Comparison of propranolol and metoprolol on isolated frog heart

Bharatha Ambadas¹, Gurudatta Moharir¹, Anthireddy Srinivas², Kondam Ambareesh³ and, Sangishetty Vijay Prasad⁴,

¹Department of Pharmacology, BLDEU’s Shri B M Patil Medical College, Bijapur, Karnataka
² Department of Pharmacology, Fathima Institute of Medical Sciences, Kadapa, A.P
³ Department of Physiology Meenakshi Medical College and Research Institute, Kanchipuram, Tamilnadu
⁴Department of Pharmacology, PDVVPF Medical College Ahmednagar, Maharashtra

Abstract—Sympathomimetic drugs and drugs that block adrenoceptors have important effects, some of which are of great clinical value. These effects vary dramatically according to the drug’s selectivity for α and β receptors. β₁ and β₂ adrenoceptors coexist in the heart of various animal species, including man. Competitive radio-ligand binding studies performed in membranes from homogenized hearts have shown that only 20-30% of the total β-adrenoceptors are of the β₂-subtype in adult mammalian ventricular tissue. This number is even further reduced when purified cardiac myocytes rather than homogenized tissues are used. Unlike the mammalian heart, β-adrenoceptor population in the frog heart is composed of a majority (~ 80%) of β₂-receptors. Frogs, weighing 150 – 250g, double pith a frog and fasten it to a frog board, ventral side up, midline incision was given on the abdomen. Pectoral girdle was removed and the heart was exposed, Pericardium was removed carefully ‘v’ shaped cut was given in inferior vena cava and the tip of syme’s cannula was passed into it. It was tied firmly with inferior vena cava to assure the cannula in place. Immediately the aorta were cutted and carefully heart along with cannula were isolated from the animal, The effect of cardio-selective β adrenergic blocker Metoprolol was added to the biophase in addition to 1 µg of adrenaline and the contraction of heart till the 70-80% of inhibition is produced and the difference from normal contraction (inhibition in height of contraction) was recorded. The procedure was repeated by adding β-blocker Metoprolol in the dose of 2.5 µg, 5 µg, 10 µg, 20 µg, 40 µg, 80 µg, 160 µg and 320 µg respectively. Individual findings are recorded on smoked drum cylinder, fixed with resin (colophony) and the recordings are measured. The ID₃₀ of propranolol and metoprolol was found to be 1.3 and 44 µg respectively and interestingly propranolol was more efficient in inhibiting the contraction of adrenaline than metoprolol. These interesting facts can be supported by the finding that β₂ receptors predominate in the myocytes of Rana tigrina and this overwhelms the more efficient post-receptor coupling of β₁ blockers.

Keywords—Metoprolol, Propranolol, adrenaline, Frog heart.

I. INTRODUCTION

Sympathetic nervous system plays a vital role in a wide variety of physiologic and pathophysiologic responses such as stress, exercise in the body. So sympathomimetic drugs and drugs that block adrenoceptors have important effects, some of which are of great clinical value. These effects vary dramatically according to the drug’s selectivity for α and β receptors. β₁ and β₂ adrenoceptors coexist in the heart of various animal species, including man. Both receptors are positively coupled to the adenylyl cyclase system and participate in the mediation of the positive chronotropic and inotropic effects of catecholamines. However, the relative amount of each receptor subtype as well as the post receptor cellular signaling pathways may differ significantly depending on the cardiac tissue, the animal species, the pathophysiological state, the age or the developmental stage.

Competitive radio-ligand binding studies performed in membranes from homogenized hearts have shown that only 20-30% of the total β-adrenoceptors are of the β₂-subtype in adult mammalian ventricular tissue. This number is even further reduced when purified cardiac myocytes rather than homogenized tissues are used. Yet, selective activation of β₂–adrenoceptors produces a large increase in the amplitude of contraction in intact mammalian cardiac muscle as well as in isolated ventricular myocytes. Moreover, selective β₂-adrenoceptor activation was found to produce a stimulation of the L-type Ca²⁺ channel current (I_{Ca,L}) in guinea-pig atrial myocytes, and in rat, guinea-pig and dog ventricular myocytes. When compared to the effect produced by non-selective β-adrenoceptor agonists such as isoprenaline, the β₂-response may present 25-100% of the
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isoprenaline response. This suggests that the two receptors may differ in their signaling cascade or in the post receptor amplification mechanisms.

Unlike the mammalian heart, β-adrenoceptor population in the frog heart is composed of a majority (~80%) of β₁-receptors. Thus, one may question the functional role of β₁-adrenoceptors in this preparation and their contribution to the sympathetic control of heart function.

For this reason, the present study undertaken to evaluate the effect of Propranolol (non-selective β-antagonist) and Metoprolol (selective β₁-antagonist) on adrenaline (β-agonist) induced isolated frog heart.

II. MATERIAL & METHODS

Frogs, weighing 150 – 250g, double pith a frog and fasten it to a frog board, ventral side up; midline incision was given on the abdomen. Pectoral girdle was removed and the heart was exposed. Pericardium was removed carefully and few drops of frog ringer were poured over the heart by holding with the forceps pericardial sac was cutted carefully away from the heart using with scissors, A thread was passed under inferior vena cava and 'v' shaped cut was given in inferior vena cava and the tip of syme’s cannula was passed into it. It was tied firmly with inferior vena cava to assure the cannula in place. Immediately the aorta were cutted and carefully heart along with cannula were isolated from the animal, horizontal arm of syme’s cannula was connected to the perfusion bottle containing frog ringer while the vertical arm is fixed with the clamp, Thin pin hook was passed throug aorta and the tip of syme’s cannula was passed into it. It was tied firmly with inferior vena cava to assure the cannula in place. Immediately the aorta were cutted and carefully heart along with cannula were isolated from the animal, horizontal arm of syme’s cannula was connected to the reservoir containing frog ringer solution and the flow was adjusted such that the level of fluid in the vertical arm remains constant. The heart was stabilized for 15min prior to the administration of drug. All the drug containing solutions were freshly prepared before the experiments: Propranolol and Metoprolol (1, 10 and 100µg/ml) respectively and Adrenaline (1µg/ml). Responses were recorded on a smoked drum using a starling’s heart lever.

