Full Paper

Effect of \textit{bis}-1,4-Dihydropyridine in the Kidney of Diabetic Rats

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Received October 29, 2012; Accepted May 2, 2013

Abstract. The in vivo effectiveness of 4-dihydropyridine (\textit{bis}-1,4-DHP), a new calcium-channel blocker, as a nephroprotector in isolated perfused kidney was evaluated by determining its effects on parameters associated with renal injury in diabetic rats. Diabetes in male Wistar rats, control, diabetic, control + \textit{bis}-1,4-DHP, and diabetic + \textit{bis}-1,4-DHP, was induced by a single administration of STZ (55 mg kg\textsuperscript{-1}, i.p.). In the drug-treated groups, treatment with \textit{bis}-1,4-DHP (10 mg kg\textsuperscript{-1} day\textsuperscript{-1}) started one week before diabetes induction; \textit{bis}-1,4-DHP was dissolved in DMSO (0.3%) and suspended in drinking water with carboxymethyl cellulose (3%). Parameters evaluated were body weight, blood glucose, albuminuria, proteinuria, creatinine, urea excretion, kidney’s weight / body weight ratio, and kidney perfusion pressure in all rat groups at different times of diabetes (2, 4, 6, and 10 weeks). Kidney weight of diabetic rats significantly increased vs. control, control + \textit{bis}-1,4-DHP, and diabetic + \textit{bis}-1,4-DHP rats at different times of diabetes. The ratios % kidney weight / 100 g body weight were different between control, control + \textit{bis}-1,4-DHP, and diabetic + \textit{bis}-1,4-DHP rats vs. diabetic rats (\textit{P} < 0.05). Kidney perfusion pressure was decreased by diabetes, while it was partially recovered by \textit{bis}-1,4-DHP treatment in response to phenylephrine. \textit{Bis}-1,4-DHP had a tendency to decrease hyperglycemia vs. diabetic rats, even though glycemia was too high as compared with controls, and it ameliorated albuminuria, creatinine, and urea excretion, suggesting a favorable effect on renal haemodynamics. \textit{Bis}-1,4-DHP, by inhibiting Ca\textsuperscript{2+} entrance, induced vasodilation in renal vascular bed and thus may have a nephroprotective effect against diabetes-induced renal dysfunction, but does not have significant impact on hyperglycemia.

Keywords: \textit{bis}-1,4-dihydropyridine derivative, calcium-channel blocker, nephroprotector, diabetes, kidney

Introduction

Diabetes mellitus (DM) and hypertension are commonly associated diseases (1). Diabetics have a greater prevalence of high blood pressure than the non-diabetic population, while hypertension occurs two times more frequently in diabetic individuals than in non-diabetic ones and aggravates complications of diabetes (1 – 3). Patients with both conditions have a higher risk of developing chronic renal failure and poorer prognosis than those with diabetes alone (4). In this regard, one of the most important factors that prevent the progression of renal damage in DM is definitively blood pressure control (5 – 8). Furthermore, kidneys are a main target of hypertension (6); they undergo vascular changes and develop areas of ischemic cortical atrophy, accompanied by renal atrophy with glomerular lesions and tubular changes (6). Hypertension-induced micro-anatomic changes in the kidney elicit functional impairment with hypoperfusion and both elevation of glomerular pressure

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Published online in J-STAGE on July 2, 2013
doi: 10.1254/jphs.12248FP
Numerous antihypertensive agents have been developed to blunt the progression of chronic kidney disease, including 1,4-dihydropyridines (1,4-DHPs). 1,4-DHPs are well known since their pharmacological profile show them as the most important Ca\(^{2+}\)-channel antagonists and are widely used as first-line antihypertensives in diverse patient groups, like those with chronic renal failure, and do not show adverse short-term effects on kidney function (7 – 10). It is known that 1,4-DHPs-induced reduction in blood pressure is associated with maintenance or increase in glomerular filtration rate (GFR) and renal blood flow due to vasodilation controlled by preglomerular (afferent) and postglomerular (efferent) arterioles, as well as by mesangial ultrafiltration, which may result in nephroprotection (6, 11).

