JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 515–520

Received July 9, 2004
Accepted December 20, 2004
DOI 10.1211/0022357055768
ISSN 0022-3573

Selective β_1-adrenoreceptor blocking activity of newly synthesized acyl amino-substituted aryloxypropanolamine derivatives, DPJ 955 and DPJ 890, in rats

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Abstract

The in-vivo β-adrenoreceptor antagonistic activity of test compounds DPJ 955 and DPJ 890 was assessed against β-adrenoreceptor agonist (isoprenaline) induced tachycardia in anaesthetized rats. The selectivity to block isoprenaline responses on different β-adrenoreceptor subtypes (β_1, β_2 and β_3) of the test compounds was carried out on isolated rat right atria, isolated rat uterus and isolated rat colon preparations, respectively. Intravenous injection of isoprenaline alone in anaesthetized rats caused hypotension and tachycardia. DPJ 955 or DPJ 890 alone produced a fall in mean arterial pressure and bradycardia in a dose-dependent manner. Administration of isoprenaline to anaesthetized rats pre-treated with test compounds significantly blocked both the tachycardial and hypotensive responses induced by isoprenaline. The test compounds shifted the concentration response curves of isoprenaline towards the right for isolated rat right atrial preparations, rat uterus and rat colon, indicating β_1, β_2 and β_3 adrenoreceptor blockade, respectively. The selectivity ratio for β_1/β-adrenoreceptors to DPJ 955 and DPJ 890 was 64.6 and 83.2, respectively. DPJ 890 was more potent in blocking β_1-adrenoreceptors and was more selective towards β_1 receptors than to other β-adrenoreceptor subtypes. In conclusion, DPJ 955 and DPJ890 have β-adrenoreceptor blocking activity with high selectivity for the β_1-adrenoreceptor subtype.

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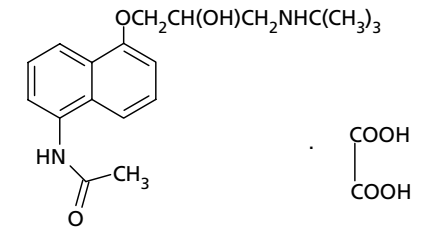
Acknowledgements: The authors would like to thank M/s Khandewal Laboratory Ltd, Mumbai, India, for providing the gift sample of atenolol hydrochloride. We are also thankful to Dr S. S. Kadam, Poona College of Pharmacy, Pune, India, for providing the necessary facilities for this research work.

Introduction

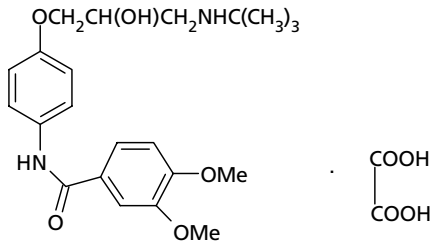
Hypertension is a major risk factor for various cardiovascular diseases. However, it is easily diagnosed and can be treated effectively. Treatment of high blood pressure has consistently been reported to reduce the risk of cardiovascular disease (Staessen et al 2001). β-Blockers and diuretics are regarded as a first-line treatment for hypertension by the Joint National Committee (Anonymous 1997) and the British Hypertensive Society (Ramsay et al 1999), and a first-line alternative along with various antihypertensives by the World Health Organization (Anonymous 1999).

In addition to the treatment of hypertension, β-blockers are also widely used in the treatment of angina pectoris, tachyarrhythmias, acute and post-myocardial infarction, congestive heart failure and left ventricular dysfunction (Hjalmarson 2000). They are also indicated in non-cardiovascular disorders such as migraine, hyperthyroidism, anxiety, tremor and glaucoma (Hoffman 2001). Numerous β-blockers have been synthesized having various properties such as intrinsic sympathomimetic activity, high lipophilicity, cardioselectivity, α-adrenergic blocking, peripheral vasodilation, antioxidant and antiproliferative activity. These properties have improved the choice and selection of β-blockers for various cardiovascular diseases.

Non-selective β-blockers pose a risk of bronchoconstriction, hypoglycaemia, hypokalaemia and altered lipid metabolism (Hoffman 2001). Selective cardiac β_1-blockers such as atenolol and metoprolol were developed to overcome the bronchoconstriction effect and increase in peripheral resistance caused by the administration of non-selective β-blockers. However, the selectivity of these agents is only relative and is lost at therapeutic doses (Johnson 1998).



