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ALTERATIONS IN BETA-ADRENERGIC RECEPTOR DENSITY ON HUMAN LYMPHOCYTES IN RESPONSE TO CHRONIC EXERCISE

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ALTERATIONS IN BETA-ADRENERGIC RECEPTOR DENSITY ON HUMAN LYMPHOCYTES IN RESPONSE TO CHRONIC EXERCISE

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INTRODUCTION

A number of cardiovascular adaptations have been shown to occur in healthy individuals as a result from regular, chronic exercise training. These changes include, but are not limited to, a lower resting heart rate, a lower heart rate at any given submaximal workload, an increase in stroke volume, an increase in maximal cardiac output due primarily to an increase in contractility, a decreased peripheral vascular resistance (increased peripheral vascular conductance), an overall increase in vascularity, an increase in left ventricular mass, and an increase in total body oxygen extraction (Raven, 1994). Some of these adaptations are also known to commonly occur in patients with coronary artery disease enabling them to increase their total work capacity. Therefore, exercise apparently adapts the heart to better cope with the adverse effects of coronary artery disease and helps to prevent the aforementioned disease from developing in healthy individuals.

The beta-adrenergic receptor (β-AR) is essential for the activation of many aspects of the cardiovascular system during dynamic exercise (1). The catecholamines epinephrine and norepinephrine are released from the adrenal medulla and postganglionic fibers of the sympathetic nervous system respectively in response to dynamic exercise. Epinephrine and other beta-adrenergic receptor agonists bind and activate the β–AR on the cell membrane thus allowing it to couple with the stimulatory GTP-binding regulatory protein Gs. This step initiates the activation of adenylate cyclase and the synthesis of cyclic adenosine 3',5' monophosphate (cyclic AMP), a key intracellular second

messenger. Cyclic AMP ultimately activates cyclic AMP-dependent protein kinase (PKA), an enzyme that phosphorylates a number of different intracellular proteins that subsequently influence cell metabolism and function.

Alterations in the activity of the adrenergic system seen in several clinical and physiological situations, including exercise, are directly associated with changes in lymphocytic β–AR density or function (2). Moreover, it has been suggested that the changes in receptor density on lymphocytes correlate closely with cardiovascular responsiveness to catecholamines in humans (3-6). Additionally, changes in catecholamine concentration within the physiological range have a regulatory effect on β–AR density and function (7). One particular study established an inverse relationship between plasma and urine catecholamine concentrations and lymphocytic β–AR density in man (8).

It is the intent of this review to describe some of the cardiovascular adaptations that occur as a result of chronic exercise and how these changes could be caused by alterations in β–AR density and responsiveness. Additionally, the comparisons and contradictions between chronic heart failure and chronic exercise will be made. The role of the beta-adrenergic system in mediating the effects of exercise will be introduced. The structure of the β–AR will be described and how its molecular structure dictates its function. A brief synopsis will be presented on the mechanism in which β–AR operates subsequent to ligand binding. Alterations of the β–AR, particularly its overexpression in the heart, through transgenics will then be reviewed to show how this receptor could be responsible for some of the aforementioned adaptations to chronic exercise. In this, some

of the differences between the β_1- and β_2-AR will be described as well as some of the therapeutic implications that could result from overexpression of the β–AR. Following this, alterations in the density of the β–AR after both short-term and long-term exposure to catecholamines will be examined. Included in this section will be the detailed description of the mechanism of receptor desensitization that precedes receptor down-regulation. A brief review will then be given on the effects of chronic exercise on β–AR density. The use of human lymphocytes as model cells will then be described. Binding theory will be explained as it will be the basis of methodology used in any subsequent studies. Along with this, [125 Iodo] cyanopindolol (^{125}I-CYP) will be introduced and its advantages and disadvantages as a β–AR ligand probe will be discussed.

CARDIAC ADAPTATIONS TO CHRONIC EXERCISE

Several cardiovascular changes occur as the result of regular exercise training. In addition, similar cardiovascular changes are also observed in patients with chronic heart failure. Below, some of the mechanisms underlying these adaptations will be explored as well as whether or not the changes in β–adrenergic responsiveness that may result from chronic exercise could be a regulator of some of these adaptations.