On the top of their antihypertensive efficacy, 1,4-DHPs do have various pharmacological activities such as Ca\(^{2+}\)-channel antagonistic, antiangiogenic, antitumor, anti-inflammatory, analgesic, antithrombotic, vasodilator, anticonvulsant, stress protective, and cardio-depressant effects; and they cause neither sodium retention, water retention, hyperkalemia, nor reduction of bone mass (ostepenia) (9, 12 – 16). According to these multitude of actions, extensive efforts have been made to modify the 1,4-DHP ring to gain insight into pharmacological benefits (17). In this regard, the last generation of 1,4-DHP-type Ca\(^{2+}\) antagonists (mibefradil and efonidipine) exert blockade on L-type and T-type Ca\(^{2+}\) channels, eliciting vasodilation of afferent and efferent arterioles, increase renal blood flow without elevation of GFR, and decrease renal vascular resistance in rats (10). In addition, 1,4-DHPs dilated not only glomerular afferent but also efferent arterioles, reducing glomerular capillary pressure and injury, affording nephroprotection (10, 18); so, in this line of thought we have synthesized bis-1,4-DHP and tested them as antihypertensives in spontaneously hypertensive rats, as well as in precluding Ca\(^{2+}\)-induced contraction on isolated rat aorta (19).

Since the specific role played by 1,4-DHPs-mediated changes in renal vascular tone associated with diabetes has not yet been established, we aimed to study the effects in kidney perfusion pressure evoked by bis-1,4-dihydropyridine (bis-1,4-DHP), a novel Ca\(^{2+}\) channel antagonist, in the diabetic rat.

**Materials and Methods**

*Experimental animals and pharmacological treatment*

Animals obtained from our facilities (F.E.S. Iztacala, U.N.A.M.) were divided into four groups: control (C), diabetic (D), control + bis-1,4-DHP (CT), and diabetic + bis-1,4-DHP (DT). Body weight was determined every 2 weeks. Diabetes was induced in 9 – 10-week-old male Wistar rats (240 – 250 g) by a single administration of streptozotocin (STZ, 55 mg·kg\(^{-1}\), i.p.; Sigma-Aldrich, St. Louis, MO, USA), dissolved in citrate buffer (pH 4.5). Forty-eight hours later, tail blood samples were obtained and glucose concentration was measured using a one-touch glucometer (Accu-Chek sensor; Roche, Mannheim, Germany). Diabetes was considered established when glycemia was higher than 300 mg·dl\(^{-1}\) (time 0). Control and CT rats were not injected with STZ but were kept under identical conditions: at constant temperature (22°C ± 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).

Treatment with bis-1,4-DHP (Fig. 1), synthesized as described (14, 19), started one week before diabetes induction; the compound was dissolved in dimethyl sulfoxide (0.3%) and suspended in drinking water with carboxymethyl cellulose (3%), and given at doses of 10 and 31.6 mg·kg\(^{-1}\)·day\(^{-1}\) to both CT and DT rats.

Blood pressure and heart rate were monitored every week in the conscious state, by tail cuff plethysmography (PanLab, Letica, Barcelona, Spain); once blood pressure was measured, rats were placed in metabolic cages for 24 h. Urine excretion was measured and collected in order to quantify protein, albumin, urea, and creatinine.

*Tissue preparation*

Animals were subjected to surgery at 2, 4, 6, and 10 weeks after diabetes induction. Rats were anesthetized with sodium pentobarbital (65 mg·kg\(^{-1}\), i.p.), and after a midline laparotomy, the renal artery and right kidney were isolated as described (20). A cannula was placed in the mesenteric artery and advanced across the abdominal aorta into the renal artery. The kidney was removed and immediately perfused with oxygenated Krebs-Henseleit buffer at 37°C and constant flow of 10 ml·min\(^{-1}\); after at least 30 min of perfusion and once a stable perfusion
pressure was achieved, contractile responses to phenylephrine (0.032 – 100 nmol, spaced by a factor of 10:1/2; Sigma-Aldrich) were obtained for all groups of rats. At the conclusion of the experiment, the wet weight of the kidneys was recorded.

Protein was measured with the Folin reagent (ref. 21, Sigma-Aldrich), albuminuria was determined by enzyme immunoassay (Sigma-Aldrich albuminuria kit), creatinine was measured by the picric acid reaction in alkaline conditions (Sigma-Aldrich creatinine kit), and urea excretion was measured by the Sigma-Aldrich urea kit.