DPJ 955



DPJ 890

Figure 1 Structure of DPJ 955 and DPJ 890.

We have been involved in the development of cardioselective β_1-blocking agents based on the structure of practolol by introducing a para amidic functional group in the phenyl or naphthyl ring system (Jindal et al 2002). In this study, we report the in-vivo β-blocking activity of two compounds, *N*[5-(3-*tert*-butylamino-2-hydroxy-propoxy)-naphthalene-1-yl]-acetamide oxalate (DPJ955) and *N*[4-(3-*tert*-butylamino-2-hydroxy-propoxy)-phenyl]-3,4-dimethoxy benzamide oxalate (DPJ 890) (Figure 1) and their affinity to different β-adrenoreceptor subtypes in-vitro.

Materials and Methods

Animals

Healthy Wistar rats (200–250 g) of either sex were obtained from the National Toxicology Centre, Pune, India. They were maintained at a temperature of $25 \pm 1°C$ and relative humidity of 45–55% in a clean environment under a 12-h light/dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India) and water. The experimental protocols were approved by the Institutional Animal Ethics Committee of Poona College of Pharmacy, Pune, India.

In-vivo β-adrenoreceptor blocking activity

Male Wistar rats, 200–250 g, were anaesthetized with urethane ($1.25\,g\,kg^{-1}$, i.p.; Fluka Chemika GmbH, Buchs, France). The trachea was intubated to facilitate spontaneous respiration. The heart rate was recorded using an electrocardiogram via subcutaneously placed electrodes. Mean arterial pressure was measured from the left carotid artery via a pressure transducer (SS 13L). The electrode lead set of the electrocardiogram (SS 2L) and the pressure transducer were connected to a four-channel physiological data acquisition system (MP 30; BIOPAC Systems, Inc., Santa Barbara, CA, USA) and recorded. The left jugular vein was cannulated for intravenous administration of drugs in a volume of $1\,mL\,kg^{-1}$. After 15–30 min of equilibration, during which the cardiovascular parameters were allowed to stabilize, experiments were performed.

The dose–response curve to isoprenaline (isoprenaline hydrochloride; Sigma Chemical Co., St Louis, MO, USA) was constructed for increase in heart rate (tachycardia) and fall in mean arterial pressure after intravenous bolus injections of 0.3, 1 and $3\,\mu g\,kg^{-1}$ of isoprenaline. Next, a single dose of the test drug was administered intravenously. At 15–20 min later, a further injection of isoprenaline (0.3, 1 and $3\,\mu g\,kg^{-1}$, i.v) was given and the changes in heart rate and mean arterial pressure were measured.

β-Adrenoreceptor antagonism and selectivity

β_1-Antagonism was studied in isolated rat right atria. In brief, male Wistar rats were killed; the heart was rapidly removed and the right atrium was dissected. The atrial strips were mounted in a 40-mL organ bath containing a physiological salt solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25, glucose 11.1, ascorbic acid 0.03, and sodium salt of EDTA 0.03. Tissues were maintained at a temperature of $37 \pm 1°C$, aerated with 95% O_2 and 5% CO_2. A resting load of 0.5 g was applied and the tissue was allowed to equilibrate for 1 h. Spontaneous atrial frequency was measured with a force displacement transducer (T-305) connected to a Student's Physiograph (Bio-Devices, Ambala, India). Concentration–response curves to isoprenaline were constructed from the increase in atrial frequency produced by cumulative addition of isoprenaline in absence and presence of antagonists. Antagonists were added to the bath after 30 min of isoprenaline response alone. Antagonists were incubated with the atrium for 30 min before addition of cumulative concentrations of isoprenaline.