Heart Rate

Although the heart beat is intrinsic in nature and does not require input from the central nervous system, an increase or decrease in heart rate is dictated by both the sympathetic and parasympathetic nervous system respectively. The sympathetic branch, through the β–AR, increases the heart rate whereas the parasympathetic branch, through the vagus nerve, slows the heart. The majority of studies to date support the conclusion that the bradycardia that results from regular exercise training is due primarily to an increase in parasympathetic activity (Raven, 1994). Focusing on the sympathetic side of the autonomic nervous system, studies have shown that there is a decrease in the concentration of catecholamines released in trained individuals (95,96). This observation could be a contributing factor in the lower resting heart rate seen in athletes. In theory, a decrease in β–adrenergic responsiveness, as seen with chronic exercise, could also be a factor in a lower resting heart rate. Also, transgenic overexpression of the β–AR results in faster heart rates in mouse models (97).

Cardiac Output

Although maximum cardiac output is increased with training, at any submaximal workload, it is unchanged if not lowered slightly. To maintain cardiac output, stroke volume must increase in the trained individual to compensate for the decreased heart rate. The result is an increased "efficiency" of the heart at any submaximal workload as well as a decreased arterial pressure. Accordingly, an important benefit of chronic exercise training is the reduced work performed by the heart at any submaximal workload (98).

Nearly all of the increase in maximal oxygen delivery (VO_2max) and maximal cardiac output that results from physical training is due to the increase in maximal stroke volume (99). Stroke volume can be increased by two cardiac adaptations an increase in chamber volume due to chronic exercise or an increase in contractility of the myocardium. Both are seen in the trained heart and the latter is dictated by the phosphorylation of myofilaments by a cyclic AMP-dependent protein kinase that is activated through the β–AR (97). Phosphorylation events increase the availability of calcium to bind to troponin which then activate more cross bridges allowing for a greater contractile force. It is unclear if a decrease in β–AR responsiveness has any effect on the contractile properties of the heart because in theory, a decrease in responsiveness of the beta-receptor would lead to a decreased availability of calcium to bind to the cardiac myofilaments causing a decrease in contractility. Therefore, it is probable that another event related to chronic exercise training is responsible for the increase in contractility of the heart. A recent study demonstrated that overexpression of the β–AR 200 times the normal amount in the mouse heart increased contractility (97). The enhanced contractile

performance was present under basal, unstimulated conditions demonstrating that the β–AR has intrinsic activity when overexpressed (97).

Peripheral Vascular Resistance

An increase in vascular conductance is seen with endurance training and is affected by a reduced peripheral resistance during submaximal and maximal exercise (Raven, 1994). This decrease in vascular resistance allows the endurance athlete to obtain a much higher cardiac output than the untrained individual. Although the mechanism responsible for the decreased resistance is unclear, the increase in overall vascularity seen with chronic exercise training is an important factor. Also, beta-adrenergic receptors, β_2-AR specifically, found on smooth muscle cells of blood vessels increase the levels of intracellular cyclic AMP causing a vasodilation of the vessels. A decrease in density of the β–AR that could result from chronic exercise, a topic that will be reviewed later, could not explain this vasodilation since a decrease in receptor number would be expected to lead to a decreased intracellular cyclic AMP level.

Coronary Blood Flow

Trained animals show a greater capillary-to-fiber ratio than untrained animals and this adaptation could be an important means of delivering more oxygen to myocardial cells. One study found that when heart rate, afterload and perfusion pressure were all held constant in isolated rat hearts, coronary blood flow was much greater in the hearts of trained animals probably due to an increase in overall myocardial vascularity (98).

Whether or not training has any effect on coronary blood flow in humans is not known. Angiogenesis, the growth of new vessels, is thought to be responsible for the increased vascularity seen in the trained heart. There is no current evidence that links alterations in β–AR density or responsiveness to angiogenesis.

Left Ventricular Mass

An increase in left ventricular mass is seen in endurance trained athletes with a concomitant increase in chamber size. This type of hypertrophy, termed eccentric hypertrophy, is distinct from the hypertrophy seen in diseased hearts in that while the diseased heart shows an overall increase in left ventricular mass, the chamber size does not increase. The increase in maximal stroke volume seen in chronic exercise training is directly correlated with the increase in left ventricular chamber size. It is not known whether or not the β–AR and its alterations in the trained individual have any effect on the hypertrophy of the left ventricle. One particular study demonstrated that the overexpression of the β–AR in the heart resulted in delayed cardiomyopathy and cardiac hypertrophy (100). Therefore, a down-regulation of the β–AR as a result of chronic exercise could not explain the enlarged heart seen in the trained athlete.

