Pharmacological Characterization of Pp-17, a \( \alpha/\beta \)-adrenoceptor Blocking Agent with Antihypertensive Effect

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ABSTRACT

Background: The purpose of the current study was to investigate the various pharmacological characteristics of a newly synthesized PP-17 [3-(2-hydroxy-3-isopropylamino-propoxy) benzaldehyde oxime] under the in vitro and in vivo conditions. Methods: Potency of PP-17 was investigated towards different \( \alpha \), \( \beta_1 \), \( \beta_2 \), and \( \alpha_1 \) adrenoceptor subtypes by using rat isolated right atria, uterus, distal colon, thoracic aorta preparations. Antihypertensive and metabolic activity was tested in left renal artery ligated and fructose induced hypertension model. Results: \( \text{pA}_2 \) values of PP-17 for \( \beta_1 \), \( \beta_2 \), \( \alpha_1 \) and \( \alpha_2 \) adrenoceptor were 7.0±0.1, 5.7±0.1, 6.0±0.1 and 7.0±0.1, respectively. The \( \beta_1/\beta_2 \) selectivity ratio was calculated and shows in the order of atenolol > PP-17 > labetalol > propranolol while \( \beta_2/\alpha_1 \) selectivity ratio was in the order of labetalol > PP-17. PP-17 (10 and 30 mg kg\(^{-1}\), p.o.) significantly decreased the mean arterial blood pressure and heart rate in both left renal artery ligated and fructose induced hypertension model. In addition, treatment with PP-17 (30 mg kg\(^{-1}\), p.o.) showed significant decrease in plasma TC and VLDL level. Conclusion: Our results suggest that PP-17 is a dual \( \alpha_1 \) and \( \beta_2 \)-adrenoceptor receptor antagonist having antihypertensive property.

Key words: Adrenoceptor receptor, fructose, mean arterial blood pressure, heart rate


INTRODUCTION

Hypertension is a circulatory disease characterized by sustained elevation of blood pressure. It is often defined as mild (borderline) or severe depending on the blood pressure levels. This disease can be genetic in origin (also termed primary or essential) or may occur as a secondary product of cardiac diseases such as congenital heart diseases or interactions with environmental factors, such as a high salt diet\(^1\).

\( \beta \)-Adrenoceptor antagonists are widely used in the treatment of hypertension and coronary heart disease. For the treatment of cardiovascular diseases, they may generally be divided into first, second and third-generation agents. The classic first-generation agents such as propranolol or timolol are nonselective for \( \beta_1 \) or \( \beta_2 \)-receptors and have no obvious ancillary cardiovascular effects. Second-generation compounds are those that exhibit selectivity for \( \beta \)-receptor subtypes, such as \( \beta_1 \)-selective compounds atenolol, metoprolol and betaxolol. The term "third-generation \( \beta \)-blocker" refers to the kind of blocking drugs that possess an ancillary cardiovascular action other than \( \beta \)-blockade. Examples include the \( \beta \)-blocker/vasodilator agent’s labetalol and carvedilol. Both labetalol and carvedilol are vasodilators because of \( \alpha \)-adrenergic blockade. Labetalol is characterized by similar affinities for \( \alpha \) and \( \beta \)-receptors. Carvedilol is also a \( \alpha \)-blocking agent but with approximately 2 to 3 fold selectivity for \( \beta_2 \)-versus \( \alpha_1 \)-receptors. Nevertheless, this degree of \( \alpha_1 \)-blockade is responsible for the moderate vasodilating properties of carvedilol.

During the past few years, studies demonstrated that most antihypertensive agents modify insulin sensitivity in parallel with alterations in the atherogenic lipid profile. \( \alpha \)-blockers and angiotensin converting enzyme inhibitors were found to improve insulin resistance and the profile of atherogenic lipids, whereas most of the calcium channel blockers were found to be metabolically inert. The diuretics and \( \beta \)-adrenoceptor antagonists further decrease insulin sensitivity and worsen...
3-Cl-hydroxy-3-isopropylamino-propoxy)-benzaldehyde oxime