Vaginal smears from female rats were daily monitored at 0800 hours. Rats in oestrus were killed and the uterine horns were mounted in a 40-mL organ bath filled with Locke's solution of the following composition (mM): NaCl 154, KCl 5.6, $NaHCO_3$ 6.0, $CaCl_2$ 2.2, glucose 11.1, ascorbic acid 0.03, and sodium salt of EDTA 0.03. The tissues were maintained as described above for the atrial preparations. Spontaneous contractions were recorded under a constant load of 1 g. Concentration–response curves to isoprenaline were constructed by cumulative addition (0.5 log unit increments) to the contracting uterus at intervals of 3 min until the uterus completely relaxed. The tissues were washed for 30 min with Locke's solution and allowed to equilibrate for 30 min in the presence of antagonists. The β_2 adrenoreceptor antagonistic activity

was determined by constructing concentration–response curves to isoprenaline in the presence of antagonists.

The distal colon (3 cm) from male rats was mounted in organ baths containing Krebs solution (composition and conditions as described above for atrial preparations). Each tissue was placed under 1 g resting tension and equilibrated for 40 min before contraction with 50 mM KCl for 15 min followed by a 30-min wash. The tissues were again contracted with a sub-maximal concentration of KCl (30 mM) and washed for 30 min. Tissues were then incubated with appropriate concentrations of antagonists for 30 min, with control tissues receiving saline treatment. The tissues were then contracted again with KCl (30 mM) and cumulative concentration–response curves to relaxants were obtained after contractions stabilized. The ability of antagonists to block β_3-receptors was estimated from this experiment.

Propranolol (propranolol hydrochloride; Sigma Chemical Co., St Louis, MO, USA) and atenolol (atenolol hydrochloride; Khandewal Laboratory Ltd., Mumbai, India) were used as standard drugs for comparison.

Calculations and data analysis

Responses to isoprenaline were calculated as percentage decrease in mean arterial pressure and percentage increase in heart rate with or without test compounds. Changes in sinus frequency were expressed as percentage of maximum increase in beating rate caused by isoprenaline. Responses to isoprenaline were calculated as percentage relaxation in isolated rat uterus and in KCl-induced contraction on isolated rat colon. All the values are represented as mean ± s.e.m.

In the in-vivo studies, effective dose 50% (ED50) values of isoprenaline were calculated in the presence and absence of test or standard compounds for isoprenaline-induced tachycardia and hypotension. The antagonistic potency of the β-adrenoreceptor towards hypotensive and tachycardic responses to isoprenaline was calculated according to the formula:

$$\log_{10}[([E']/[E]) - 1] - \log_{10}[B]$$

where [E′] and [E] are the ED50 values of isoprenaline with or without previous administration of the antagonist, respectively, and [B] is the dose of the antagonist.

In the in-vitro studies, mean concentration curves to isoprenaline were analysed using non-linear regressions (Graph Pad Prism, version 4.0; Graph Pad Inc., San Diego, CA, USA). The EC50 and pEC50 (negative logarithm of EC50) values of isoprenaline were obtained in the presence and absence of the antagonist. The concentration ratios (CR) were determined from the EC50 values. The plot of log(CR – 1) versus log[antagonist] (Arunlakshana & Schild 1959) was analysed by linear regression. Antagonism was considered to be competitive in nature if the slope of the regression line was not significantly different from unity. In such cases, a mean pA_2 value was obtained from the equation:

$$pA_2 = \log(CR - 1) - \log[\text{antagonist}]$$

In cases where the slope or regression line significantly differed from unity, the value obtained from the above equation is pK_B rather than pA_2 value.

Statistically significant differences between two means were analysed using repeated one-way analysis of variance followed by Tukey's test where comparison was made to the same control group. For comparing unpaired data one-way analysis of variance followed by Tukey's test was performed. $P < 0.05$ was considered significant.

Results

In-vivo β-adrenoreceptor blocking activity

Isoprenaline (0.3, 1 and 3 $\mu g\,kg^{-1}$, i.v.) produced a dose-dependent increase in heart rate (tachycardia) and decrease in mean arterial pressure (hypotension). Intravenous injection of test compounds (DPJ 955 and DPJ 890) or standard drugs (propranolol and atenolol) alone produced bradycardia and hypotension in a dose-dependent manner. Propranolol caused a temporary increase in mean arterial pressure, followed by a decrease in blood pressure. The rank order of potency of these compounds to produce bradycardia was atenolol > propranolol > DPJ 890 > DPJ 955 (Figure 2).

