Chronic T-type Ca\textsuperscript{2+} channel blockade with mibefradil in hyperinsulinemic, insulin-resistant and hypertensive rats

Subodh Verma, Sanjay Bhanot, Alan Hicke, John H. McNeill

Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, B.C. V6T 1Z3, Canada

Received 30 September 1996; accepted 8 January 1997

Abstract

Objectives: To determine the effects of calcium antagonists on hyperinsulinemia, hypertriglyceridemia and hypertension, we examined the long-term effects of a new calcium channel blocker, mibefradil, on plasma insulin levels, plasma triglyceride levels and systolic blood pressure in insulin-resistant and hyperinsulinemic fructose-hypertensive FH rats. To this aim, both prevention and reversal protocols were employed.

Methods: Prevention study: Male Sprague-Dawley rats were procured at 6 weeks of age and were divided into: control (C, n = 6), control-treated (CT, n = 5), fructose (F, n = 7) and fructose-treated (FT, n = 6). Baseline measurements of plasma glucose, insulin and systolic blood pressure were conducted in all groups. At week 7, chronic mibefradil treatment (30 mg/kg/day, orally for 6 weeks) was initiated in the CT and FT groups. At week 8, the rats in the F and FT groups were started on a 66% fructose diet to induce hyperinsulinemia and hypertension. Weekly measurements of plasma insulin, plasma triglycerides and systolic blood pressure were conducted for the following 4 weeks.

Reversal protocol: In a separate study, 8-week-treated FH rats and their age-matched controls were used to examine the effects of mibefradil on reversing fructose-induced hyperinsulinemia and hypertension.

Results: The F group exhibited hyperinsulinemia (3.2 ± 0.1 vs. C 2.3 ± 0.07 ng/ml, P < 0.05), hypertension (148 ± 3 vs. C 121 ± 1 mmHg, P < 0.002) and elevated triglyceride levels (5.4 ± 0.8 vs. C 1.6 ± 0.3 mM, P < 0.005). Chronic mibefradil treatment prevented the development of hyperinsulinemia (1.6 ± 0.08 ng/ml, P < 0.004 vs. F) and hypertension (123 ± 1 mmHg, P < 0.001 vs. F) and attenuated the development of hypertriglyceridemia. In the reversal study, mibefradil treatment reversed the development of hyperinsulinemia, hypertriglyceridemia and elevated BP in FH rats. Treatment did not affect the plasma glucose levels in any group prevention or reversal.

Conclusions: Long-term treatment with the calcium antagonist, mibefradil, both prevents and reverses the development of hyperinsulinemia, hypertriglyceridemia and hypertension in FH rats. These data indicate beneficial effects of mibefradil on carbohydrate and lipid metabolism in hyperinsulinemic and insulin-resistant states.

Keywords: Mibefradil; Calcium channel blockers; Hypertension, fructose; Triglycerides; Rat

1. Introduction

The intriguing association between insulin and hypertension has been extensively explored during the past decade. Interest in the link between insulin and high blood pressure (BP) was fuelled by two distinct observations: (1) the lack of effective antihypertensive drugs to reduce the increased incidence of coronary artery disease in hypertensive subjects and (2) the realization that essential hypertension per se is frequently associated with insulin resistance (resistance to the glucoregulatory actions of insulin) and hyperinsulinemia. These observations led to the so-called ‘insulin hypothesis’ of hypertension where it was postulated that insulin resistance and/or hyperinsulinemia may be causally linked to the development of hypertension. This was an attractive proposition which could help explain the apparent inability of conventional antihypertensive drugs (thiazides, beta-blockers) to decrease the incidence of coronary ischemic events, since these drugs did not improve but rather worsened insulin action (for review, see Ref. [1]).

In a series of experiments, we recently examined the proposition that insulin resistance and hyperinsulinemia contribute causally to the development of hypertension.
Essentially, if these defects were intrinsically linked to high BP, then agents that specifically improved insulin action should decrease BP. Indeed, we found that multiple drug interventions that exhibited the common property of attenuating hyperinsulinemia (metformin, the trace element vanadium, pioglitazone) lowered BP in two experimental models of hypertension [2,3]. Furthermore, the antihypertensive effects of these agents could be reversed by simply increasing the plasma insulin levels in the drug-treated rats to pre-treatment levels. These results indicate that there exists a strong and close association between hyperinsulinemia and hypertension in rodent models of hypertension.

