

Insulin resistance and changes in chronotropic responses to adrenergic and cholinergic agonists in isolated rat atria

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ABSTRACT

Objectives: To determine the effects of β -adrenoceptor and muscarinic agonists on the contractile rate of isolated right atria of rats fed a high fructose diet with or without rosiglitazone (RSG).

Materials and Methods: Male Sprague Dawley rats were assigned to four groups and given ad libitum access to one of the following diets: standard chow, standard chow supplemented with 4 mg/kg/day RSG, a high fructose diet and a high fructose diet with 4 mg/kg/day RSG. All the groups were maintained on these regimens for three weeks with weekly measurements of systolic blood pressure and body weight. At the end of the three weeks, the rats were exsanguinated and the hearts were rapidly removed following which blood glucose, insulin and lipid profiles were estimated. The right atria were isolated from the heart and their responsiveness to sympathetic and parasympathetic agonists was studied.

Results: Basal, spontaneous, isolated atrial pacemaker rate was diminished in fructose-fed rats. The maximum pacemaker rate to isoproterenol or norepinephrine was unchanged in fructose-fed rats. Further, the increase in atrial rate and half maximal effective concentration values for each agent were also unaffected. The sensitivity to negative chronotropic action of acetylcholine was enhanced in fructose fed rats, whereas the response to carbachol was unchanged. The increased sensitivity to acetylcholine was restored by RSG treatment.

Conclusion: High fructose diet induced insulin resistance and hypertension with alterations in basal spontaneous pacemaker, enhanced sensitivity to cholinergic agonist without any changes in the response to adrenergic and cholinergic receptor activation. Treatment with insulin sensitizer rosiglitazone was able to prevent all these changes. The present study suggests that rosiglitazone may have effect on the cardiovascular system in addition to the insulin sensitising action.

KEY WORDS: Insulin resistance, β -adrenoceptor, muscarinic receptor, isolated atria, insulin sensitizer

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Clinical evidence suggests that insulin resistance (IR) increases cardiovascular risk in patients with type-2 diabetes.^[1,2] Detectable cardiac dysfunction has been reported to occur as early as the glucose intolerance phase characterized by hyperinsulinemia and dyslipidemia that follows insulin resistance.^[3] Cardiac muscles exhibit insulin resistance in various pathological conditions such as obesity, hypertension and coronary heart diseases.^[4-6] Recently, it has been shown that hypertrophied muscle, which develops as a compensatory mechanism during heart failure, also exhibits IR^[7] and contributes to the progressive deterioration in myocardial function.^[8] Insulin directly activates the sympathetic nervous system^[9] hence, compensatory hyperinsulinemia developed

during IR may contribute towards increased heart rate and hypertension.^[10,11] Further, chronic hyperinsulinemia is found to be accompanied by reduced cardiac vagal activity.^[12] However, to our knowledge, no data is available regarding the influence of IR on autonomic receptor responsiveness of the heart.

Diabetes and the metabolic syndrome (hypertension, IR and obesity) adversely affect the cardiac autonomic function and are associated with increased risk of cardiovascular events.^[13-15] Elevated fasting insulin levels have been shown to increase sympathetic activity and heart rate.^[16] The IR syndrome also predisposes individuals to cardiovascular hyperresponsiveness to sympathetic stimulation and has been shown to reduce heart rate variability.^[17] Further, chronic

hyperinsulinemia is associated with heightened sympathetic tone and decreased vagal tone.^[18,19] It would be interesting to know whether IR could change the atrial pacemaker rate and autonomic responsiveness of the cardiac tissue. Hence in the present study, a high fructose diet was chosen as a model to induce IR to determine the effect of β -adrenoceptor and muscarinic agonists on the spontaneous rate of isolated right atria and the effect of rosiglitazone (RSG) on IR-induced changes in atrial pacemaker rate changes and cardiovascular responsiveness to the above agonists.

Materials and Methods

All the experimental procedures were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India. The study was approved by the Institutional Animal Ethics Committee, K.L.E.S's College of Pharmacy, Belgaum, India.

Twelve week-old male Sprague Dawley rats obtained from the National Centre for Laboratory of Animal Sciences, National Institute of Nutrition, Hyderabad, India, were housed individually in a controlled environment with 12-h light / dark cycles and free access to food and water. After a seven day acclimation period, they were randomly assigned to different experimental groups.