Administration of isoprenaline (0.3, 1 and 3 $\mu g\,kg^{-1}$, i.v.) to rats pre-treated with DPJ 955 (0.3, 1 and 3 $mg\,kg^{-1}$, i.v.), DPJ 890 (0.1, 0.3 and 1.0 $mg\,kg^{-1}$, i.v.), propranolol (0.5, 1 and 2 $mg\,kg^{-1}$, i.v.) or atenolol (0.1, 0.3 and 1.0 $mg\,kg^{-1}$, i.v.) caused a significant decrease in isoprenaline-induced tachycardia and hypotension (Figures 3 and 4). The blockade of the cardio-stimulant and hypotensive effects of isoprenaline by the test compounds indicated β-adrenoreceptor blocking activity. However, the potency ratio to block tachycardial and hypotensive responses of isoprenaline by the test and standard compounds varied (Table 1).

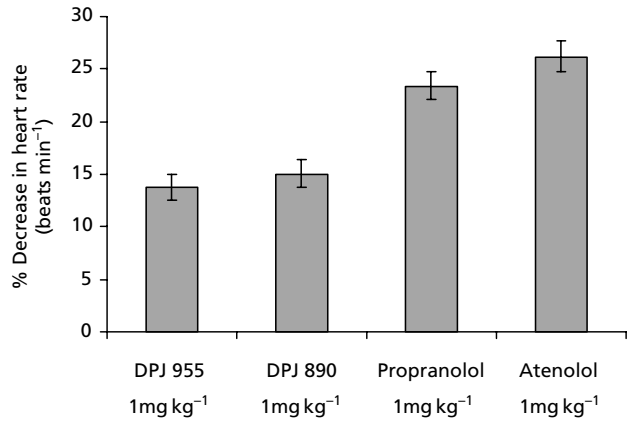


Figure 2 Effect of test compounds, propranolol and atenolol on heart rate. Each bar represents the mean ± s.e.m from five rats.

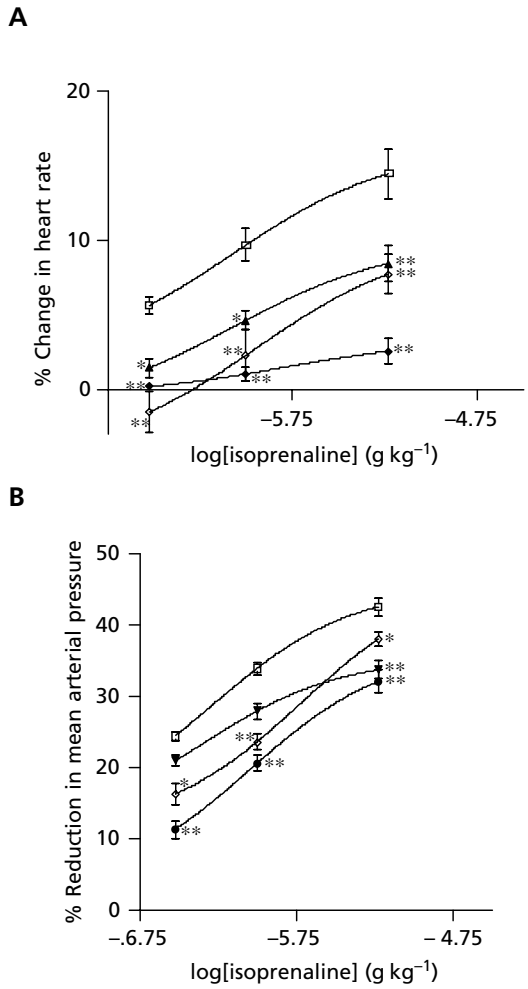


Figure 3 Effect of isoprenaline alone (□) and in the presence of 0.3 (▲), 1 (◇) and 3 mg kg^{-1} (●) DPJ 955 on heart rate (A) and mean arterial blood pressure (B). Values are expressed as mean ± s.e.m. from five rats. $*P < 0.05$, $**P < 0.01$, significantly different compared with isoprenaline alone (repeated one-way analysis of variance followed by Tukey's test).