In addition to drugs that specifically attenuate hyperinsulinemia (such as metformin, vanadium), recent studies indicate that insulin sensitivity is increased by several different classes of antihypertensive agents such as angiotensin-converting enzyme inhibitors, angiotensin-II receptor antagonists and peripheral alpha-adrenergic antagonists [4,5]. Calcium channel antagonists are used as major therapeutic agents in the treatment of systemic hypertension and ischemic heart disease; however, significant variation in their effects on carbohydrate and lipid metabolism has been reported. In contrast to diuretics and beta-blockers (that worsen insulin sensitivity), calcium antagonists have generally been considered to be metabolically neutral (nifedipine, verapamil, diltiazem) or slightly positive (isradipine, pioglitazone) in their effects on glucose and insulin [13–17]. On the other hand, there is some debate as to whether the clinical use of these agents is associated with suppression of insulin secretion and the development of insulin resistance and diabetes mellitus [18–21]. Therefore, the present study was initiated to further characterize the effects of a new calcium antagonist, mibefradil, on insulin, glucose, triglycerides and systolic blood pressure in insulin-resistant and hyperinsulinemic rats.

Mibefradil (Ro 40-5967) is a new calcium channel blocker that has been demonstrated to have potent antihypertensive and anti-ischemic effects [6] and is currently in phase III clinical trials. Mibefradil appears to be the only calcium antagonist that completely blocks the T-type calcium channels [6,22–24]. Although the available calcium blockers exert their effects on the L-type (long-lasting, high-voltage-activated) calcium channels, the T-type (transient, low-voltage activated) calcium channel is found in relatively high density in spontaneously active vascular muscle and may play a role in vascular hypertrophy and remodeling [24–27]. To our knowledge, there is no information on the effects of chronic inhibition of T-type calcium channels on carbohydrate and lipid metabolism in states of insulin resistance and hypertension, which was the objective of the present study. To this aim, the effects of chronic mibefradil treatment were studied in hyperinsulinemic, hypertensive FH rats. The FH rat model has been extensively used to study the inter-relationship between hyperinsulinemia, insulin resistance and hypertension, wherein feeding normal Sprague-Dawley rats a high fructose diet results in hyperinsulinemia, marked insulin resistance, elevated triglycerides and high BP [3,5,7,12].

2. Methods

2.1. Animals and experimental design

2.1.1. Prevention protocol

Male Sprague-Dawley rats were procured locally (180–200 g body weight) at 6 weeks of age. The animals were divided into 4 experimental groups: control-untreated (C, n = 6), control mibefradil-treated (CT, n = 5), fructose-untreated (F, n = 7) and fructose mibefradil-treated (FT, n = 6). After recording basal values of plasma insulin, glucose, triglycerides and systolic BP chronic mibefradil treatment was initiated in the CT and FT groups. Treatment was initiated at a concentration of 30 mg/kg/day (p.o. via daily oral gavage) as previously described [8]. One week after initiating mibefradil treatment, the animals in the F and FT groups were started on a 66% fructose diet. The fructose diet (66% fructose, 12% fat and 22% protein; Teklad Labs, Madison, WI, USA) had an electrolyte, protein and fat content very comparable to the standard rat chow. Beginning at week 9, BP, plasma insulin, glucose and triglycerides were measured each week for the next 4 weeks. Insulin sensitivity (IS) was estimated by comparing the ratios of plasma insulin to glucose (5 h fasted) in the experimental groups at week 12.

2.1.2. Reversal protocol

In this study the effects of mibefradil were examined after the development of hyperinsulinemia and hypertension in FH rats. Male Sprague-Dawley rats that had been treated with fructose diet for 8 weeks (and their age- and weight-matched control rats) were divided into: C (n = 6), CT (n = 6), F (n = 6) and FT (n = 6). Mibefradil treatment was started in the CT and FT groups (30 mg/kg/day p.o. via daily oral gavage) for 2 weeks. Plasma glucose, insulin, triglycerides and BP were determined before and after treatment.

2.2. BP measurement

Systolic BP was measured in conscious rats using the indirect tail-cuff method without external preheating [28]. The animals were preconditioned to the experimental procedure before the actual measurements were conducted. In this method, the reappearance of pulsations on gradual deflation of the BP cuff are detected by a photoelectric sensor and are amplified and recorded digitally as the systolic BP. An average of 5 such readings was taken to obtain the individual systolic BP. We have validated readings obtained by this method by comparison with those
obtained by direct intra-arterial cannulation. Recorded pressures were similar (within 5 mmHg) to those obtained by direct cannulation; similar results have been reported by other laboratories [7,28,29].

2.3. Biochemical measurements

Blood samples (5-h-fasted) were centrifuged for 15 min at 15,000 rpm using a desktop centrifuge (Biofuge 17R, model 2752, Baxter) to separate plasma. Plasma samples were stored at −70°C until further analyzed. Plasma glucose was measured by the glucose oxidase method, using a diagnostic kit from Boehringer Mannheim (Laval, Quebec, Canada). Plasma triglycerides were determined colorimetrically using a kit from Boehringer Mannheim. Plasma insulin was assayed using a double-antibody radioimmunoassay, using a kit from Linco Research Inc. Diagnostics Inc. (St. Louis, MO, USA) using a guinea-pig anti-rat insulin antibody and rat insulin standards.

2.4. Statistical analysis

Differences among groups were evaluated using multivariate analysis of variance (MANOVA), using a Number Cruncher Statistical Program (NCSS). Values are expressed as means ± s.e. In this study, a probability of \( P < 0.05 \) was taken to indicate a significant difference between the means. Once the MANOVA detected a significant difference in the mean vector, the individual variables were analyzed by employing the Newman-Keuls test for multiple comparisons. Comparisons of insulin/glucose ratios were done using a Student’s \( t \)-test (Fig. 2).

3. Results

3.1. General characteristics

The F group exhibited hyperinsulinemia and hypertension when compared to the C group (average of weeks

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>CT</th>
<th>F</th>
<th>FT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma glucose (mM)</strong></td>
<td>Week 6</td>
<td>8.1±0.5</td>
<td>7.8±0.2</td>
<td>7.5±0.1</td>
</tr>
<tr>
<td></td>
<td>Avg. weeks 9–12</td>
<td>7.4±0.1</td>
<td>7.6±0.1</td>
<td>7.4±0.1</td>
</tr>
<tr>
<td><strong>Plasma insulin (ng/ml)</strong></td>
<td>Week 6</td>
<td>2.8±0.1</td>
<td>2.5±0.08</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td></td>
<td>Avg. weeks 9–12</td>
<td>2.3±0.07</td>
<td>1.4±0.3  ( ^b )</td>
<td>3.2±0.1 ( ^a )</td>
</tr>
<tr>
<td><strong>Plasma triglycerides (mM)</strong></td>
<td>Week 6</td>
<td>1.4±0.3</td>
<td>1.3±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td></td>
<td>Avg. weeks 9–12</td>
<td>1.6±0.3</td>
<td>1.2±0.2</td>
<td>5.4±0.8   ( ^* )</td>
</tr>
</tbody>
</table>

\( ^a \) \( P < 0.05 \), different from C, CT and FT. \( ^b \) \( P < 0.05 \), different from C, CT and F. \( ^* \) \( P < 0.05 \), different from C and F.

Fig. 1. Plasma insulin levels (A) and systolic BP (B) in the 4 experimental groups: (C, ○), (CT, ●), (F, △) and (FT, ▲). Values are mean ± s.e. 9–12, insulin: 3.2 ± 0.1 vs. C 2.3 ± 0.07 ng/ml, \( P < 0.05 \), Fig. 1A, systolic BP: 148 ± 3 vs. untreated C 121 ± 1 mmHg, \( P < 0.002 \), Fig. 1B). The presence of elevated insulin levels in the face of normal glucose levels (Table

Fig. 2. Insulin sensitivity estimated by comparing the insulin to glucose ratios (5-h-fasted values) at week 12 of the prevention study. \( ^* \) \( P < 0.05 \) different from C, CT and FT.
1) is indicative of insulin resistance; we have previously demonstrated that FH rats are markedly insulin-resistant when assessed using the euglycemic hyperinsulinemic clamp [3]. In this study, IS was estimated by comparing the insulin/glucose ratio among groups. Using this index, the F rats exhibited a decreased IS when compared to the C group (Fig. 2). The F group also exhibited increases in triglycerides when compared to the C group (Table 1, Fig. 3).

3.2. Effects of mibefradil treatment before the development of hyperinsulinemia and hypertension in FH rats (prevention study)

Chronic mibefradil treatment of the F group caused marked and sustained decreases in both plasma insulin levels (average of weeks 9–12: 1.6 ± 0.08 ng/ml, \( P < 0.004 \)) and systolic BP (average of weeks 9–12: 123 ± 1 mmHg, \( P < 0.001 \)) (Fig. 1A). This was associated with an improvement in IS in the FT group (Fig. 2). Although the drug decreased insulin levels in the CT group, it did not affect BP (discussed later). Additionally, chronic mibefradil treatment attenuated the development of hypertriglyceridemia in the FT group (Table 1, Fig. 3).