Animal model

The animals were randomized to four groups and given ad libitum access to one of the following diets: standard chow (control, n = 7), standard chow supplemented with 4 mg/kg/day RSG (RSG, n = 8), a diet high in fructose (fructose-fed, n = 9), a diet high in fructose with 4 mg/kg/day RSG (fructose + RSG, n = 9).^[20] The fructose content provided 60% of the total calories in the diet prepared in the laboratory as follows: (g/kg) casein high protein 207.0; DL-methionine 3.0; fructose 600.0; lard 50.0; cellulose 79.81; mineral mix 50.0; zinc carbonate 0.04; vitamin mix 10. The amount of RSG to be mixed with chow was calculated depending upon the average quantity of chow diet consumed by each rat in 24 hours.

Animals were maintained on these regimens for three weeks and the systolic blood pressure and body weights were measured weekly. At the end of the third week, food was withdrawn for 16 h and about 3-4 ml of blood was collected through the retro-orbital plexus under light ether anaesthesia. Blood samples were immediately centrifuged (3000 g for 20 min) and commercial kits used to assay plasma for glucose by GOD / POD method [Span Diagnostic Ltd. Sachin, Surat] as well as for cholesterol and triglycerides by an enzymatic method [Accurex Bio systems, Mumbai and Crest Bio-systems, Goa respectively]). Plasma insulin was measured using a standard radioimmunoassay kit (07260102; ICN Pharmaceuticals Costa Mesa). The degree of IR was estimated using Homeostasis Model Assessment (HOMA) as follows:

Index of insulin resistance = [insulin (in μ U) X glucose (in mmol/L)]/22.5.^[21]

The rats were then exsanguinated and the hearts isolated for autonomic responsiveness study.

Measurement of blood pressure

Systolic blood pressure was measured in conscious rats by the tail cuff method.^[22] All the rats were preconditioned

to the experimental conditions before actual measurements were conducted. At the time of the experiment, the rats were placed in a constant temperature chamber (32°C) for 30 min. Thereafter, each animal was placed in a rat holder, the tail cuff and pulse sensor was placed on the tail and connected to a blood pressure transducer (Harvard Apparatus), which in turn was connected to a computer that displayed the blood pressure. Systolic blood pressure was measured at the point where the reappearance of pulsations was detected by the pulse sensor. Eight readings were obtained for each rat. The highest and the lowest measurements were rejected and the average of the remaining was taken as systolic blood pressure of that individual rat.

Isolated right atrial preparation

The hearts were rapidly removed from the exsanguinated rats and placed in a beaker containing Krebs-Henseleit buffer. After expressing the blood from the heart, the ventricle tissue, fat and connective tissue were trimmed off. The right atrium was isolated and immediately placed in a beaker containing ice-cold oxygenated Krebs-Henseleit buffer, pH 7.4 and gassed with 95% O₂ : 5% CO₂. The composition of the Krebs-Henseleit buffer was (mmol/L) NaCl 124; KCl 4.75; MgCl₂ 1.30; CaCl₂ 2.25; NaHCO₃ 25.0; NaH₂PO₄ 0.6 and Dextrose 10.0. The right atrium was then suspended vertically in a 20 ml tissue bath containing the above medium. One end of the atria was attached to a tissue holder and the other end was mounted by a silk thread to an isometric force transducer (BioPac TSD 104 A Santa Barbara, California) connected to a computerized data acquisition system (BioPac Systems, Inc. MP100A-CE Santa Barbara, California). The atria were allowed to beat spontaneously and were equilibrated at 37°C for 60 min. The Krebs bicarbonate solution was replaced every 15 min. Resting tension of 1 g in the tissue was maintained throughout the experiment.

Experiments were designed to evaluate the effect of IR on the chronotropic responsiveness of the atrial tissue to adrenergic and cholinergic agonists. Cumulative dose-response curves for the adrenergic agonists (norepinephrine and isoproterenol) and the cholinergic agonists (acetylcholine and carbachol) were obtained by sequential addition of drug to the bathing medium. The changes in atrial rate due to the adrenergic and cholinergic drugs were recorded. Preparations used for adrenergic and cholinergic studies were isolated from the same animals. When cumulative dose-response curves were being obtained, the next higher concentration of the agonist was added to the bath solution only after the tissue gave a steady-state response at the previous level for about 60-90 seconds.

