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Comparison of propranolol and metoprolol on isolated frog heart

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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. β_1 and β_2 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 β_2-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 β_2-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 ID_{50} 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 β_2 receptors predominate in the myocytes of Rana tigrina and this overwhelms the more efficient post-receptor coupling of β_1 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. β_1 and β_2 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 β_2-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 β_2–adrenoceptors produces a large increase in the amplitude of contraction in intact mammalian cardiac muscle as well as in isolated ventricular myocytes. Moreover, selective β_2-adrenoceptor activation was found to produce a stimulation of the L-type Ca^{2+} channel current (I_{Ca}) 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 β_2-response may present 25-100% of the

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 β_2-receptors. Thus, one may question the functional role of β_1-adrenoceptors in this preparation and their contribution to the sympathetic control of heart function.

For this reason, this present study undertaken to evaluate the effect of Propranolol (non-selective β-antagonist) and Metoprolol (selective β_1-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 through the tip of the ventricle and with the help of a fine thread attached to the hook, it is tied to the free limb of the heart lever, which is fixed to a stand and the tension adjusted with the spring such that it gives maximum contraction. Proper tension and magnification was adjusted by altering the height of the lever. The cannula 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 (ID_{50}): Propranolol 1.3 µg and metoprolol 44µg

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

Dose(µg)	Log dose(µg)	Frog 1		Frog 2		Frog 3		Frog 4		Frog 5		Frog 6			
		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	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	12	3.8

Table: 2. Metoprolol on the contraction of heart, Mean % inhibition and probit

Dose(µg)	Log dose(µg)	Frog 1		Frog 2		Frog 3		Frog 4		Frog 5		Frog 6			
		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	-	9	0	16	0	11	0	11	0	6	0	7	0	0	3.7
1	0.0	9	0	15	6	11	0	9	18	4	33	7	0	0	3.8
2.5	0.4	9	0	14	13	10	9	9	18	4	33	7	0	0	4.0
5	0.7	9	0	14	13	9	18	9	18	4	33	6	14	14	4.1
10	1.0	9	0	13	19	9	18	9	18	4	33	6	14	14	4.6
20	1.3	6	33	11	31	8	27	8	27	3	50	4	43	43	4.9
40	1.6	3	67	10	38	7	36	7	36	3	50	3	57	57	5.6
80	1.9	2	78	5	69	4	64	3	73	2	67	1	86	86	6.1
160	2.2	1	89	1	94	2	82	1	91	1	83	1	86	86	6.2
320	2.5	1	89	1	94	2	82	1	91	1	83	1	86	0	3.7

Table: 3 statistically by multiple regression method

SOURCE	SS	df	MS	F
Reg (X)	77.36801	1	77.36801	0.121447
Residual	21659.69	34	637.0496	
Reg (X,Z)	15883.43	2	7941.716	44.7717
Residual	5853.622	33	177.3825	
Reg (X,Z,XZ)	18040.7	3	6013.567	52.06054
Ridual	3696.354	32	115.5111	

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 ID_{50} of them were calculated from the DRC by interpolation method. Unexpectedly, the ID_{50} of Metoprolol, the cardio-selective β-blocker drug was greater than that of Propranolol. Propranolol was a non-specific β-blocker which blocks both β_1 and β_2 adrenergic receptors while Metoprolol was a cardio-selective β-blocker whose effect is confined to blocking of β_1 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 β_2 receptors. This finding is also consistent with the fact the sympathetic nerves carry adrenaline in the frog and the β_2-receptors is, by definition, an 'adrenaline receptor'. However, there is evidence for the presence of β_1-receptors in frog cardiac myocytes and their relative proportion is comparable to that of β_2-receptors in mammalian cardiac myocytes. These findings may explain the low ID_{50} 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 β_1 and β_2 blocker) propranolol and human cardio-selective β-blocker (β_1 blocker) metoprolol. The ID_{50} 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 β_2 receptors predominate in the myocytes of *Rana tigrina* and this overwhelms the more efficient post-receptor coupling of β_1 blockers. There was lack of parallelism of DRC of the two drugs may be explained by the fact overwhelming number

of β_2 receptors and elevation Cyclic AMP in a compartment more efficiently coupled to L-type Ca^{++} channels than β_1-receptors. However, more experiments to be done to establish this fact conclusively.

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