3.3. Effects of mibefradil treatment after the development of hyperinsulinemia and hypertension in FH rats (reversal study)

The effects of mibefradil treatment on various parameters determined in this study are described in Table 2. Treatment of FH rats (after 8 weeks of fructose feeding) reversed the elevated BP, insulin and triglyceride levels in the FT group. Treatment had no effect on the glucose levels in either group.

4. Discussion

This is the first report to demonstrate that the antihypertensive effects of a specific T-type calcium channel blocker, mibefradil, are associated with sustained and marked re-

---

Table 2

General characteristics of rats in the 4 experimental groups in the reversal study

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>CT</th>
<th>F</th>
<th>FT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma glucose (mM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>7.3 ± 0.4</td>
<td>7.1 ± 0.4</td>
<td>7.9 ± 0.4</td>
<td>7.5 ± 0.1</td>
</tr>
<tr>
<td>After</td>
<td>7.4 ± 0.1</td>
<td>7.6 ± 0.1</td>
<td>7.4 ± 0.1</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td><strong>Plasma insulin (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.4 ± 0.3</td>
<td>2.1 ± 0.2</td>
<td>3.8 ± 0.3 ( b )</td>
<td>3.6 ± 0.2 ( b )</td>
</tr>
<tr>
<td>After</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>3.6 ± 0.3 ( a )</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td><strong>Plasma triglycerides (mM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.8 ± 0.3</td>
<td>1.6 ± 0.1</td>
<td>4.2 ± 0.3</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>After</td>
<td>1.6 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>5.4 ± 0.8 ( a )</td>
<td>2.6 ± 0.5 ( b )</td>
</tr>
<tr>
<td><strong>Systolic BP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>132 ± 2</td>
<td>128 ± 2</td>
<td>153 ± 4 ( b )</td>
<td>148 ± 3 ( b )</td>
</tr>
<tr>
<td>After</td>
<td>130 ± 2</td>
<td>128 ± 2</td>
<td>149 ± 4 ( a )</td>
<td>121 ± 3</td>
</tr>
</tbody>
</table>

Fructose hypertensive rats (8 weeks fructose-fed) and their age and weight matched control rats were divided into the 4 experimental groups as shown above. Plasma glucose, insulin, triglycerides (5-h-fasted values) and systolic blood pressure were determined before and after (2 weeks) mibefradil treatment. Values are means ± s.e. \( a P < 0.05 \), different from corresponding C, CT and FT. \( b P < 0.05 \), different from corresponding C and CT.
ductions in plasma insulin and improvement in IS. The treatment was effective in lowering the triglyceride levels in FT rats in both the prevention and reversal protocols employed. These data indicate that mibefradil exhibits beneficial effects on carbohydrate and lipid metabolism in addition to its antihypertensive effects.

Several mechanisms have been proposed to link hyperinsulinemia to hypertension including insulin-induced antinatriuresis, stimulation of the sympathetic nervous system, alterations in calcium transport systems and insulin-mediated alterations in vascular smooth muscle (VSM) tone (for review, see Ref. [1]). Of these mechanisms, interest has focused on the action of insulin on the VSM [9]. Recent reports indicate that insulin may attenuate the contractile responses to a variety of vasoactive agents and cause decreases in VSM tone and reactivity [11]. This has led to the hypothesis that in states of insulin resistance, insulin-induced vasodilation may be blunted, which may contribute to the development or maintenance of elevated BP [9]. In support of this view, we have recently found that insulin-mediated attenuation of vasoactive responses to angiotensin II are blunted in insulin-resistant FH rat aortae [30].

Interestingly, a decrease in plasma insulin levels in the control-mibefradil-treated rats did not cause a decrease in BP in the prevention study. The question arises as to why hyperinsulinemia causes hypertension in insulin-resistant rats without doing so in insulin-sensitive control animals. One possibility is that insulin-sensitive tissues would increase glucose utilization in response to the increase in insulin levels, which may initiate vasodilatory responses in order to increase local blood flow. By contrast, insulin resistance could blunt such vasodilatory effects [30]. As stated in the earlier paragraph, in support of this we have recently demonstrated that insulin attenuates the vasoconstrictor effects of angiotensin II in control rat arteries; however, this effect is blunted in FH rats [30]. As altered blood flow in resting muscle is considered to represent a possible factor responsible for the development of insulin resistance, it is possible that in insulin-sensitive cases the insulin–glucose system may respond to the vasodilation evoked by calcium channel blockers in a different way from insulin-resistant hypertensive states vs. the former control situation [35].