Materials

Acetylcholine chloride, carbachol chloride, dl-isoproterenol hydrochloride and dl-norepinephrine bitartrate were purchased from Sigma Chemicals (St.Louis, MO, USA). RSG was a gift sample from Dr. Reddy's Laboratory Ltd., Hyderabad, India. All other chemicals were reagent grade. Stock solutions of adrenergic and cholinergic agonists were prepared in 1% ascorbic acid and 5% sodium dihydrogen orthophosphate solution respectively; further dilutions were made with Krebs-Henseleit solution.

Statistical analysis

Data are expressed as the mean \pm SE (standard error).

Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test or by Student's t-test wherever appropriate. The maximum response produced by the agonists (Emax) and half-maximal effective concentration (EC50) values were obtained by graphical evaluation of individual dose-response curves using statistical software GraphPad Prism (Graph pad Software). $P < 0.05$ was considered to be statistically significant.

Results

General characteristics of animals

All four treatment groups gained weight to a similar extent over the study period without any significant differences [Table 1]. At the end of the experimental period, fructose-fed rats showed a significant increase in levels of fasting insulin ($P < 0.001$) and triglyceride ($P < 0.05$) as well as the IR index ($P < 0.001$). Treatment with RSG completely restored these values in fructose-fed rats. Fasting glucose and total cholesterol levels did not differ in control and fructose-fed rats. In fructose-fed rats, the systolic blood pressure increased significantly from the second week onwards and remained elevated till the end of the experimental period. Intake of RSG even with the high fructose diet completely prevented the development of hypertension in fructose-fed rats. RSG did not however, affect any of these parameters in control rats.

Responsiveness of Spontaneous Pacemaker

Basal rate of spontaneous pacemaker

There was a reduction in the basal pacemaker rate (right atria) in fructose-fed rats ($P < 0.05$) [Table 2]. The depression in the atrial rate associated with insulin resistance was reversed with RSG treatment. However, here was no alteration in the atrial rate in RSG-supplemented control chow-fed rats [Table 2].

Adrenergic responsiveness

Both norepinephrine and isoproterenol produced similar chronotropic responses when applied to the atria of the rats of the four groups. While there were no significant differences in the maximal atrial pacemaker rates after application of norepinephrine and isoproterenol in the control, chow + RSG and fructose-fed rats, administration of RSG along

with the high fructose diets results in an increase in these atrial rates.

Cholinergic responsiveness

The isolated atrial preparation from high fructose-fed rats showed an enhanced sensitivity to acetylcholine as seen with the leftward shift in the dose-response curve [Figure 1; Table 3]. The increased sensitivity to acetylcholine was restored to control levels by RSG treatment. In contrast, responsiveness to carbachol (a cholinergic agonist not readily metabolised by acetylcholinesterase) was not affected by high fructose diet-induced IR or RSG treatment as seen with acetylcholine. The maximum spontaneous rates were found to be unchanged in control and fructose-fed rats during exposure to the cholinergic agonists. However, there was a slight increase in these rates after application of acetylcholine and carbachol in RSG-treated rats as compared to the rats of the corresponding groups not receiving RSG.

Figure 1: Effect of acetylcholine on spontaneous rate in right atria isolated from control, rosiglitazone-treated, fructose-fed and fructose plus rosiglitazone treated insulin resistant rats. Preparations ($n = 7-9$ /group) were isolated three weeks after feeding the respective diets. Dose-dependent effects of acetylcholine were examined by cumulative addition; vertical bars represent \pm SE