After taking the normal recordings for about 2 - 3cm. Adrenaline 1µg was added and response was recorded. The effect of non-selective β adrenergic blocker propranolol was added to the biphasic in addition to 1 µg of adrenaline and the contraction of heart till the 70-80% of inhibition is produced and the difference from normal contraction (inhibition in height of contraction) was recorded. The procedure was repeated by adding β-blocker propranolol in the dose of 1.0 µg, 2 µg, 4 µg and 8 µg respectively. The effect of cardio-selective β adrenergic blocker Metoprolol was added to the biophase in addition to 1 µg of adrenaline and the contraction of heart till the 70-80% of inhibition is produced and the difference from normal contraction (inhibition in height of contraction) was recorded. The procedure was repeated by adding β-blocker Metoprolol in the dose of 2.5 µg, 5 µg, 10 µg, 20 µg, 40 µg, 80 µg, 160 µg and 320 µg respectively. Individual findings are recorded on smoked drum cylinder, fixed with resin (colophony) and the recordings are measured.

III. RESULTS

Shown in table no 1, 2 & 3. Median inhibitory Dose (ID₅₀): Propranolol 1.3 µg and metoprolol 44µg

Table: 1. Propranolol on the contraction of heart, Mean % inhibition and probit

| Dose(µg) | Log dose(µg) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Difference in mm | Inhibition (%) | Mean inhibition % | probit |
|---------|-------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|---------------|
| 0       | -           | 13              | 0              | 8               | 0              | 5               | 0              | 6              | 0              | 10             | 0              | 10             | 0              | 10             | 0              | 10             | 0              | 10             | 0              | 10             | 0              | 10             | 0              | 12             | 3.8           |
| 0.5     | -0.3        | 10              | 23             | 8               | 0              | 4               | 20             | 6              | 0              | 8              | 20             | 9              | 10             | 43             | 4.8            |
| 1       | 0.0         | 6               | 54             | 5               | 38             | 2               | 60             | 5              | 17             | 5              | 50             | 6              | 40             | 80             | 5.8            |
| 2       | 0.3         | 2               | 85             | 2               | 75             | 1               | 80             | 1              | 83             | 2              | 80             | 2              | 80             | 87             | 6.1            |
| 4       | 0.6         | 1               | 92             | 1               | 88             | 1               | 80             | 1              | 83             | 1              | 90             | 1              | 90             | 87             | 6.1            |
| 8       | 0.9         | 1               | 92             | 1               | 88             | 1               | 80             | 1              | 83             | 1              | 90             | 1              | 90             | 87             | 6.1            | 12             | 3.8           |
There was lack of parallelism of DRC of the myocytes of R. tigrina than metoprolol. These interesting facts can be supported by the finding that respectively and interestingly propranolol was more efficient in inhibiting the contraction of adrenals than metoprolol. The ID₅₀ of propranolol and metoprolol was found to be 1.3 and 44 μg respectively and interestingly propranolol was more efficient in inhibiting the contraction of adrenaline than metoprolol. These interesting facts can be supported by the finding that β₂ receptors predominate in the myocytes of Rana tigrina and this overwhelms the more efficient post-receptor coupling of β₁ blockers.

IV. DISCUSSION

The study showed that there was dose dependent inhibition of adrenaline induced contraction of frog’s heart by both Propranolol and Metoprolol and the ID₅₀ of them were calculated from the DRC by interpolation method. Unexpectedly, the ID₅₀ of Metoprolol, the cardio-selective β-blocker drug was greater than that of Propranolol. Propranolol was a non-specific β-blocker which blocks both β₁ and β₂ adrenergic receptors while Metoprolol was a cardio-selective β-blocker whose effect is confined to blocking of β₁ receptors in the normal dose range.

It was a matter of great interest that unlike human heart, the frog cardiac myocytes is exclusively mediated by β₂ receptors. This finding is also consistent with the fact the sympathetic nerves carry adrenaline in the frog and the β₂-receptors is, by definition, an ‘adrenaline receptor’. However, there is evidence for the presence of β₁-receptors in frog cardiac myocytes and their relative proportion is comparable to that of β₂ receptors in mammalian cardiac myocytes. These findings may explain the low ID₅₀ of Propranolol in comparison to metoprolol.

V. CONCLUSION

Dose-dependent inhibition of adrenaline-induced contraction of myocytes of Rana tigrina were studied for non-specific β-blocker (both β₁ and β₂ blocker) propranolol and human cardio-selective β-blocker (β₁ blocker) metoprolol. The ID₅₀ of propranolol and metoprolol was found to be 1.3 and 44 μg respectively and interestingly propranolol was more efficient in inhibiting the contraction of adrenaline than metoprolol. These interesting facts can be supported by the finding that β₂ receptors predominate in the myocytes of Rana tigrina and this overwhelms the more efficient post-receptor coupling of β₁ blockers. There was lack of parallelism of DRC of the two drugs may be explained by the fact overwhelming number
of β₂ receptors and elevation Cyclic AMP in a compartment more efficiently coupled to L-type Ca** channels than β₁-receptors. However, more experiments to be done to establish this fact conclusively.

REFERENCES: