depolarized Rat Aorta Without Endothelium ± SEM

Compound [M]	Calcium EC_{50} [M]
Vehicle	$5.26x10^{-4}$
DHP-03 [10^{-7} M]	$3.84x10^{-4}$
DHP-03 [10^{-6} M]	$2.52x10^{-2}$
DHP-04 [10^{-8} M]	$2.43x10^{-4}$
DHP-04 [10^{-7} M]	$1.53x10^{-3}$
Nifedipine [10^{-9} M]	$1.31x10^{-3}$
Nifedipine [10^{-8} M]	$1.68x10^{-2}$

DISCUSSION

Calcium is involved in several major cellular signaling processes, including contraction, secretion and neuronal activity. In this sense, several studies have shown that certain 1,4 dihydropyridines (Hantzsch esters) alter cardiac and smooth muscle contraction by blocking or antagonizing Ca^{2+} entry through channels in the myocyte membrane. In gen-

eral, the intravenous injection of Ca^{2+} antagonists produces a more pronounced hypotensive effect in hypertensive than in normotensive animals [22]. In order to contribute to this field, in this study we assayed a series of four molecules Fig. (**1**), obtained under the green chemistry context, in which the nitro was substituted by a second 1,4-dihydropyridinic moiety [15]. We investigated whether or not these molecules possess hypotensive and antihypertensive properties in normotensive and SHR rats respectively. Thereafter, a study was done in relation to the effects of such molecules on aortic rings precontracted with KCl and noradrenaline, as well as on the Ca^{2+}-evoked contraction of aortic rings depolarized by KCl incubated in a Ca^{2+}-free medium.

This study shows that the *bis*-DHPs produced significant hypotension in both normotensive and SHR rats. Of these four *bis*-DHPs, the *meta* regioisomers (*bis*-DHP-03 and 04) and nifedipine induced a higher decrease on diastolic and systolic blood pressure than the isomers in the *para* position (*bis*-DHP-01 and 02). Furthermore, the four *bis*-DHPs and nifedipine induced bradycardia, most likely explained by the blockage of Ca^{2+} channels in the sinoatrial node.

Godfraind [5,23,24] showed that the membrane permeability to calcium of vascular smooth muscle in hypertensive rats is greater than that in normotensive rats. In addition, it has been shown that the calcium content of cardiovascular tissue is increased in SHR rats [10,25,26]. Such findings support the hypothesis that the basic change occurring in vascular smooth muscle with the development of hypertension is an increase in cell membrane permeability to calcium [5,22,23,24]. The results in this study are in agreement with this assertion, since the effect of *bis*-DHP-03 and 04 on systolic and diastolic blood pressure was observed in both SHR and normotensive rats, being greater in the former.

Rat aorta has been used as a model to elicit the mechanism(s) of action of DHPs in contractile experiments, since the major clinical uses of Ca^{2+} entry blockers are in the domain of hypertension. Depolarization of vascular smooth muscle is known to increase cell membrane permeability to Ca^{2+} by opening of membrane potential-dependent Ca^{2+} channels [5]. Thus, KCl stimulation evokes an increase in the rate of Ca^{2+} influx through channels sensitive to Ca^{2+} antagonists, which suggests that depolarization induces a conformation of Ca^{2+} channels with increased affinity for dihydropiridines. It is important to mention that only *bis*-DHP-03 and 04 evoked a vasodilator effect on aortic rings precontracted with KCl and noradrenaline.

On the other hand, Godfrain [24] reported that in the absence of endothelium, Ca^{2+} entry blockers are apparently more potent than in its presence. However, the experiments in this study showed that the relaxant effect of *bis*-DHP-03 and 04 were independent of the presence of endothelium.

In rat aorta, stimulation of α_1-adrenoceptors by noradrenaline increases cytosolic free Ca^{2+} and produces contraction. This contraction may be characterized by an initial fast component followed by a sustained tonic component. It has been shown that the initial fast component of the contraction evoked by a high concentration of noradrenaline persisted in the absence of extracellular Ca^{2+}, implying that noradrenaline releases Ca^{2+} from intracellular stores. The activation of α_1-adrenoceptors causes the hydrolysis of inositol phospholipids by activation of phospholipase C. Inositol 1,4,5-trisphosphate released into the cytosol induces the release of calcium from internal stores, which is responsible for the initial phase of the contraction. The tonic contraction is partly dependent on the presence of Ca^{2+} in the bathing solution. Noradrenaline has been reported to enhance Ca^{2+} influx [5]. This observation indicates that the stimulation of α_1-adrenoreceptors opens channels in the membrane, allowing Ca^{2+} to enter the cell in accordance with its electrochemical gradient. The assumption of the existence of receptor-operated channels, different from voltage-operated channels implies that an agonist such as noradrenaline evokes a contraction without depolarizing the membrane because it does not activate voltage-operated Ca^{2*} channels. Calcium antagonists prevent the increased Ca^{2+} entry produced by stimulation of vascular smooth muscle without affecting resting Ca^{2+} fluxes or intracellular Ca^{2+} release. It has also been reported that Ca^{2+} antagonists are less potent against calcium entry when stimulated by receptors than by high potassium depolarization [23]. It is important to mention that the IC_{50} was higher for noradrenaline than for KCl. This supports the hypothesis that *bis*-DHPs act as a voltage dependent Ca^{2+} channel blocker.

The addition of Ca^{2+} to the depolarizing solution evokes an increase in tension that is concentration dependent. In the presence of the *bis*-DHP-03, 04 and nifedipine, concentration-effect curves were shifted to the right. The inhibitory effect is typical of that observed with other calcium entry blockers [23-26]. The EC_{50} of calcium showed that *bis*-DHP-03 and 04 were less potent than nifedipine on vascular smooth muscle.

One of the main conclusions that can be drawn from these experiments is that the mechanism of action of the molecules under study is very probably through the blockage of calcium channels, based on the fact that *bis*-DHP-03 and 04 decreased blood pressure and induced bradycardia. This suggestion is indeed reinforced when considering the inhibitory effect of the contraction induced by KCl, noradrenaline and Ca^{2+}. Obviously, this hypothesis requires further investigation, but the results of this study are in agreement with the pharmacological profiles reported for several calcium antagonists. It's important to point out that only bis-03 and 04 had activity as antihypertensive agents, indicating a high isomeric selectivity to the receptive binding site.

In conclusion, we synthesized a set of four *bis*-1,4 dihydropyridines (01-04), of which molecules 03 and 04 showed a vasorelaxant effect in vascular smooth muscle. These two latter molecules could have potential in the treatment of hypertension or coronary artery disease. In addition, it is important to mention that the studied compounds are not photosensitive and consequently they could represent an advantage over nifedipine. Finally, it would be interesting to synthesize a series of Hantzsch esters in which the main substituents in the aromatic ring are pyrimidic moieties, aza-analogues of dihydropyridines.

The fact that the *meta* isomers (*bis*- DHP 03 and 04) had better pharmacological response than *para* isomers (*bis*-

DHP 01 and 02) could be due to the geometry of the ligand in the receptor binding site.

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