Fig. 1: Chemical structure of PP-17

Dyslipidemia. The mechanisms by which β-adrenoceptor antagonist treatment exert its side effects are not fully understood but several possibilities exists such as significant body weight gain, reduction in enzyme activities (muscle lipoprotein lipase and lecithin cholesterol acyltransferase), alterations in insulin clearance and insulin secretion and, probably most important, reduced peripheral blood flow due to increase in total peripheral vascular resistance. Recent metabolic studies found beneficial effects of the newer vasodilating β-blockers, such as dilevalol, carvedilol and celiprolol, on insulin sensitivity and the atherogenic risk factors. In many hypertensive patients, elevated sympathetic nerve activity and insulin resistance are deleterious combination. Although conventional β-blocker treatment was able to take care of the former, the latter got worse: the newer vasodilating β-blocker generation seems to be capable of successfully treating both of them.

These advantages have initiated the search for some novel potential vasodilating β-blocker of greater selectivity toward β-receptor which also improves insulin sensitivity and dyslipidemia. We have been involved in development of new β-blockers for past few years. Recently we developed new chemical entity namely PP-17, having a propanolamine group at para position in benzaldehyde oxime ring PP-17 [3-(2-hydroxy-3-isopropylamino-propoxy)-benzaldehyde oxime] (Fig. 1), was synthesized from combination of m-hydroxybenzaldehyde and arylexypyrrolidone (the basic structure with β-blocking activity). This study was aimed to investigate the various pharmacological characteristics of PP-17 in different animal model of hypertension.

MATERIALS AND METHODS

Animals: Adult Wistar rats (100-250 g) of either sex were purchased from National Toxicology Center, Pune, India. Animals were housed in clean environment under 12:12 light-dark cycle at a temperature of 25±2°C and relative humidity of 55±5%. Food and water were available ad libitum. All procedures involving animals were approved by the Institutional Animal Ethical Committee of Poona College of Pharmacy.

β1-adrenoceptor assay: Male Wistar rats were used to isolate distal colon (2.5 cm in length). Distal colon was mounted in 25 mL organ baths (INCO, Ambala, India) containing Krebs bicarbonate buffer solution of composition (mM) NaCl (118), KCl (4.7), MgSO4 (1.2), KH2PO4 (1.2), CaCl2 (2.5), NaHCO3 (25), Disodium EDTA (0.03) and glucose (11.1). Temperature of physiological solution was maintained at 37°C and aerated with carbogen (95% O2 and 5% CO2). Spontaneous responses of aorta were recorded by connecting the upper end to the force transducer (T-305) connected to student physiograph (Bio-Devices, Ambala, India). The resting tension was maintained at 0.5 g during a 30 min equilibration period. Positive chronotrophic effect of isoproterenol was determined in the presence and absence of PP-17 or atenolol or propranolol or labetalol at different concentration. Sinus rate was assessed for 15 sec after the addition of each successive concentration of isoproterenol for 1 min.

β2-adrenoceptor assay: Female Wistar rats having estrus phase were used to isolate uterine horns. Uterine horn was mounted in organ baths (INCO, Ambala, India) filled with 25 mL of Locke ringer solution of composition (in mM) NaCl (154), KCl (5.6), NaHCO3 (6.0), CaCl2 (2.2), glucose (11.1), ascorbic acid 30 µM and sodium salt of EDTA 30 µM. Temperature of physiological solution was maintained at 37°C and aerated with carbogen (95% O2 and 5% CO2). To achieve a steady spontaneous contraction, an initial tension of 2 g was applied for 30 min. After the tissue was equilibrated, relaxant effect of isoproterenol was determined in the presence and absence of PP-17 or atenolol or propranolol or labetalol at different concentration.

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to stabilize. Relaxant effect of isoprenaline was determined in the presence and absence of PP-17 or atenolol or propranolol or labetalol at different concentration\textsuperscript{13}.