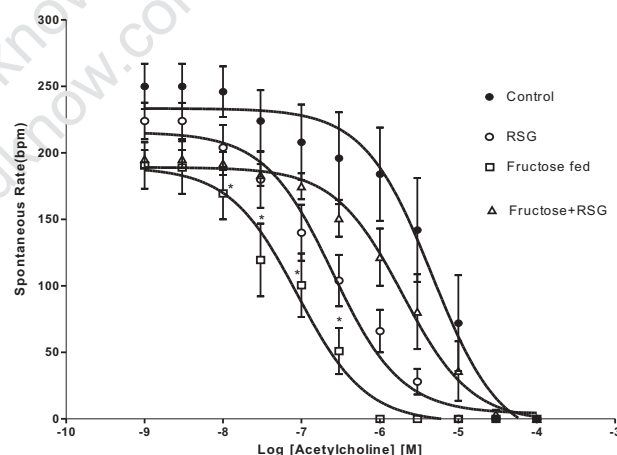


Table 1

General characteristics of the rats in the four experimental groups

Parameter	Control	Chow + RSG	Fructose-fed	Fructose + RSG	One-way ANOVA		
					F,	df,	P value
Gain in body weight, g	65.47 \pm 6.82	59.12 \pm 3.60	55.30 \pm 16.20	60.12 \pm 4.86	11.38,	3, 20,	> 0.05
Systolic blood pressure, mm Hg	90.4 \pm 16.18	110.2 \pm 6.42	148 \pm 6.70*	106.7 \pm 3.73†	102.6,	3, 20,	< 0.01
Plasma glucose, mg/dL	65.94 \pm 5.98	71.60 \pm 5.38	72.56 \pm 4.19	68.39 \pm 2.86	0.811,	3, 20,	> 0.05
Plasma Insulin, μ IU/ml	46.37 \pm 7.32	39.17 \pm 4.75	83.17 \pm 5.54*	52.33 \pm 4.31†	12.17,	3, 20,	< 0.001
Insulin resistance Index (HOMA)	6.72 \pm 0.901	7.85 \pm 1.82	16.49 \pm 1.82*	8.79 \pm 0.69†	12.24,	3, 20,	< 0.001
Plasma cholesterol, mg/dl	67.17 \pm 3.25	57.33 \pm 4.92	67.1 \pm 3.07	77.17 \pm 1.82	6.59,	3, 20,	> 0.05
Plasma triglyceride, mg/dl	99.17 \pm 4.02	86.50 \pm 7.99	115.0 \pm 7.01*	88.17 \pm 2.67†	4.99,	3, 20,	< 0.05

Values are mean \pm SE; $n = 7-9$ rats per group; HOMA: Homeostasis model assessment. * $P < 0.05$ -Significantly different from control group. † $P < 0.001$ -Significantly different from fructose-fed group

Table 2

Basal atrial rate, maximum chronotropic responses and EC₅₀ values of nor-epinephrine and isoproterenol in isolated right atria from chow fed and fructose fed rats treated with or without rosiglitazone

Variable	Control	Chow + RSG	Fructose-fed	Fructose + RSG	One-way ANOVA		
					F	df	P value
Basal rate, beats/min	205 ± 7.60	200 ± 13.7	172 ± 11.49*	206 ± 9.33†	3.67,	3, 32,	< 0.05
Norepinephrine							
Maximum rate, beats/min	312.2 ± 12.41	282.1 ± 4.63	316.9 ± 6.45	342.4 ± 7.99	9.31,	3, 29,	0.05
EC ₅₀ (x10 ⁻⁸)	5.67 ± 4.13	2.80 ± 7.99	1.14 ± 4.47	5.26 ± 1.27	3.42,	3, 27,	> 0.05
% Increase in rate, beats/min	47.89 ± 5.59	42.11 ± 1.47	90.56 ± 14.10*	65.3 ± 9.26	5.94,	3, 32,	< 0.05
Isoproterenol							
Maximum rate, beats/min	294.5 ± 10.72	288.3 ± 1.003	290.7 ± 6.85	310.6 ± 6.98	2.08,	3, 30,	> 0.05
EC ₅₀ (x10 ⁻⁷)	4.30 ± 2.02	1.79 ± 3.48	2.14 ± 5.98	2.13 ± 3.90	1.69,	3, 27,	> 0.05
% Increase in rate, beats/min	43.44 ± 4.03	44.22 ± 1.66	72.30 ± 9.09*	49.89 ± 4.43	5.70,	3, 33,	< 0.05

Values are mean ± SE; n = 7-9 rats per group. EC50, half maximal effective concentration. *P < 0.05 - Significantly different from control group. †P < 0.05 - Significantly different from fructose-fed group

Table 3

Maximum chronotropic responses and EC₅₀ values for acetylcholine and carbachol in isolated right atria from chow fed and fructose fed rats treated with or without rosiglitazone

Variable	Control	Chow + RSG	Fructose-fed	Fructose + RSG	One-way ANOVA		
					F	df	P value
Acetylcholine							
Maximum rate, beats/min	198.5 ± 8.69	211.1 ± 12.22	188.9 ± 17.96	191.5 ± 6.80	0.78,	3, 25,	> 0.05
EC ₅₀ (x10 ⁻⁸)	7.48 ± 3.24	6.14 ± 2.64	1.86 ± 5.35*	5.06 ± 2.50†	2.47,	3, 25,	< 0.05
Carbachol							
Maximum rate, beats/min	175.7 ± 15.43	220.5 ± 12.12	173.1 ± 15.03	198.5 ± 5.31	3.32,	3, 25,	> 0.05
EC ₅₀ (x10 ⁻⁸)	6.61 ± 6.25	3.24 ± 8.50	2.15 ± 1.37	1.78 ± 6.34	0.97,	3, 24,	> 0.05

