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Binding Studies of Cardiovascular Drug on β Adrenoceptors in Rat Left Ventricle using (−)-[^{3}H]-DHA, Non-β_1/β_2-selective Radioligand

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ABSTRACT

β-Adrenoceptor binding study of β-agonist ((−)NE), β-antagonists ((±) propranolol, labetalol) and PDE inhibitors (imazodan, KR-30045, KR-30075 etc.) was performed using (−)-[^{3}H]-DHA, as a non-β_1/β_2 selective radioligand. In saturation studies, K_d and B_{max} of (−)-[^{3}H]-DHA to β-adrenoceptors in rat left ventricle in which both β_1 and β_2 receptors coexist were determined to be 1.5±0.43 nM and 22.0±0.9 fmol/mg protein, respectively. (±) Propranolol, labetalol and (−)NE competed for (−)-[^{3}H]-DHA binding sites in an essentialy monophasic manner with Ki=17.0±0.40 nM, 57.3±1.30 nM and 1.57±0.95 μM, respectively. All of PDE inhibitors inhibited the (−)-[^{3}H]-DHA binding by only below 10% even at the high concentration of 10^{-3}M. The present results suggest that propranolol, labetalol and NE are non-β_1/β_2 selective antagonists and agonist, respectively.

Additionally, this study shows that imazodan and new synthesized PDE inhibitors may hardly have the affinities to β-adrenocetors in cardiac muscle.

Key Words: (−)-[^{3}H]-DHA, non-β_1/β_2 selective radioligand, Rat left ventricular muscle, Coexistence of β_1/β_2 receptors

Abbreviation: NE; norepinephrine, PDE; phosphodiesterase, DHA; dihydroalprenolol

INTRODUCTION

β-Adrenoceptors are stimulated by the activation of adenylate cyclase via a regulating coupling protein that binds to nucleosides (Stiles *et al.*, 1984). The activation of adenylate cyclase leads to an increase in intracellular c-AMP which mediates many of the physiological effects such as the stimulation of β-receptors by agonists in cardiac muscle (Reuter *et al.*, 1983). All of the β-receptors, i.e., β_1-and β_2-receptors, are thought to be closely associated with adenylate cyclase (Brodde *et al.*, 1984; Liang *et al.*, 1986). However, in general, the positive inotropic effect of catecholamines is thought to be related to β_1-receptors in ventricular muscles in spite of the evidence for the coexistence of β_1- and β_2-receptors in the muscles.

Recently some highly selective β_1- and β_2-agonists became available. Selectivity to β-receptors can be evaluated by the receptor competition binding studies with membranes which contain β_1- and β_2-adrenoceptor. In this respects, membranes of rat heart and lung have been proved to

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be very useful (Annerose *et al.*, 1990) for the study because of their varing β_1/β_2-receptor ratio of about 65/35 and 20/80, respectively (Nahorski *et al.*, 1981). Using rat heart or lung membranes, the selective β-adrenoceptor drugs have been identified (Daemmgen *et al.*, 1982; Klockow *et al.*, 1986).

Intracellular concentration of c-AMP can be increased by PDE inhibitors as well as by β-stimulants. Especially, selective inhibitors of PDE III, a low Km, c-AMP specific form of the enzyme, have been developed for potential cardiotonic agents (Ahn *et al.*, 1986; Weiss *et al.*, 1977; Lee *et al.*, 1989). Of course, it is possible that positive inotropic effects of these agents can also be associated with β-adrenergic effects.

In this study, we attempted to examine whether the new synthesized c-AMP-PDE inhibitors have the affinities to β_1- or β_2- receptors in cardiac muscle in addition to the binding experiments of β-adrenoceptor agonist (NE) and antagonists (propranolol, labetalol). Non β_1/β_2- selective radioligand [^{3}H]-DHA and rat ventricular muscles were used to investigate the competition properties of drugs.

MATERIALS AND METHODS

Radioligand (−)-[^{3}H]-DHA was purchased from Amersham Bucher. (−)-Isoproterenol bitartrate, (±) propranolol, (−) norepinephrine, labetalol were obtained from Sigma Chemical Co. Imazodan and new PDE inhibitors of pyridazinone structure (KR-30045, KR-30075) or tetrazole structure (KR-3017, KR-30120), were synthesized by Medicinal Chemistry Department, Korea Research Institute of Chemical Technology (KRICT). All reagents used were the parest grade available.

Male wistar rats weighing 250-300 g were used in this experiment, Rats were sacrificed by decapitation, after which the hearts were Minneman *et al.* (1979), and stored at −70°C before use. Proteins were assayed by the method of Lowry *et al.* (1951).

The procedures of saturation and competition are essentially identical to those described previously (Jones *et al.*, 1979). Non specific binding was observed in the presence of 100 nM (−) isoprenolol (Jones *et al.*, 1980). For the competition experiments, the final concentration of (−)-[^{3}H]-DHA was 3 nM.