\textbf{\textit{\textalpha}_{1} Adrenoceptor assay:}} Thoracic aorta from male wistar rats were dissected and cleaned to remove fat and connective tissue. Endothelium was removed by gently rubbing the intimal surface with fine wires. The 3 mm length ring was mounted under 0.5 g of resting tension in organ baths containing Krebs solution of the following composition (mM): NaCl (118), KCl (4.7), CaCl$_2$ (1.25), KH$_2$PO$_4$ (1.4), MgSO$_4$ (1.1), glucose (10), NaHCO$_3$(25). Temperature of physiological solution was maintained at 37°C and aerated with carbogen (95% O$_2$ and 5% CO$_2$). Tissue was allowed to equilibrate for 60 min with periodic washes before starting experiments. Removal of functional endothelium was checked by the lack of any relaxation to acetylcholine (1 mM) in rings pre-contracted with noradrenaline (0.1 mM). Vasocontraction effect of phenylephrine was determined in the presence and absence of PP-17 or atenolol or propranolol or labetalol at different concentration\textsuperscript{13}.

\textbf{\textit{In vivo evaluation of \textbeta Adrenoceptor antagonistic activity:}} Wistar rats were anesthetized with urethane (1.25 g kg$^{-1}$, i.p.). Jugular vein was cannulated for administration of PP-17 or atenolol or labetalol and Carotid artery was cannulated for blood pressure measurement. Body temperature was maintained at 37°C with a help of thermal blanket. The carotid artery was connected to pressure transducer (SS13L, BIOPAC Systems, Inc., Santa Barbara, CA) for measurement of MABP and heart rate. Dose response curve of isoprenaline was constructed for increase in heart rate (tachycardia) and fall in mean arterial blood pressure (MABP) after intravenous injection (0.3, 1 and 3 \textmu g kg$^{-1}$) in presence and absence of PP-17 or atenolol or labetalol\textsuperscript{13}.

\textbf{\textit{Left renal artery ligated hypertension:}} Male Wistar rats (175-200 g) were anesthetized with Ketamine (75 mg kg$^{-1}$, i.p.) and Xylazine (10 mg kg$^{-1}$, i.p.) and Left Renal Artery (LRA) was ligated. After renal ligation, the animals were housed and provided with 1% sodium chloride solution instead of normal drinking water. Animals were divided into following 7 groups after 4 weeks of LRA ligation. Group I: normal control animals, Group II: vehicle (0.05% Tween-80 + 0.5% CMC, 3 mL kg$^{-1}$), Group III: PP-17, 3 mg kg$^{-1}$, Group IV: PP-17, 10 mg kg$^{-1}$, Group V: PP-17, 30 mg kg$^{-1}$, Group VI: atenolol, 10 mg kg$^{-1}$ and Group VII: labetalol, 10 mg kg$^{-1}$. All treatments were given orally for next 2 weeks. At the end of 6th week, MABP and Heart rate was recorded by cannulating the carotid artery as previously described\textsuperscript{13}.

\textbf{\textit{Fructose induced hypertension:}} Wistar rats (200-220 g) were divided into seven groups according to body weight. Normal control group was given ordinary drinking water \textit{ad libitum} throughout the whole treatment course and the remaining group was given 10% fructose solutions to drink \textit{ad libitum}\textsuperscript{14}. Nine weeks later, the fructose-treated animals were assigned the following treatment regimen; Group I: normal control animals, Group II: vehicle (0.05% Tween-80 + 0.5% CMC, 3 mL kg$^{-1}$), Group III: PP-17, 3 mg kg$^{-1}$, Group IV: PP-17, 10 mg kg$^{-1}$, Group V: PP-17, 30 mg kg$^{-1}$, Group VI: atenolol, 10 mg kg$^{-1}$ and Group VII: labetalol, 10 mg kg$^{-1}$. Vehicle, PP-17 and drug treatment was initiated after 9 weeks of fructose feeding and treatment was continued for next 2 weeks. At the end of 11th week, MABP and Heart rate was recorded by cannulating the carotid artery as previously described.

\textbf{\textit{Measurement of mean arterial pressure and heart rate:}} Wistar rats were anesthetized with urethane (1.25 g kg$^{-1}$, i.p.). Carotid artery was cannulated with the help of polyethylene catheter containing heparin dissolved in isotonic saline (50 IU ml$^{-1}$). Body temperature was maintained at 37°C with a help of thermal blanket. The cannulated artery was connected to pressure transducer (SS13L, BIOPAC Systems, Inc., Santa Barbara, CA) for measurement of MABP and heart rate.