Values are mean ± SE; n = 7-9 rats per group. EC50, half maximal effective concentration. *P < 0.05 - Significantly different from control group. †P < 0.05 - Significantly different from fructose-fed group

Discussion

A high fructose diet leads to development of hyperinsulinemia, IR and hypertension within several weeks in rats.^[23] Sympatho-excitation has been reported to play an early and integral role in the final expression of elevated plasma insulin levels and blood pressure in rats fed with a high fructose diet.^[24] Further, acute insulin administration causes hemodynamic changes^[9] and an increase in cardiac output.^[25] The present study shows that a high fructose diet induces IR and hypertension; is associated with a decline in the basal pacemaker rate and enhanced sensitivity of right atria to acetylcholine but not with any changes in the responsiveness to adrenergic receptor activation.

Several recent reports have suggested that IR in cardiac tissue can be manifested by changes in the function of ion channels. Shimoni *et al.*^[26] have demonstrated that ventricular myocytes from insulin-resistant rats exhibited resistance to the normal ability of insulin to enhance delayed rectifier K⁺ currents. Further, Dutta *et al.*^[27] have reported that sucrose-induced insulin resistance prolonged electrically stimulated Ca²⁺ transients in rat cardiomyocytes. The present data provides little information regarding the mechanism for the decline

observed in the basal spontaneous pacemaker rate of right atria isolated from fructose-fed rats. Thus, it would seem that changes in ionic currents of the sinoatrial node may modify the spontaneous pacemaker rate.

It is well established that overstimulation of adrenoceptors may induce their desensitization^[28] leading to a decrease in receptor density and / or reduction of agonist efficacy.^[29] The high plasma epinephrine levels found in this experimental model of hypertension^[11] would therefore be expected to alter adrenoceptor-mediated chronotropic responses. However, current data suggests that fructose diet-induced IR does not affect the responsiveness to adrenergic agonists. The maximum spontaneous rate observed in the presence of the selective β-agonist, norepinephrine, and the nonselective β-agonist, isoproterenol, was not altered. In addition, no changes in EC₅₀ values of these agonists were observed. This data is consistent with the observations of Kamide *et al.*^[30] wherein the β-adrenoceptor density was not altered in the left ventricle of fructose-fed insulin resistant rats.

Atrial tissue isolated from insulin resistant rats showed a significant reduction in EC₅₀ values for acetylcholine, whereas the sensitivity to carbachol [a cholinergic agonist not readily metabolised by acetylcholinesterase (AChE)] was not

significantly affected. In addition, the maximum response to both the agonists was not changed in these tissues indicating no significant change in receptor density or efficacy. An earlier study had suggested that AChE activity can be measured by the pretreatment with di-isopropylfluorophosphate (DFP), an irreversible inhibitor of acetylcholinesterase.^[31] DFP treatment decreased the basal spontaneous pacemaker rate and abolished the enhanced sensitivity to acetylcholine in diabetic and insulin-treated diabetic rats. This data suggests that the enhanced sensitivity to the negative chronotropic action of acetylcholine in fructose-fed rats may be a result of diminished AChE activity.

The autonomic nervous system influences blood pressure and heart rate through baroreceptor mechanisms. A fructose-fed rat exhibits insulin resistance, hyperinsulinemia, normal fasting glucose concentration and hypertension.^[13] In addition, chronic fructose feeding of rats induces autonomic dysfunction due to impaired vagal reflex activity leading to a decrease in the parasympathetic drive to the heart.^[32] Previous reports have shown that chronic hyperinsulinemia is accompanied by cardiac vagal withdrawal in rats^[33] and humans.^[34] These observations suggest that increased sensitivity of right atria to acetylcholine observed in the present study could be a result of vagal withdrawal. Chronic vagal withdrawal induces muscarinic supersensitivity, developed either to augment or to preserve vagal control of heart.

Dunlap *et al.*^[35] showed that changes in vagal control of the heart at the presynaptic level could lead to upregulation of muscarinic receptors and reduced activity of AChE in the sinoatrial node. We speculate that hypersensitivity of the right atria to acetylcholine in the present study may be the result of decreased AChE activity in response to vagal withdrawal. However, no significant change in muscarinic receptor density was observed in the present study. The use of a muscarinic partial agonist like pilocarpine, whose actions are more sensitive to changes in the receptor number and / or coupling mechanisms,^[36] may throw more light on the mechanism of the increased sensitivity of the right atria to acetylcholine in fructose-fed rats.