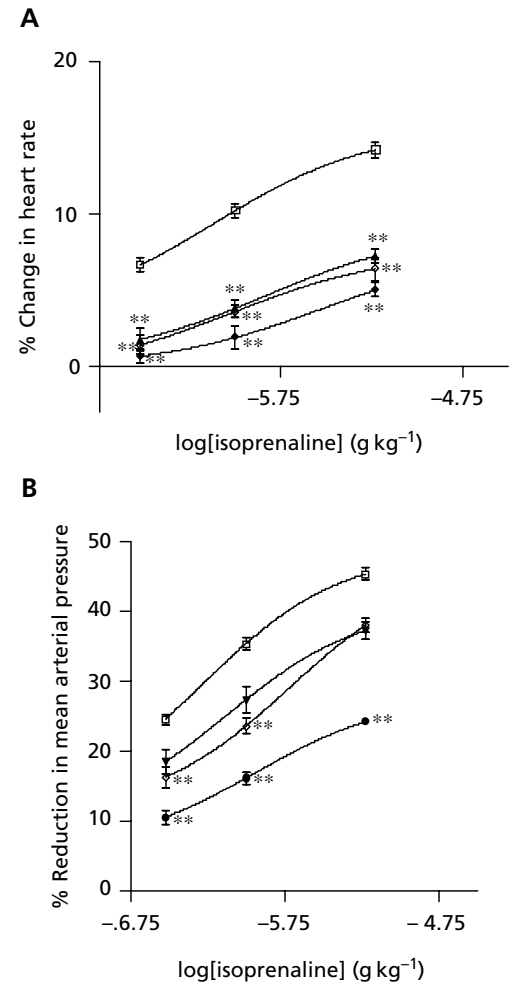


Figure 4 Effect of isoprenaline alone (□) and in the presence of 0.1 (▲), 0.3 (◇) and 1 mg kg^{-1} (●) DPJ 890 on heart rate (A) and mean arterial blood pressure (B). Values are expressed as mean ± s.e.m from five rats. $**P < 0.01$, significantly different compared with isoprenaline alone (repeated one-way analysis of variance followed by Tukey's test).

β-Adrenoreceptor antagonism and selectivity

Isoprenaline produced an increase in the chronotropic effect on isolated rat right atrial preparations, a relaxant effect on isolated rat uterus, and a relaxation of KCl-induced contractions in isolated rat colon in a dose-dependent manner, with changes in pEC50 values in the three isolated preparations. The test drugs shifted the concentration–response curves of isoprenaline towards the right for all three preparations. Schild regression obtained from the results of antagonism between the test drugs or standard drugs and isoprenaline yielded a line with a slope close to unity in isolated atrial preparations and isolated rat uterus preparations, indicating that the drugs are competitive antagonists of β_1 and β_2 receptor subtypes. However, the slope of the Schild plot was significantly different from unity for the test drugs and standard drugs on isolated rat colon, indicating non-competitive antagonism of β_3-adrenoreceptors. Table 2

Table 1 Potency ratio of DPJ 955, DPJ 890, propranolol and atenolol on heart rate and mean arterial pressure in rats

Test compound/standard drug	Potency ratio to antagonize isoprenaline-induced:	
	tachycardia	hypotension
DPJ 955	2.94 ± 0.39	2.32 ± 0.49
DPJ 890	3.45 ± 0.30	2.51 ± 0.25
Propranolol	3.73 ± 0.37	3.66 ± 0.63
Atenolol	3.33 ± 1.04	2.71 ± 0.15

Values are the mean ± s.e.m calculated from three different dose levels of each antagonist against isoprenaline.

Table 2 pA_2 values of test compounds and standard β-adrenoreceptor antagonists in in-vitro studies

β-Blocker	β_1	β_2	β_3	β_1/β_2 ratio[a]	β_1/β_3 ratio[a]
	pA_2 value (slope) Right atrium	pA_2 value (slope) Uterus	pA_2 value (slope) Colon		
DPJ 955	7.24 ± 0.01 (0.99 ± 0.03)	5.43 ± 0.05 (1.04 ± 0.16)	6.00 ± 0.31 (1.78 ± 0.76)	64.6	17.4
DPJ 890	8.25 ± 0.13 (1.01 ± 0.22)	6.33 ± 0.03 (1.07 ± 0.05)	4.98 ± 0.13 (0.68 ± 0.31)	83.2	1862.1
Atenolol	7.16 ± 0.09 (1.18 ± 0.10)	5.57 ± 0.06 (0.99 ± 0.11)	4.62 ± 0.33 (0.15 ± 0.05)	39.2	346.4
Propranolol	8.29 ± 0.02 (1.02 ± 0.06)	8.18 ± 0.02 (0.96 ± 0.01)	6.64 ± 0.16 (0.54 ± 0.17)	1.3	44.2