\textbf{\textit{Biochemical measurements:}} Concentrations of glucose, cholesterol, LDL, HDL, VLDL and triglycerides were measured in plasma samples at the end of the experiment using a Hitachi 912 fully automated random access biochemistry analyzer Roche. Plasma insulin was determined with an ELISA (linco research).

\textbf{\textit{Statistical analysis:}} Data in table and figure are expressed as Mean±Standard Error of Mean (SEM). One-way Analysis of Variance (ANOVA; Graph Pad PRISM\textsuperscript{TM}, Version 4.0, San Diego, CA, USA) followed by Dunnett’s test was applied to determine differences between the groups. A value of \textit{p}<0.05 was considered significant.

\textbf{\textit{RESULTS}} \textbf{\textit{\textbeta Adrenoceptor blockade and selectivity:}} PP-17 antagonized the isoproterenol induced positive chronotropic effects on the isolated wistar rat right atria. PP-17 also caused a dose-dependent parallel shift to the right of the isoproterenol concentration response curves yielding $\textit{pA}_2$ values of 7.0±0.2 (Fig. 2a). $\textit{pA}_2$ values of
Fig. 2 (a-c): Effects of PP-17 on responses in Wistar rat atria, uterus and colon. Mean cumulative concentration-response curves are shown for the (a) positive chronotropic responses to isoprenaline in spontaneously beating rat right atrium, (b) relaxant effects of isoprenaline in rat uterus (c) relaxant effects of isoprenaline in rat colon in the absence or presence of PP-17. Each point represents the mean±S.E.M. of six individual experiments.

PP-17, atenolol, labetalol and propranolol treatment, calculated from Schild plots are shown in Table 1. In all cases, the slopes of Schild plots were not significantly different from 1.0. PP-17 antagonized isoprenaline induced relaxation of spontaneously contracting uterus and KCl induced contraction in rat colon. PP-17 also caused a rightward shift of concentration response curve of isoprenaline in isolated rat uterus and isolated rat colon indicating β2 and β2 adrenoceptor antagonistic activity (Fig. 2b,c). The apparent pA2 values of PP-17 for β2 and β2 adrenoceptor were found to be 5.7±0.1 and 6.0±0.1 respectively (Table 1). The β1/β2-selectivity ratio was calculated from the antilogarithm of the difference between the pA2 values obtained from rat right atria and uterus. The estimated β1/β2-selectivity ratio value (20.0) indicated that PP-17 had more affinity to β2 adrenoceptor than to β2 adrenoceptor subtypes. The relative order of β1/β2-selectivity was found to be in the order of Atenolol>PP-17>Labetalol>Propranolol.

α1-Adrenoceptor blocking activity: PP-17 antagonized phenylephrine induced vasoconstrictions effects in
Table 1: α and β-Adrenergic receptor blocking potency and selectivity of PP-17

<table>
<thead>
<tr>
<th></th>
<th>α &amp; β blocker</th>
<th>β₂, Right atria (Slope)</th>
<th>β₁, Uterus (Slope)</th>
<th>β₁, Colon (Slope)</th>
<th>α₁, Aorta (Slope)</th>
<th>α₁ / β₁</th>
<th>β₁ / β₂</th>
<th>α₁ / α₂</th>
<th>α₁ / β₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP-17</td>
<td>7.0±0.2</td>
<td>5.7±0.1</td>
<td>6.0±0.1</td>
<td>7.0±0.1</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>(1.0±0.4)</td>
<td></td>
<td>(1.0±0.1)</td>
<td>(0.9±0.1)</td>
<td>(0.9±0.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>8.3±0.1</td>
<td>8.2±0.1</td>
<td>6.6±0.2</td>
<td>NT</td>
<td>1</td>
<td>-</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1.0±0.2)</td>
<td></td>
<td>(1.0±0.3)</td>
<td>(0.9±0.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atenolol</td>
<td>7.2±0.1</td>
<td>5.6±0.1</td>
<td>4.6±0.3</td>
<td>NT</td>
<td>40</td>
<td>-</td>
<td>298</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.9±0.1)</td>
<td></td>
<td>(1.0±0.1)</td>
<td>(1.0±0.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labetalol</td>
<td>7.9±0.1</td>
<td>7.1±0.1</td>
<td>6.5±0.2</td>
<td>7.5±0.2</td>
<td>6</td>
<td>3</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1.0±0.1)</td>
<td></td>
<td>(1.1±0.2)</td>
<td>(1.0±0.1)</td>
<td></td>
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</tbody>
</table>