In the current study, chronotropic response to β -agonists was unchanged in the right atria of fructose-fed rats but the percentage increases in spontaneous rate (beats/min) after application of the β -agonists were significantly higher compared to chow-fed rats. These observations indicate an enhanced responsiveness of β -adrenoceptors to agonists, which could be due to decreased spontaneous basal rate and enhanced sensitivity to parasympathetic stimulation. This notion is supported by the fact that cardiac tissue from a failing heart shows enhanced sensitivity towards sympathetic activation.^[34]

Steady state hypertension [mean arterial pressure (MAP) > 150 mm Hg] is also known to impair baroreflex activity.^[37,38] Contribution of hypertension to impaired vagal reflexes in fructose-fed rats is unlikely because a high fructose diet does not raise 24 h MAP^[39] beyond a mild to moderate extent (MAP < 150 mm Hg).^[40] Furthermore, a high fat diet was found to induce hypertension^[41] while pressure load-induced cardiac hypertrophy^[42] has been reported to downregulate cardiac muscarinic receptors. However, no change in E_{max} was observed in the present study for both Carbachol and Acetylcholine

indicating that there was no change in the muscarinic receptor density of atria from fructose-induced insulin resistant hypertensive rats. Taken together, it is conceivable that supersensitivity of right atria to acetylcholine in fructose-fed rats could be due to insulin resistance or hyperinsulinemia. This view is supported by our observations in RSG-treated normal and fructose-fed rats. Treatment with RSG prevented fasting hyperinsulinemia and hypertension in fructose-fed rats. Basal spontaneous rate and autonomic responsiveness of right atrial pacemaker from fructose plus RSG rats were identical to those of the control rats. Furthermore, RSG had no effect on these parameters in control rats. This data supports the view that insulin resistance or hyperinsulinemia may be the cause for observed changes in pacemaker activity.

Insulin resistance is etiologically related to hypertension. In the present study, rosiglitazone was found to decrease blood pressure substantially, which is consistent with the hypothesis that rosiglitazone decreases the blood pressure by improving the insulin resistance.^[43] Potential mechanisms for the antihypertensive effect of this drug (other than improvement of insulin resistance) include improved endothelium-dependent vasodilation, decrease in calcium influx and calcium sensitivity of the contractile apparatus^[44,45] and inhibition of endothelin-1 expression and secretion (in bovine vascular endothelial cells through activation) of PPAR- γ .^[46] Rosiglitazone appears to interrupt these sequences and prevents the development of insulin resistance, which probably explains why hypertension did not develop in RSG-treated animals.

Chronic fructose feeding in rats induces hyperinsulinemia, hypertriglyceridemia, insulin resistance and hypertension.^[23] Fructose-induced hypertriglyceridemia probably occurs due to decreased catabolism of very low-density lipoprotein-triglyceride^[47] and / or increased secretion of very-low density lipoprotein-triglyceride in the liver.^[48] Further, hypertriglyceridemia induces insulin resistance in skeletal muscle and adipose tissue.^[49] Consistent with the actions of the PPAR- γ pathway, rosiglitazone, a PPAR- γ agonist is reported to increase lipid uptake by the adipose tissue thus reducing triglyceride levels, which leads to increased insulin sensitivity in skeletal muscle and liver.^[50]

Regardless of the exact mechanism, the supersensitivity of right atria to acetylcholine as indicated by reduction of EC_{50} values to acetylcholine may be an index of altered control of the vagus on heart rate and this finding may exhibit important clinical implications. Imbalance of the sinoatrial node sensitivity to autonomic neurotransmitters and increased sympathetic activity in insulin resistance may favor the development of cardiac arrhythmia and such conditions might contribute to the increased risk of cardiac death in the state of insulin resistance.^[51]

To conclude, the fructose-induced insulin resistance and hypertension model presented in this study did not exhibit any changes in the potency or efficacy of β -adrenoceptors. However, a decrease in basal spontaneous pacemaker rate and enhanced sensitivity to cholinergic agonists might reflect an alternation in the ionic current and cholinesterase enzyme activity respectively. Furthermore, treatment with a clinically relevant insulin sensitiser (RSG) prevents the changes in spontaneous pacemaker rate associated with insulin resistance. Further

studies are needed to characterize the underlying cellular consequences and factors that contribute to the diminished basal pacemaker rate and enhanced sensitivity to cholinergic agonists.

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