[a]Ratios were obtained from the anti-logarithm of the difference between the mean pA_2 values from in-vitro studies. pA_2 values were obtained from the formula: $pA_2 = \log(\text{concentration ratio} - 1) - \log \text{molar concentration of antagonist}$.

gives the pA_2 values of DPJ 955, DPJ 890, propranolol and atenolol calculated from the Schild plots.

β_1-Adrenoreceptor selectivity was determined using β_1/β_2 and β_1/β_3 selectivity ratios. The estimated β_1/β_2 selectivity ratios for DPJ 955 and DPJ 890 were 64.6 and 83.2, respectively. The results suggested that DPJ 955 and DPJ 890 had greater selectivity for β_1-adrenoreceptor than for β_2-adrenoreceptor subtypes. The relative order of β_1/β_2 selectivity was in the order DPJ 890 > DPJ 955 > atenolol > propranolol.

Discussion

Isoprenaline is a potent cardio-stimulant with high affinity for all β-adrenoreceptors. Intravenous administration of isoprenaline produced tachycardia and hypotension. The tachycardia produced by isoprenaline is primarily due to β-adrenoreceptor activity in the heart and the hypotensive effect is due to the β_2-adrenoreceptors in the blood vessels (Kannan et al 1996; Chiu et al 2000). Although β_1, β_2 and β_3 adrenoreceptors are all present in mammalian heart, the positive ionotropic and chronotropic effects of isoprenaline in-vivo are brought about by β_1-adrenoreceptors (Piercy 1988; Wellstein et al 1988). Catecholamine-induced positive chronotropic and ionotropic effects were completely absent in β_1-deficient mice (Rohrer et al 1996). Isoprenaline-induced tachycardia alone was blocked by DPJ 955 ($0.3\,mg\,kg^{-1}$), DPJ 890 (0.1 and $0.3\,mg\,kg^{-1}$) and atenolol ($0.1\,mg\,kg^{-1}$), indicating blockade of cardiac β-adrenoreceptors at these dose levels. However, DPJ 955 (1 and $3\,mg\,kg^{-1}$), DPJ 890 ($1\,mg\,kg^{-1}$), atenolol (0.3 and $1\,mg\,kg^{-1}$) and propranolol (0.5, 1 and $2\,mg\,kg^{-1}$) blocked both the tachycardial and hypotensive effects of isoprenaline, suggesting blockade of β-adrenoreceptors in the heart and also in blood vessels by the test compounds and standard drugs at the stated dose levels. The in-vivo experiments suggested that the test drugs possess cardio-selective β-blockade; however, it was relative and was lost at higher doses. In-vitro experiments were carried out to determine the β_1/β_2 and β_1/β_3 selectivity ratios. For receptor selectivity of β-adrenoreceptor antagonists, comparison of their affinity towards different β-adrenoreceptor subtypes is required, and the pA_2 value was used for this purpose (Arunlakshana & Schilid 1959). In-vitro tissue bath experiments showed that test compounds and standard drugs competitively antagonized the isoprenaline-induced chronotropic response in rat right atria and the relaxant effect in rat uterus. Test compounds and standard drugs also antagonized the inhibition of the relaxant effect of isoprenaline in isolated rat colon pre-contracted with KCl. However, the antagonistic effect of all β-blockers tested in rat colon was found to be non-competitive in nature. The pA_2 values from the three in-vitro preparations (functional studies) indicated that the test drugs and standard drugs had different affinity for β-adrenoreceptor subtypes. The anti-logarithm of the difference between the mean pA_2 values of right atrium and uterus can be used as a quantitative estimation of the degree of β_1 and β_2 adrenoreceptor selectivity (Chiu et al 2000). The ratio of β_1/β_2 selectivity for DPJ 955, DPJ 890, atenolol and propranolol was 83.2, 64.6, 39.2 and 1.3, respectively, indicating that the test compounds have more affinity for β_1-adrenoreceptors (Table 2). The test drugs were found to possess greater selectivity for β_1-adrenoreceptors than the standard β_1-blocker, atenolol. Previous results of radioligand binding studies of the test compounds from turkey erythrocyte membrane and rat lung homogenate (Jindal et al 2002) correlate well with the present functional studies. However, the β_1/β_3 selectivity was in the order DPJ 890 > atenolol > propranolol > DPJ 955.