The selectivity ratio values were obtained from the antilogarithms of the difference between the mean pA₂ values from in vitro studies. The pA₂ values were obtained from the formula pA₂ = [log (DR-1) x log molar concentration antagonist] and the slope values were calculated from individual Schild plot by regression analysis. Data are expressed as Mean±SEM, n = 6 at each individual experiments. NT: Not Tested.

Fig. 3: Inhibitory effects of PP-17 on the concentration-response curves of phenylephrine for the contractions in rat aorta strips. Data are expressed as Mean±SEM, n = 6 at each individual experiments.

isolated Wistar rat thoracic aorta rings. PP-17 also caused a dose-dependent parallel shift to the right of the phenylephrine concentration-response curves (Fig. 3). The apparent pA₂ value for PP-17 on aorta rings was 7.0±0.1 as indicated in Table 1.

In vivo evaluation of β-adrenoceptor antagonistic activity: Administration of isoprenaline (0.3, 1 and 3 μg kg⁻¹, i.v.) produced a dose dependent increase in heart rate and decrease in the MABP. Administration of isoprenaline (0.3, 1 and 3 μg kg⁻¹, i.v.) in the presence of PP-17 (10 and 30 μg kg⁻¹, i.v.), labelol (30 μg kg⁻¹, i.v.) and atenolol (30 μg kg⁻¹, i.v.) significantly reduced isoprenaline induced increase in heart rate (Fig. 4a) and decrease in the MABP (Fig. 4b). Inhibition of cardioaccelerator and hypotensive responses to isoprenaline by PP-17 revealed β adrenoceptor blocking activity.
Table 2: Effect of PP-17 on mean arterial blood pressure, heart rate, plasma levels of glucose, insulin, cholesterol, triglycerides, HDL, LDL and VLDL in Fructose-fed rat

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vehicle</th>
<th>PP-17: (3 mg kg⁻¹)</th>
<th>Atenolol (10 mg kg⁻¹)</th>
<th>Labetalol (10 mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2 mg kg⁻¹)</td>
<td>(10 mg kg⁻¹)</td>
<td>(20 mg kg⁻¹)</td>
<td>(10 mg kg⁻¹)</td>
<td>(10 mg kg⁻¹)</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>95 ± 4.3</td>
<td>155 ± 3.7*</td>
<td>149 ± 3.0</td>
<td>141 ± 2.4</td>
<td>105 ± 2.1*</td>
</tr>
<tr>
<td>Heart rate (Beats min⁻¹)</td>
<td>306 ± 12</td>
<td>338 ± 13</td>
<td>326 ± 11</td>
<td>306 ± 10</td>
<td>282 ± 5.2</td>
</tr>
<tr>
<td>Glucose (mg dL⁻¹)</td>
<td>98 ± 10</td>
<td>140 ± 8*</td>
<td>130 ± 8</td>
<td>125 ± 7</td>
<td>122 ± 11</td>
</tr>
<tr>
<td>Insulin (mg mL⁻¹)</td>
<td>1.2 ± 0.2</td>
<td>3.8 ± 0.3*</td>
<td>3.6 ± 0.2</td>
<td>2.9 ± 0.4</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Cholesterol (mmol L⁻¹)</td>
<td>1.3 ± 0.1</td>
<td>1.6 ± 0.04</td>
<td>1.5 ± 0.07</td>
<td>1.4 ± 0.04</td>
<td>1.2 ± 0.08</td>
</tr>
<tr>
<td>Triglycerides (mmol L⁻¹)</td>
<td>0.8 ± 0.01</td>
<td>1.5 ± 0.00*</td>
<td>1.4 ± 0.02</td>
<td>1.3 ± 0.02</td>
<td>1.1 ± 0.03*</td>
</tr>
<tr>
<td>HDL (mmol L⁻¹)</td>
<td>0.38 ± 0.01</td>
<td>0.32 ± 0.04</td>
<td>0.36 ± 0.02</td>
<td>0.37 ± 0.02</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>LDL (mmol L⁻¹)</td>
<td>0.38 ± 0.02</td>
<td>0.58 ± 0.04*</td>
<td>0.53 ± 0.02</td>
<td>0.49 ± 0.02</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>VLDL (mmol L⁻¹)</td>
<td>0.36 ± 0.03</td>
<td>0.66 ± 0.04*</td>
<td>0.59 ± 0.04</td>
<td>0.55 ± 0.04</td>
<td>0.48 ± 0.02*</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± SEM, n = 8 at each individual treatment. *p < 0.05 as compared to control group, **p < 0.05 as compared to vehicle group.