As discussed earlier, multiple drug interventions that possess insulin-sensitizing properties lead to concurrent decreases in plasma insulin levels and BP in FH rats [1]. By contrast, diverse antihypertensive-vasodilator agents (angiotensin-receptor blockers, peripheral alpha antagonists, angiotensin-converting enzyme inhibitors) have also been shown to improve insulin sensitivity and decrease plasma insulin levels [4,5]. In the former case, the antihypertensive effects of insulin sensitizers have been attributed to their ability to counter hyperinsulinemia and thereby correct the hypertensinogenic mediator(s) linking insulin to BP (discussed above). On the other hand, the ability of vasodilator-antihypertensive agents to increase insulin sensitivity may be an indirect consequence of drug-induced vasodilation and hence an increased blood flow to insulin-sensitive tissues [10]. On the basis of these observations, it has been speculated that some common mechanism (such as increases in intracellular VSM calcium) may underlie both the expression of insulin resistance/hyperinsulinemia and vasoconstriction [10]. Thus, on the one hand, insulin resistance results in vasoconstriction, while on the other hand vasoconstriction results in decreases in blood flow to insulin target tissues, which further worsens insulin resistance. As discussed elegantly by Kotchen [10], if the cycle is interrupted by agents that directly improve insulin sensitivity or by vasodilators that improve blood flow, the final outcome is an improvement in insulin sensitivity, a decrease in plasma insulin levels and a decrease in BP. A diagrammatic representation of this hypothesis is presented in Fig. 4.

![Fig. 4. Hypothetical model linking insulin resistance, vascular smooth muscle tone and elevated blood pressure.](image-url)
An issue that merits attention are studies that have associated calcium channel antagonists with the development of diabetes, impaired glucose tolerance and insulin resistance, presumably through blocking calcium-induced insulin secretion [18–21]. However, the balance of published work suggests that at hemodynamically active doses, they do not normally interfere with insulin release nor impair glucose tolerance in man [31–33]. This is further supported by animal studies that report that calcium channel blockers inhibit insulin secretion in vitro but not in vivo [18]. Comparative studies indicate that diltiazem, verapamil, nifedipine and flunarizine are neutral towards carbohydrate metabolism [31], while the calcium channel blocker, proglitazone, markedly attenuates the development of hyperinsulinemia in rats [17]. By marked contrast, L-NAME, L50358 markedly inhibits insulin secretion in vivo [31]. Thus, to reach a general consensus regarding the effects of calcium antagonists on insulin sensitivity is difficult as the effects vary depending on the agent investigated.

If one assumes that the beneficial effects of vasodilators compounds (ACE inhibitors, angiotensin II antagonists, peripheral alpha antagonists) on insulin sensitivity lie in their ability to increase skeletal muscle blood flow and counter insulin resistance, then it is difficult to construe why there is such marked variation in the effects of calcium channel blockers on insulin sensitivity. The current data on dihydropyridine-type calcium channel blockers (e.g., nifedipine) is conflicting [37,38] while the non-dihydropyridine derivative calcium antagonists (verapamil, diltiazem) appear to neither improve nor worsen insulin sensitivity in essential hypertension [15,37]. It is important to note that mibefradil [(1,5,2,5)-2(2-(3-(benzinidazolyl)propyl)methyl-amino)ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphthyl methoxyacetate dihydrochloride] represents a novel non-dihydropyridine calcium channel blocker that binds to the same site as verapamil, yet does not reduce myocardial force in either normal or diseased hearts [22,23]. It appears to be the only calcium antagonist that completely blocks the T-type calcium channels. Thus, it is possible that the unique pharmacological properties of this compound may distinguish it from other calcium antagonists; however, this remains to be determined.