Receptor binding data were analyzed by a computer system using the Mcpherson program (1985). Values presented were mean±SEM of N separate experiments.

RESULTS

For saturation experiments, membrane were incubated with increasing concentrations of (−)-[^{3}H]-DHA from 0.2 nM to 20 nM. The results from a typical saturation experiment are shown in Fig. 1. A specific binding was decreased from about 80% to 40% of the total binding in proportion to the concentration of radioligand. At 3.0 nM (−)-[^{3}H]-DHA, a specific binding was approximately 75% of the total binding.

The scachard plot of a typical experiment dealing with the binding of (−)-[^{3}H]-DHA to the membrane preparation are shown in Fig. 2. A

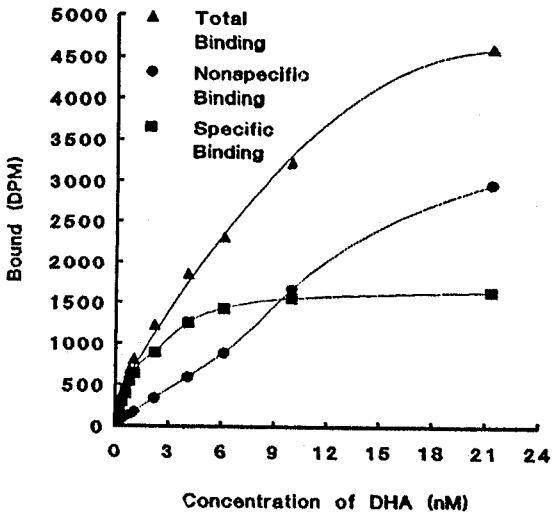

Fig. 1. A typical single experiment of specific (−)-[^{3}H]-DHA binding to a membrane preparation from rat left ventricle (septum plus wall). A specific binding to β-adrenergic receptors is defined as the difference between the total amount of radioactivity bound in the presence of (−)-[^{3}H]-DHA alone and the nonspecific binding observed in the presence of (−)-[^{3}H]-DHA plus (−) isoprenolol.

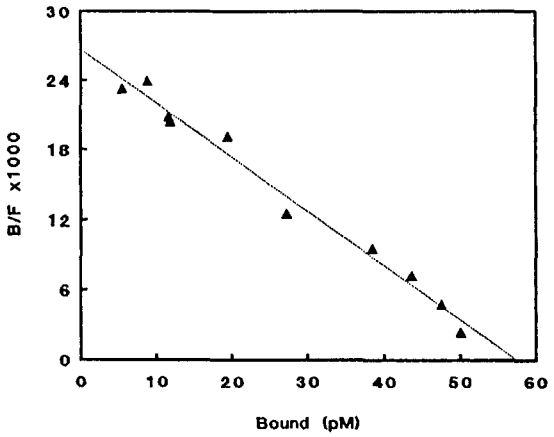

Fig. 2. A scatchard plot of a saturation experiment. Mean of the Kd and B_{max} values of (−)-[^{3}H]-DHA were $1.5\pm0.43\times10^{-9}$ M and 22.0 ± 0.9 fmol/mg protein (mean of 5 experiments), respectively.

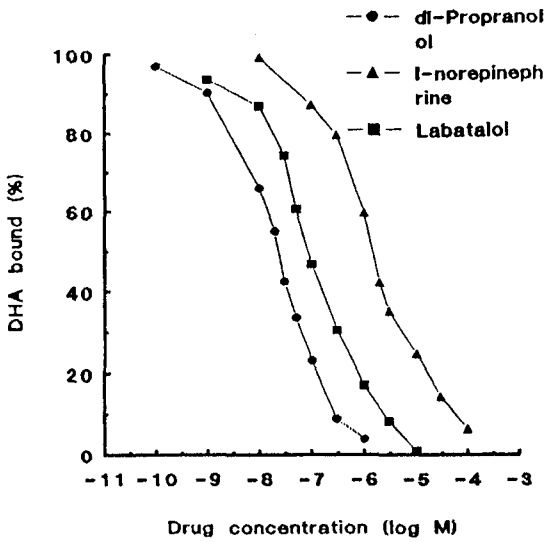

Fig. 3. Competition curves by (±)-propranolol, labetalol and (−)-NE for (−)-[^{3}H]-DHA binding to rat left ventricular membranes. A representative experiment, replicated three or four times, is shown.

Table 1. Apparent affinities of β-adrenoceptor drugs in competition with (−)-[^{3}H]-DHA in rat left ventricle

Radioligand	Unlabeled drug	Ki (M)	IC_{50} (M)
(−)-[^{3}H]-DHA	(±) propranolol	$1.70\pm0.40\times10^{-8}$	$1.91\pm1.12\times10^{-8}$
	(−)-norepinephrine	$1.57\pm0.95\times10^{-6}$	$2.08\pm2.0\times10^{-6}$
	labetalol	$5.73\pm1.30\times10^{-8}$	$7.30\pm2.95\times10^{-8}$

Ki values were determined from the equation, $Ki=IC_{50}/(1+(-)[^3H]\text{-DHA/Kd})$, described by Cheng and Prosoff (1973), by the computer supported analysis. Each value shown is the mean±SEM of values from 3-4 separate experiments.