Fig. 5(a-b): Effect of PP-17 (3.10 and 30 mg kg⁻¹, p.o.), atenolol (10 mg kg⁻¹, p.o.) and labetalol (10 mg kg⁻¹, p.o.) on Mean Arterial Blood Pressure (MABP) and heart rate of LRA ligated rats after 14 days of treatment. (a) Change in mean arterial blood pressure. (b) Change in heart rate. Data are expressed as mean ± SEM, n = 8 at each individual treatment. *p < 0.05 as compared to normal control group, **p < 0.05 as compared to vehicle group.

LRA ligated hypertension: The MABP and heart rate significantly increased in LRA ligated vehicle group when compared with normal control group. Treatment with PP-17 (3, 10 and 30 mg kg⁻¹, p.o.) produced a dose dependent decrease in MABP and heart rate of LRA ligated rats. PP-17 (10 and 30 mg kg⁻¹, p.o.), labetalol (10 mg kg⁻¹, p.o.) and atenolol (10 mg kg⁻¹, p.o.) showed significant decrease in MABP and heart rate. The fall in MABP produced by PP-17 (10 mg kg⁻¹, p.o.) was less than the atenolol (10 mg kg⁻¹, p.o.) and labetalol (10 mg kg⁻¹, p.o.) shown in Fig. 5.

Fructose induced hypertension: Plasma concentrations of glucose, insulin, cholesterol, LDL, HDL, VLDL and TG levels was estimated after 12 h fasting in normal and fructose fed rats (Table 2). After 11 weeks, fructose-fed rats showed significant increase in plasma glucose, insulin, TG, LDL and VLDL level when compared with normal control rats. Treatments with PP-17 (10 and 30 mg kg⁻¹, p.o.) showed significant decrease in MABP as compared with vehicle group. Treatment with PP-17 (30 mg kg⁻¹, p.o.) showed significant decrease in plasma TG and VLDL level as compared with vehicle group.

DISCUSSION
This study demonstrates that PP-17 is dual a dual α and β-adrenoceptor receptor antagonist. PP-17 significantly decreased blood pressure and heart rate in high renin and fructose induced hypertensive model. The similarities between the PP-17, atenolol and labetalol end with their cardiovascular effects, however; their metabolic effects are different. PP-17 showed more decrease in fasting plasma glucose and insulin levels than labetalol whereas atenolol did not show any change in metabolic parameters. This indicates that PP-17.
improves insulin sensitivity more than labetalol whereas atenolol did not. The results of this study indicated that PP-17 differ from classical β blocker.

Isoprenaline induced increase in the atrial rate was competitively antagonized by PP-17, labetalol, atenolol and propranolol, suggesting that PP-17 possessed β₁-adrenoceptor antagonist activity. The rank order potency for β₁ adrenoceptor was found to be atenolol > PP-17 > labetalol > propranolol. The presence of vast majority of β₁-adrenoceptor in rat uterus was confirmed by autoradiography localization and the length of gestation influenced the β₂-adrenoceptor mediated response⁶. PP-17 weakly antagonized relaxation response of isoprenaline in spontaneous contraction of uterus indicates that PP-17 possessed weak β₁ adrenoceptor antagonist activity. The presence of β₁-adrenoceptor has been confirmed in human and rodent colon¹ⁱ. PP-17 weakly antagonized relaxation response of isoprenaline on KCl mediated contraction in rat colon suggesting that PP-17 possessed weak β₁ antagonist activity. The relative order of β₁/β₂-selectivity was found to be in the order of Atenolol > PP-17 > Labetalol > Propranolol.