The discussion of the insulin hypothesis of hypertension cannot be complete without highlighting the relationship between hyperinsulinemia and the sympathetic nervous system. Although hyperinsulinemia causes marked sympathetic activation, it paradoxically attenuates the vasoconstrictor effects in vitro and causes vasodilation in vivo [39]. Preliminary studies from our research group have evaluated the role of the sympathetic nervous system in modulating FH in rats. Interestingly, we have observed that removing the sympathetic influence (by chemical sympathectomy) completely obliterates the development of both hyperinsulinemia and hypertension in fructose-fed rats [36]. Furthermore, sympathectomy restores the ability of insulin to regulate vascular smooth muscle tone (S. Verma, S. Bhanot, L. Yao, I. Laher and J. McNeill, unpublished observations, September 1996). These data suggest that an intricate relationship exists linking hyperinsulinemia, insulin resistance, elevated sympathetic discharge, and the vascular effects of insulin in states of hypertension (independent of obesity). Given the complexity of mechanism(s) and their interdependence, the dissection of cause and effect is difficult; however, it is possible that the beneficial effects of mibefradil may be secondary to a decrease in sympathetic nervous system activity and a correction of the aforementioned pathways.

It may be argued that the effects of mibefradil on plasma insulin levels are secondary to inhibition of calcium channels in the pancreatic beta cells and a reduction in insulin secretion. As discussed earlier, in vitro studies do demonstrate that calcium antagonists may inhibit pancreatic insulin release; however, this effect does not appear to occur in vivo at hemodynamically effective doses. Furthermore, if mibefradil were to decrease insulin release, it would in turn lead to hyperglycemia, which was not the case in either the CT or FT groups. Thus, it is reasonable to suggest that the effects of the drug on insulin levels were independent of changes in pancreatic insulin release.

Finally, a recent report on fructose-induced hypertension has documented an interesting finding that deserves mention. The study deals with the effects of dietary fructose versus dietary magnesium deficiency in the etiology of hypertension in this model. The authors hypothesize that it was the magnesium deficiency in the fructose diet that lead to fructose-induced insulin resistance [34]. The fructose diet is reasonably matched for sodium and potassium with control rat chow but is low in magnesium (one third of control chow). The authors reported that when rats were fed a high fructose diet that had a magnesium content similar to that present in normal rat chow, the rats did not develop insulin insensitivity or an increase in BP. This was in contrast to the rats fed the conventional fructose diet, which did exhibit insulin resistance, hyperinsulinemia and hypertension. Although insulin sensitivity was assessed by the hindquarter perfusion method (which is not an accurate measure of in-vivo insulin sensitivity), the results were interesting, especially since several recent reports suggest that magnesium deficiency may cause insulin resistance. In our experiments, the fructose diet was deficient in magnesium and since serum magnesium levels were not measured, we cannot rule out the possibility that dietary magnesium deficiency could be partly/fully responsible for causing insulin resistance in fructose-fed rats. However, regardless of the mechanism causing insulin resistance in FH rats, the consequent metabolic and hemodynamic abnormalities that occur are prevented by both insulin sensitizers and vasodilators [this study]. Furthermore, results from our insulin implant studies [2,3] clearly indicate that insulin resistance, hyperinsulinemia and hypertension are
very closely related in these rats. Therefore, the findings from the present study mentioned above do not contradict the hypothesis in any way but rather shed light on one of the possible mechanisms underlying the severe insulin insensitivity observed in FH rats.

In summary, the present study is the first report of long-term administration of a T-type calcium channel blocker in hyperinsulinemic, insulin-resistant and hypertensive rats. Chronic mibebradil treatment of FH rats both prevents and reverses the development of hyperinsulinemia, hypertriglyceridemia and elevated BP. Whether the decrease in insulin levels is due to a direct effect of mibebradil to improve insulin sensitivity or is secondary to an improvement in blood flow to insulin target tissues, is an important question that needs to be further examined. As a large number of essential hypertensive subjects have defects in carbohydrate and lipid metabolism (that fail to respond to conventional therapy), further experimental and clinical studies documenting the beneficial effects of mibebradil on these parameters merit attention.

Acknowledgements

The study was supported by a grant from the Heart and Stroke Foundation of B.C. and Yukon. Subodh Verma is a Medical Research Council of Canada Fellow. Sanjay Bhanot is the recipient of a Heart and Stroke Foundation of Canada post-doctoral Fellowship. The gift of mibebradil from Hoffmann La Roche (Basel, Switzerland) is greatly appreciated. The assistance of Aspasia Michoulas and Dr. Linfu Yao with the reversal study is greatly appreciated. The authors thank Ms. Violet Yuen for her help with the hypothetical model depicted herein.

References

[31] Gristwood RW, Furman BL, Lienas J, Jauregui J, Berga P. The calcium channel blocker LAS 30538, unlike nifedipine, verapamil,