Statistical analyses

All data are presented as means ± S.E.M. The difference between changes in body, biochemical and pressure parameters, and times were compared by two-way ANOVA, followed by the Student Newman-Keuls test. The \( \Delta \) perfusion pressure elicited in the groups, times, and response to phenylephrine were compared by three-way ANOVA, also followed by Student Newman-Keuls test. 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, USA).

Results

The following parameters were evaluated to confirm that rats were kept diabetic for 10 weeks: body weight, blood glucose, water intake, and urinary volume.

The initial body weight of rats was 245 ± 5 g. Just as it was expected, the body weights of D and DT rats were lower than in those in the C and CT groups along the experiment (\( P < 0.05 \)); when comparing the increase in body weight throughout time, there were no significant differences among C and CT, nor between D and DT rats. Data recorded during the course of the experiment for all rat groups, at the dose of 10 mg kg\(^{-1}\) bis-1,4-DHP

<table>
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<tr>
<th>Parameter</th>
<th>Group</th>
<th>0</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>10 weeks</th>
</tr>
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<tr>
<td>BW (g)</td>
<td>Control</td>
<td>243.9 ± 6.6*</td>
<td>277 ± 11*</td>
<td>293 ± 9*</td>
<td>310 ± 9*</td>
<td>379 ± 7*</td>
</tr>
<tr>
<td></td>
<td>Control treated</td>
<td>251.1 ± 5.7*</td>
<td>289 ± 17*</td>
<td>275 ± 11*</td>
<td>308 ± 15*</td>
<td>356 ± 9*</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>248.8 ± 6.7*</td>
<td>236 ± 3†</td>
<td>233 ± 10†</td>
<td>238 ± 9†</td>
<td>241 ± 3†</td>
</tr>
<tr>
<td></td>
<td>Diabetic treated</td>
<td>248.9 ± 7.9*</td>
<td>242 ± 21‡</td>
<td>223 ± 6‡</td>
<td>239 ± 16‡</td>
<td>240 ± 8‡</td>
</tr>
<tr>
<td>H(_2)O consumed (ml/day)</td>
<td>Control</td>
<td>32.9 ± 1.5*</td>
<td>33 ± 2*</td>
<td>39 ± 1*</td>
<td>35 ± 3*</td>
<td>42 ± 5*</td>
</tr>
<tr>
<td></td>
<td>Control treated</td>
<td>34.9 ± 0.1*</td>
<td>35 ± 1*</td>
<td>47 ± 7*</td>
<td>36 ± 2*</td>
<td>41 ± 5*</td>
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<tr>
<td></td>
<td>Diabetic</td>
<td>90 ± 0.6(1^)</td>
<td>124 ± 5(‡)</td>
<td>110 ± 8(†)</td>
<td>114 ± 13(†)</td>
<td>167 ± 3(‡)</td>
</tr>
<tr>
<td></td>
<td>Diabetic treated</td>
<td>70 ± 9.4(1^)</td>
<td>111 ± 9(†)</td>
<td>91 ± 8(‡)</td>
<td>99 ± 11(‡)</td>
<td>90 ± 13(‡)</td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
<td>Control</td>
<td>5.3 ± 0.3(1)</td>
<td>3 ± 0.3(*)</td>
<td>4 ± 2*</td>
<td>6 ± 1*</td>
<td>15 ± 3*</td>
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<td>6.1 ± 0.3(1)</td>
<td>6 ± 1*</td>
<td>7 ± 2*</td>
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<td>14 ± 5*</td>
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<tr>
<td></td>
<td>Diabetic</td>
<td>85.7 ± 1(1)</td>
<td>86 ± 11(†)</td>
<td>99 ± 16(†)</td>
<td>109 ± 7(‡)</td>
<td>115 ± 10(‡)</td>
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<tr>
<td></td>
<td>Diabetic treated</td>
<td>63.8 ± 10.9(1)</td>
<td>64 ± 11(†)</td>
<td>46 ± 8(‡)</td>
<td>34 ± 8(‡)</td>
<td>90 ± 13(†)</td>
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<tr>
<td>Proteinuria (mg / 24 h)</td>
<td>Control</td>
<td>73 ± 4</td>
<td>73 ± 4</td>
<td>74 ± 4</td>
<td>70 ± 4</td>
<td>90 ± 10</td>
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<tr>
<td></td>
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<td>74 ± 4</td>
<td>85 ± 4(1)</td>
<td>118 ± 78</td>
<td>78 ± 11(†)</td>
<td>116 ± 7</td>
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<tr>
<td></td>
<td>Diabetic</td>
<td>74 ± 4</td>
<td>190 ± 25(‡)</td>
<td>399 ± 31(†)</td>
<td>315 ± 14(‡)</td>
<td>385 ± 12(‡)</td>
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<tr>
<td></td>
<td>Diabetic treated</td>
<td>74 ± 4</td>
<td>78 ± 3(1)</td>
<td>137 ± 24(†)</td>
<td>129 ± 22(‡)</td>
<td>129 ± 22(‡)</td>
</tr>
<tr>
<td>Creatinine (mg / 24 h)</td>
<td>Control</td>
<td>5 ± 0.3</td>
<td>5 ± 0.2</td>
<td>8 ± 1</td>
<td>7 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td></td>
<td>Control treated</td>
<td>5 ± 0.3</td>
<td>5 ± 1</td>
<td>9 ± 1</td>
<td>8 ± 1</td>
<td>7 ± 2</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>4 ± 0.4</td>
<td>14 ± 12(‡)</td>
<td>24 ± 5(‡)</td>
<td>19 ± 2(‡)</td>
<td>25 ± 6(‡)</td>
</tr>
<tr>
<td></td>
<td>Diabetic treated</td>
<td>4 ± 0.4</td>
<td>10 ± 2(†)</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Urea (mg / 24 h)</td>
<td>Control</td>
<td>29 ± 3</td>
<td>29 ± 4</td>
<td>27 ± 1</td>
<td>25 ± 1</td>
<td>28 ± 4</td>
</tr>
<tr>
<td></td>
<td>Control treated</td>
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<td>32 ± 4</td>
<td>162 ± 13(‡)</td>
<td>237 ± 16*</td>
<td>122 ± 11*</td>
<td>110 ± 9*</td>
</tr>
<tr>
<td></td>
<td>Diabetic treated</td>
<td>31 ± 2</td>
<td>140 ± 17(‡)</td>
<td>96 ± 9(†)</td>
<td>44 ± 6(‡)</td>
<td>46 ± 7(‡)</td>
</tr>
</tbody>
</table>