The effect of PP-17 against α₁-adrenoceptor was evaluated with the thoracic aorta isolated from the wistar rats. In isolated rat aorta, PP-17 was found to competitively antagonize increases in contraction induced by phenylephrine, suggesting that PP-17 possessed α₁-adrenoceptor antagonist activity.

Isoxsuprine is a potent β-adrenoceptor agonist with high affinity for all β adrenoceptor, devoid of any action on α-adrenoceptor. Intravenous infusion of isoprenaline stimulates cardiac β-adrenoceptor and lowers peripheral vascular resistance³. The tachycardia produced by isoprenaline is primarily due to the β₁-adrenoceptor activity in heart and the hypotensive effect is primarily due to the β₂-adrenoceptor in the blood vessels. In our study, administration of isoprenaline produced tachycardia and hypotension in anesthetized rats.

PP-17 (10 μg kg⁻¹, iv.) blocked isoprenaline induced tachycardia without affecting blood pressure which indicate that PP-17 having selective β₁-adrenoceptor (β₁) antagonistic activity this dose levels. However, PP-17 (30 μg kg⁻¹, iv.) blocked both tachycardial and hypotensive effect of isoprenaline, suggesting blockade of both β₁ and β₂-adrenoceptor. In vivo experiments suggested that PP-17 possess cardio selective β₁-blockade at lower dose levels and selectivity was abolished at higher doses.

In rat, constriction of renal artery with an intact contralateral kidney produces hypertension with an elevated plasma renin activity (PRA). β-blockers decreases blood pressure by different mechanisms such as reduction in the cardiac output, reduction of renin release from the juxtaglomerular cells, central action reducing sympathetic activity, change in the baroreceptor sensitivity, an alteration in peripheral adrenergic neuron function and an increase in the prostacyclin biosynthesis¹². In the present investigation, PP-17 also showed significant antihypertensive effect in this model that was less than atenolol and labetalol.

The results of this study indicate that insulin resistance, hyperinsulinemia, increase in mean blood pressure and hypertriglyceridemia develop when normal rats are fed a high fructose diet. As has been seen previously¹³,¹⁴. In addition, we have shown that these metabolic changes are associated with an increase in LDL and VLDL level in blood plasma. Several antihypertensive drugs effectively prevent and reverse the increase in blood pressure induced by high fructose diets¹⁵.

Hyperinsulinemia and insulin resistance could induce elevation of blood pressure levels via a variety of mechanisms including sodium-water retention, sympathetic nerve stimulation, changes in transmembrane ion traffic and direct stimulation of smooth muscle cell growth¹⁶. Moreover, other reports have shown that reducing insulin levels in these rats leads to a reduction in blood pressure and correction of other metabolic abnormalities¹⁷. Fructose diets have been found to activate sympathetic nervous system activity and elevate blood pressure in rats. Sympathetic activation also may 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 diet¹⁸. Plasma norepinephrine has been found elevated in sucrose- and fructose-fed rats and it has been proposed to account for hypertension and insulin resistance because of its vasoconstrictor activity¹⁹. PP-17 (10 and 30 mg kg⁻¹, p.o.) significantly decreased the MABP and heart rate in fructose-fed animals. In addition, PP-17 (30 mg kg⁻¹, p.o.), significantly decreased TG and VLDL level as compared with vehicle treated rats.

The above result indicates PP-17 reduced the extent of development of hypertension induced by the left renal artery ligation and high fructose by blocking both α₁ and β₁-adrenoceptor receptor. PP-17 also improved lipid profile in fructose fed rats. Therefore, PP-17 might have beneficial effects on blood pressure without unwanted side effect.

In conclusion, this study indicates that PP-17 is a third-generation selective β₁-adrenoceptor blocker with vasorelaxant characteristics, which may be explained by its α₁-adrenoceptor blockade. PP-17 was different from such first-generation compounds as atenolol and propranolol in that it possessed ancillary vasorelaxing
activity. However, further refinement of our understanding of these cardiovascular mechanisms will be required to extend these findings to the treatment of hypertension.

REFERENCES