In conclusion, both test compounds, DPJ 955 and DPJ 890, had β-blocking activity and the β_1-adrenoreceptor selectivity was greater than atenolol and propranolol. However, the efficacy of these drugs in cardiovascular disease has yet to be determined and a detailed toxicity profile would be required to determine the suitability of the drugs for clinical use.

References

Anonymous (1997) The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure *Arch. Intern. Med.* **157**: 2413–2446

Anonymous (1999) 1999 World Health Organization – International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. *J. Hypertens.* **17**: 151–183

Arunlakshana, O., Schild, H. O. (1959) Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **14**: 48–57

Chiu, C. C., Lin, Y. T., Tsai, C. H., Liang, J. C., Chiang, L. C., Wu, J. R., Chen, I. J., Yeh, J. L. (2000) Pharmacological effects of an aldehyde type alpha/beta adrenoreceptor-blocking agent with vasodilating properties. *Gen. Pharmacol.* **34**: 391–400

Hjalmarson, A. (2000) Cardio protection with β adrenoreceptor blockers. Does lipophilicity matter. *Basic Res. Cardiol.* **95** (Suppl. 1): I41– I45

Hoffman, B. B. (2001) Catecholamines, sympathomimetic drugs and adrenergic receptor antagonists. In: Hardman, J. G., Limbird, L. E. (eds) *Goodman and Gilman's the pharmacological basis of therapeutics*, 10th edn. McGraw Hill, New York, pp 215–267

Jindal, D. P., Coumar, M. S., Bruni, G., Massarelli, P. (2002) Synthesis and β_1, β_2-adrenergic receptor binding studies of 4-acyl amino-substituted phenoxypropanolamine and 5-acyl amino-substituted naphthyloxypropanolamine derivatives. *Arzneimittelforschung* **52**: 654–663

Johnson, E. (1998) Adrenergic receptor antagonists. In: Delgado, J. N., Remers, W. A. (eds) *Wilson and Gisvold's text book of organic medicinal and pharmaceutical chemistry*, 10th edn. Lippincott Williams and Wilkins, Philadephia, pp 498–503

Kannan, J., Gridhar, R., Balaraman, R. (1996) Synthesis and preliminary cardiovascular activity of phenoxypropanolamines as β blockers. *Indian J. Pharm.* **28**: 120–122

Piercy, V. (1988) Method for assessing the activity of drugs at beta 1- and beta 2-adrenoceptors in the same animal. *J. Pharmacol. Methods* **20**: 125–133

Ramsay, L. E., Williams, B., Johnson, G. D., MacGregor, G. A., Paston, L., Putter, J. F., Poulter, N., Russell, G. (1999) Guidelines for the management of hypertension: report of the third working party of the British Hypertension Society. *J. Hum. Hypertens.* **13**: 569–592

Rohrer, D. K., Desai, K. H., Jasper, J. R., Stevens, M. E., Regula, D. P., Barsh, G. S., Bernstein, D., Kobilka, B. K. (1996) Targeted disruption of the mouse beta1-adrenergic receptor gene: developmental and cardiovascular effects. *Proc. Natl Acad. Sci. USA* **93**: 7375–7380

Staessen, J. A., Wang, J. G., Thijis, L. (2001) Cardiovascular protection and blood pressure: a meta-analysis. *Lancet* **358**: 1305–1315

Wellstein, A., Belz, G. G., Butzer, R., Polsak, R., Pett, B. (1988) Beta adrenoreceptor subtype binding activity in plasma and beta blockade by propranolol and beta-1 selective bisoprolol in humans. Evaluation with Schild-plots. *J. Pharmacol. Exp. Ther.* **246**: 328–337