Different parameters were evaluated: body weight, urine volume, water consumed; proteinuria and creatinine and urea excretion. The data are expressed as the mean ± S.E.M. (n = 5). The significance of differences between means was assessed by ANOVA two-way followed by the Student Newman-Keuls test. Body weight, consumed water, urine volume; different symbols \( P < 0.05 \), C, CT and DT vs. D at two weeks; \( I P < 0.05 \), C and CT vs. D and DT; \( II P < 0.05 \), D vs. DT, at 4, 6, and 10 weeks. Urinary proteinuria excretion: \( * P < 0.05 \), C and CT vs. D and DT; \( * II P < 0.05 \), D and DT vs. C and CT, DT vs. D. Urinary creatinine excretion: \( ** P < 0.05 \), C and CT vs. D and DT; \( ** I P < 0.05 \), D and DT vs. C, CT, DT vs. D. Urinary urea excretion: Different symbols (*, †, ‡) \( P < 0.05 \).
Table 1. The results were similar at the dose of 31.6 mg·kg$^{-1}$ bis-1,4-DHP (data not shown), at different times.

When glucose was measured, all rats showed initial glycemia within normal values (92 ± 3 mg·dl$^{-1}$); however, two days after STZ administration, both diabetic rat groups showed hyperglycemia (> 300 mg·dl$^{-1}$) and they remained high at longer times (approximately 500 mg·dl$^{-1}$, Fig. 2). Glycemia values were similar and normal in C and CT groups, but different when compared with D and DT groups, throughout the experiment ($P < 0.05$). It is noticeable that DT rats had a tendency to have lower glycemia values than D rats, being statistically significant at 6 and 10 weeks of bis-1,4-DHP ($F$; $P < 0.05$). No differences between doses were observed with bis-1,4-DHP at 31.6 mg·kg$^{-1}$ (not shown).

Along with glycemia determination, systolic blood pressure (SBP) and albuminuria were monitored throughout the experimental period. As observed in Fig. 3, bis-1,4 DHP reduced SBP after 6 weeks of treatment. Systolic blood pressure of CT rats was lower than C, D, and DT rats ($P < 0.05$). While SBP of DT rats at 4, 6, and 10 weeks diminished but was not different compared with the D group, diastolic blood pressure and heart rate values were not significantly different among the groups and times (results not shown).

When albuminuria was measured, it was found to be increased four-fold in diabetic rats as compared to the control, while bis-1,4-DHP partially decreased albumin excretion as early as 2 weeks of treatment and maintained that pattern at longer times (Fig. 4).

Fig. 2. Effect of bis-1,4-dihydropyridine on blood glucose in rats. Normal and diabetic rats with bis-1,4-DHP at 10 mg·kg$^{-1}$·day$^{-1}$ (Control, C; Control Treated, CT; Diabetic, D; Diabetic Treated, DT) at different times of treatment. Data are expressed as means ± S.E.M. (n = 5). *$P < 0.05$, C, CT vs. D, DT; †$P < 0.05$, D vs. DT.

Fig. 3. Effect of bis-1,4-dihydropyridine on systolic blood pressure in different rats groups and times at dose of 10 mg·kg$^{-1}$. Data are expressed as mean ± S.E.M. (n = 5). *$P < 0.05$, CT vs. C, D, DT groups.
Water consumption by all rats was measured along the experiment; as expected it was higher in D and DT rats than in non-diabetic rats. Water ingestion at 4, 6, and 10 weeks was C = CT < D > DT (Table 1). Results also show that water consumption in DT rats was less at the 10 mg$\times$kg$^{-1}$ dose than at 31.6 mg$\times$kg$^{-1}$, at the tested times ($P<0.05$, data not shown). On the other hand, diuresis in D and DT rats was higher than in C and CT rats ($P<0.05$), while urine excretion in DT rats was lower than in D rats ($P<0.05$, Table 1).

Albumin excretion in DT rats was lower than in D rats (Fig. 3, $P<0.05$). In these two groups, albuminuria increased from the second week and remained unchanged at longer times. Albumin excretion in C and CT groups was not significantly different from each other during the experiment; however, albuminuria was less when bis-1,4-DHP was given at 10 mg$\times$kg$^{-1}$ than at 31.6 mg$\times$kg$^{-1}$ ($P<0.05$, not shown).

Proteinuria increased in both D and DT groups ($P<0.05$), but diabetic rats had higher protein excretion than DT rats ($P<0.05$, Table 1). Notice that protein excretion in D and DT groups increased along the experiment (Table 1). The order of protein excretion observed was C ≤ CT = DT < D ($P<0.05$, Table 1). As observed for albuminuria, protein excretion was lower at 10 mg$\times$kg$^{-1}$ than at 31.6 mg$\times$kg$^{-1}$ bis-1,4-DHP (data not shown).

Creatinine excretion in urine in D rats was higher than those in C, CT, and DT rats ($P<0.05$, Table 1); in C and CT groups, it was constant throughout the study; and in DT rats, it was not different to controls at 4 and 6 weeks (Table 1).

As can be observed, the rank order of urea excretion in the different groups at 10 mg$\times$kg$^{-1}$ bis-1,4-DHP was D > DT > CT = C (Table 1).

Kidney weight × 100 g body weight ratio is an index of renal hypertrophy. Figure 3 shows the increased ratio in both D and DT rats, compared with C and CT, at all times. Bis-1,4 DHP decreased kidney hypertrophy in diabetic rats (Fig. 5). Kidney weight was higher in D rats compared to those in C, CT, and DT groups (not shown).

Kidney perfusion pressure showed the vasoconstriction due to phenylephrine in the four rat groups at different times and in proven bolus concentrations (0.032 – 100 nmol, spaced by a factor of $10^{1/2}$) of the agonist (Fig. 6). At 2 weeks of treatment there was no difference in responsiveness to phenylephrine in the kidneys among the four rat groups in the study (Fig. 6A). However, at 4 weeks phenylephrine elicited a greater response in CT rat kidneys compared with C, D, and
DT groups, even though the difference between C, D, and DT was not significant (Fig. 6B). In Fig. 6C, 6 weeks of treatment is depicted; the dose–response curves to phenylephrine for D and DT rats shifted to the right and elicited lower maximal contraction than those in the C and CT groups; it can be observed that DT kidneys showed a partial recovery when compared with D rats (P < 0.01). Figure 6D shows a similar pattern to Fig. 6C, with no difference between D and DT rats, the order of apparent responsiveness is C = CT > DT = D (Table 2).

It is worthwhile to notice that the basal perfusion pressure was higher throughout time in D rats, compared to the other groups (P < 0.05, Table 2); in contrast the maximum perfusion pressure for the D group diminished, while DT rats increased the responsiveness to phenylephrine at the different times (Table 2).

CE₅₀ indicates that the phenylephrine concentration required to induce contraction was different at different times (P < 0.05, Table 2). As observed, the order of apparent responsiveness for the agonist was C = CT > DT = D.

Discussion

Since hypertension is accompanied by renal damage characterized by albuminuria, glomerular hypertrophy, and nephrosclerosis (11), we evaluated the in vivo effect of bis-1,4-DHP, a new Ca²⁺ channel antagonist, as nephroprotector in isolated perfused kidney, as well as parameters associated with renal injury in diabetic rats.

Our results show that in CT and DT rats, bis-1,4-DHP lowered systolic blood pressure at the longest exposure times, even though reduction was discrete, which agrees with a previous report in anesthetized SHR rats (19). Considering the American Diabetes Association guidelines (22), the Ca²⁺-channel antagonists appear to be appropriate for second-line therapy to low SBP, for the treatment of hypertension in diabetes mellitus (18, 22, 23). Bis-1,4-DHP also lowered glycemia in diabetic rats, possibly by increased insulin sensitivity due to enhanced vasodilatation (24, 25), and it is also possible that bis-1,4-DHP has weak Ca²⁺-channel antagonism as has been described for other 1,4-DHPs (26).
tion, other 1,4-DHPs have been tested for antimicrobial, antioxidant, antitumor, and antihypertensive activities (13, 15); similar nephroprotection has been observed with other antioxidants (27). It is not known if bis-1,4-DHP possess those activities.

Our study shows that, despite persistent hyper-

Table 2. $\Delta$ Pressure perfusion basal (mmHg), maximal effect (nmoles) and EC$_{50}$ (nM)

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<th>Group</th>
<th>Weeks</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>10</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>10</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>10</th>
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<tr>
<td>Control</td>
<td></td>
<td>85 ± 3</td>
<td>106 ± 8</td>
<td>97 ± 1</td>
<td>94 ± 6*</td>
<td>168 ± 1</td>
<td>142 ± 3</td>
<td>182 ± 2</td>
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<td>4.2</td>
<td>3.4</td>
<td>1.7</td>
<td>1.0</td>
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<tr>
<td>Control Treated</td>
<td>69 ± 14*</td>
<td>89 ± 10</td>
<td>101 ± 7</td>
<td>85 ± 7</td>
<td>166 ± 18</td>
<td>193 ± 4*</td>
<td>173 ± 2</td>
<td>220 ± 16*</td>
<td>3.6</td>
<td>2.7</td>
<td>1.5</td>
<td>1.4</td>
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<tr>
<td>Diabetic</td>
<td>132 ± 11†</td>
<td>133 ± 12†</td>
<td>124 ± 9&quot;</td>
<td>132 ± 9&quot;</td>
<td>157 ± 6</td>
<td>126 ± 12&quot;</td>
<td>99 ± 15&quot;</td>
<td>114 ± 11†</td>
<td>2.4</td>
<td>1.6</td>
<td>18.3</td>
<td>0.2</td>
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<tr>
<td>Diabetic Treated</td>
<td>86 ± 6</td>
<td>103 ± 12</td>
<td>100 ± 7</td>
<td>111 ± 3</td>
<td>168 ± 2</td>
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The significance of differences between means was assessed by two-way ANOVA followed by the Student Newman-Keuls test. *P < 0.05, CT vs. C, D, DT; †P < 0.05, D vs. C, CT, DT; ‡P < 0.05, D, DT vs. C, CT; §P < 0.05, C vs. CT, D, DT; ¶P < 0.05, DT vs. C, CT.