Table 2. Effects of PDE inhibitors on PDE_s in a guinea pig left ventricles muscle

Agent	IC_{50} (μM)			
	Enzyme	PDEI	PDE II	PDE III
	Substrate	cAMP	cGMP	cGMP	cAMP
Imazodan		>1000	>1000	414	4.5
KR-30045		>1000	>1000	>1000	2.2
KR-30075		>1000	>1000	>1000	1.9
KR-30117		56	58	35.8	14.9
KR-30120		30	44	38	17.8

The IC_{50} values were determined from concentration-response curves, in which concentrations ranged from 10^{-8} to 10^{-3} M. Each experiment assayed in triplicate. The substrate (cAMP or cGMP) concentration used was 1 μM>

computer-assisted evaluation of the binding data exhibited a Kd=1.5±0.43 nM, and the B_{max} was 22.0±0.9 fmol/mg protein.

The data from the competition experiments of β-adrenoceptor drugs summarized in Table 1 and are shown in Fig. 3. The order of the potency of these drugs in inhibition of the binding of (−)] [^{3}H]--DHA was (±) propranolol>labetalol>> (−) NE.

As previously described (Lee *et al.*, 1989), imazodan and new drugs (KR-30045, KR-30015, KR-30117, KR-30120) are PDE inhibitors of various selectivity (Table 2). In the binding experiments to β-receptors, all of those PDE inhibitors exhibited the displacement of below 10% even at the high concentration of 10^{-3} M.

DISCUSSION

The results of 5 saturation experiment using separately prepared membranes were not significantly different from one another and are similar to the results by others (Jones *et al.*, 1980) Assuming the data from the saturation studies are reliable, we conducted the following competition experiments using the above data.

(±) Propraolol and labetalol exhibited relatively high affinities to β- receptors in rat left ventricle. (−)-NE showed a weak affinity to the membrane. In fact, it is well known that NE has much higher affinity to α- adrenoceptors than β-adrenocetors. All of these drugs inhibited (−)-[^{3}H]-DHA binding to the membranes in a concentration dependent manner, as shown Fig. 3. Competition curve evaluations of (±) propranolol, labetalol and NE were best fit to the one-binding-site. The data indicate that the these drugs have the similar affinities to β_1- and β_2- receptors because of the existence of both receptors in the rat left ventricular membrane.

Previously (Lee *et al.*, 1989), we have demonstrated that imazodan, KR-30045, KR-30075, KR-30117 and KR-30120 were potent and selective PDE inhibitors. Thus, they are expected to be useful as cardiotonic agents. In this study, these PDE inhibitors hardly inhibited the (−)-[^{3}H]-DHA binding to β- adrenoceptors in rat left ventricle. Thus it is suggested that cAMP increasing effect of these agents are due to the PDE inhibition, not by the β- adrenoceptor stimulation.

In conclusion, it was shown that (±) propranolol, labetalol and (−)-NE were β_1-, β_2- nonselective antagonists and agonist, respectively. Also we found that imazodan and new PDE inhibitors (KR-30045, KR-30075, KR-30117, KR-30120) may hardly have the affinities to both β_1- and β_2-adrenoceptors.

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=국문초록=

β_1/β_2 비선택적 Radioligand (−)-[^{3}H]-DHA를 사용한 Rat 좌심실 β-adrenoceptor에 대한 심장순환계 약물의 Binding

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β-수용체 효능약물 ((−)-NE), 길항약물 ((±)-propranolol, labetalol) 및 PDE 억제약물 (imazodan, KR-30045, KR-30075 등)에 대한 β-adrenoceptor binding 실험을 β_1/β_2 비선택적 radioligand인 (−)-[^{3}H]-DHA를 사용하여 실시하였다. Saturation 실험에서 β_1 및 β_2 수용체를 모두 갖고 있는 rat 좌심실의 β 수용체에 대한 (−)-[^{3}H]-DHA의 K_d 값은 1.5±0.43 nM, B_{max}는 22.0±0.9 fmol/mg protein이었다. (±)propranolol, labetalol 및 (−)NE는 단일상으로 (−)-[^{3}H]-DHA의 결합을 억제하였으며 Ki 값은 각각 17.0±0.43 nM, 57.3±1.30 nM, 1.57±0.95 μM로 나타났다. 실험에 사용한 모든 PDE 억제약물들은 (−)-[^{3}H]-DHA 결합을 10^{-3} M의 고농도에서도 10% 미만으로 억제했다.

실험결과, propraolol, labetalol 및 NE는 β_1/β_2 수용체에 대해 비선택적인 약물로 나타났으며, imazodan 및 신합성 PDE 억제약물들은 rat 심근에 있는 β- 수용체에 친화성이 거의 없음을 알 수 있었다.