Fig. 6. Effect of bis-1,4-dihydropyridine on kidney perfusion pressure. Control and diabetic rats were treated with bis-1,4-DHP at different times, and then the isolated kidneys were stimulated with phenylephrine and perfusion pressure determined. Data are expressed as means ± S.E.M. (n = 5). *P < 0.05, CT vs. C, D, DT at 4 weeks; †P < 0.05, D vs. C, CT, DT at 6 weeks; ‡P < 0.05, DT vs. D.
glycemia, bis-1,4-DHP ameliorates water consumption, urinary volume, albuminuria, proteinuria, creatinine, and urea excretion. In regard to water and albuminuria, the reduction in DT rats due to bis-1,4-DHP suggests a favorable effect on renal hemodynamics (7, 8, 11, 28).

It is important to mention that albuminuria is a relevant indicator of the early stage of diabetic nephropathy, where there is an increase in GFR, thickening of the glomerular and tubular basement membrane, an expansion of the mesangium (accumulation of basement membrane-like material in the mesangium) then hyperfiltration increases the albumin filtration rate, and eventually a decline in GFR (4, 29 – 31).

Urinary albumin excretion can be used as an index of renal lesions associated with DM and glomerular sclerosis (4). Different reports have shown that diminished albuminuria precedes and predicts a subsequent decrease in renal deterioration (9). Our results suggest that bis-1,4-DHP has a nephroprotective effect, since there is evidence that reduction and normalization of proteinuria and albuminuria is a key of treatment goal for renal protection (28 – 30). Also Galan et al. (32) had reported that lowering blood pressure reduces the risk for developing microalbuminuria and macroalbuminuria, which also reduce risks for renal events in type 2 diabetes. Therefore, albuminuria, creatinine, and urea are frequently used as important parameters for renal evaluation (30, 31, 33, 34) when studying potential beneficial effects of antihypertensives on the progression of renal disease (11). Our data agree with previous reports where Ca\(^{2+}\)-channel antagonists reduced diabetic nephropathy and favored renal haemodynamics and renal tubular Na\(^+\) reabsorption; also Ca\(^{2+}\)-antagonists retard progression of kidney insufficiency (8).

On the other hand, the decrement of perfusion pressure in response to phenylephrine suggests that kidney injury exists in rats, as early as two weeks with diabetes, while it was partially recovered by bis-1,4-DHP. This result suggests that bis-1,4-DHP, by antagonizing Ca\(^{2+}\) entrance, probably induces vasodilatation; however, Dworkin (35) has reported that Ca\(^{2+}\)-channel antagonists possess protector effects in chronic renal disease because they increase kidney blood flow without elevation of GFR, and decrease renal vascular resistance.

The decrement of perfusion pressure in diabetic rats in response to phenylephrine can be caused by renal ischemia, characterized by hypoperfusion or constriction of some segments of renal vasculature (4). Other ways to explain this decrease could be a fall of phenylephrine \(\alpha_1\) adrenoceptors number, minor responsiveness to those receptors, diminution of the ways of signaling to phenylephrine, and greater production of vasodilator substances (36, 37).

Kidney weight decrease in DT rats suggests that bis-1,4-DHP reduced renal injury and adds renal protection that may be related to the ability of these agents to inhibit compensatory renal growth (32). There are reports that suggest that compensatory renal hypertrophy may contribute to progressive glomerular injury, independent of adaptive changes in glomerular hemodynamics (7, 38). The protective effect of Ca\(^{2+}\)-channel antagonists could be associated with renal hypertrophy compensatory reduction; our results showed decreased ratio of kidney weight/body weight, indicating that modulation of renal hypertrophy may be related to early protective effect of Ca\(^{2+}\)-channel blockade.

Finally, our study suggests that Ca\(^{2+}\)-channel antagonists represent an alternative treatment for diabetic nephropathy; since bis-1,4-DHP improved some parameters of renal damage, it can be used as a unique or combined drug with other antihypertensives, such as converting enzyme inhibitors or diuretics. Differences in the potential mechanisms of action of these two classes of agents may have a complementary and perhaps synergistic effect on the kidney.

Acknowledgments

R. G.-P. was a postdoctoral fellow from CONACYT 3537, ICyT-GDF and CBTIS No. 160, DGETI-SEP. We thank R. Maruri-Gómez for English language assistance.

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