The increase in ventricular mass is most probably caused by cardiomyocyte hypertrophy as opposed to cardiomyocyte hyperplasia since adult ventricular cells are not thought to possess the ability to undergo cell division (119,120). Endurance exercise training elicits longitudinal myocyte hypertrophy in the absence of changes in intrinsic sarcomere length in the rat (121). It has also been suggested that in response to exercise

training, epicardial myocyte hypertrophy is greater than endocardial myocyte hypertrophy (122). The observation that whole cell capacitance, a measure of cell membrane surface area, is increased by endurance training further supports the concept that training promotes cardiomyocyte growth (123).

Myocardial Oxygen Consumption

Although the cardiac output remains relatively the same in the trained individual, heart rate decreases and stroke volume increases which equates to less work performed by the heart. One could then predict that the myocardial oxygen consumption would decrease with chronic exercise training. Catecholamines, such as epinephrine, are known to make the heart a less efficient organ and since catecholamine release is decreased with training (95,96), then one could predict a decrease in myocardial oxygen consumption with training.

β–ARs and Heart Failure

As stated previously, many of the same adaptations of the cardiovascular system to chronic exercise are seen in the failing heart. Although the trained heart and the diseased heart show many of the same adaptations, they are the antithesis of each other in efficiency. For example, the increase in exposure of β–ARs to epinephrine fuels the efficient heart whereas increased plasma catecholamines seen in chronic heart failure attempt to compensate for the failing heart. It is now generally accepted that chronically elevated stimulation of the cardiac β–adrenergic system is toxic to the heart and may

contribute to the pathogenesis of heart failure. Administration of β–adrenergic agonists as therapy for patients with heart failure has been shown to decrease survival of the patients even though they produce immediate and long-term cardiovascular benefits (101). Moreover, in human heart failure, elevated circulating catecholamines lead to decreased levels and functionality of cardiac β_1-ARs and thus to desensitization of the heart to sympathetic stimulation (101). As seen later in this review, cardiac β_2-ARs do not appear to undergo down-regulation in human heart failure.

The desensitization of the β–AR, which will be discussed in detail later in this review, involves phosphorylation of the receptor by a kinase specific to G protein-coupled receptors, (GRK)(homologous desensitization) or by the cyclic AMP-dependent protein kinase (heterologous desensitization). It is widely believed that the mechanism of desensitization protects the heart from the negative effects of beta-adrenergic therapy, including the weakening of the contractile properties of the heart. The success of β–blockers in the treatment of chronic heart failure is explained by their ability to attenuate the negative effects of chronic endogenous sympathetic stimulation seen in the failing heart (101). Therapeutic strategies involving the overexpression of the β–adrenergic-coupled system seem counterproductive since current therapies involve blocking basal levels of receptor. However, as seen later in this review, expression of the β_2-AR or an inhibitor peptide for the GRK enzyme at moderate levels in the heart can increase cardiac performance through increased contractility. Additionally, overexpression of β–ARs, as well as the a-subunit of the Gs protein, has produced a cardiomyopathic phenotype in some mouse models (100,102,103).

EXERCISE AND THE STRUCTURE AND FUNCTION OF THE β–AR

Catecholamines, primarily epinephrine, play an essential role in the activation of the cardiovascular system and in the regulation of energy metabolism during physical exercise (78). Many of these effects are mediated through β–ARs located on the cell membranes of many types of cells. Many studies have focused on physiological changes in the beta-adrenergic system during, or as a result from, acute or prolonged physical exercise as well as receptor adjustments in heart failure (78). Changes in β–AR levels and functioning have been observed in a variety of clinical conditions (1,2). To further understand the β–AR and how it mediates cellular functioning, its structure and molecular mechanism of action must be described. Below, the molecular structure of the β–AR will be reviewed as well as the mechanism of receptor activation subsequent to ligand binding and how this mediates the responses of the cardiomyocytye to exercise.

The creation of complimentary DNA libraries from numerous species (9-12), including man (13-15), has allowed deduction of the primary amino acid sequence and structural analysis of the β–AR (Figure 1). As with all members of the G protein-coupled receptor superfamily of proteins, the β–AR is composed of seven hydrophobic transmembrane domains arranged in an alpha-helical nature. Extensive research suggests that the seven transmembrane domains are involved in ligand binding, while the cytoplasmic regions of the protein interact with the Gs-protein (16).

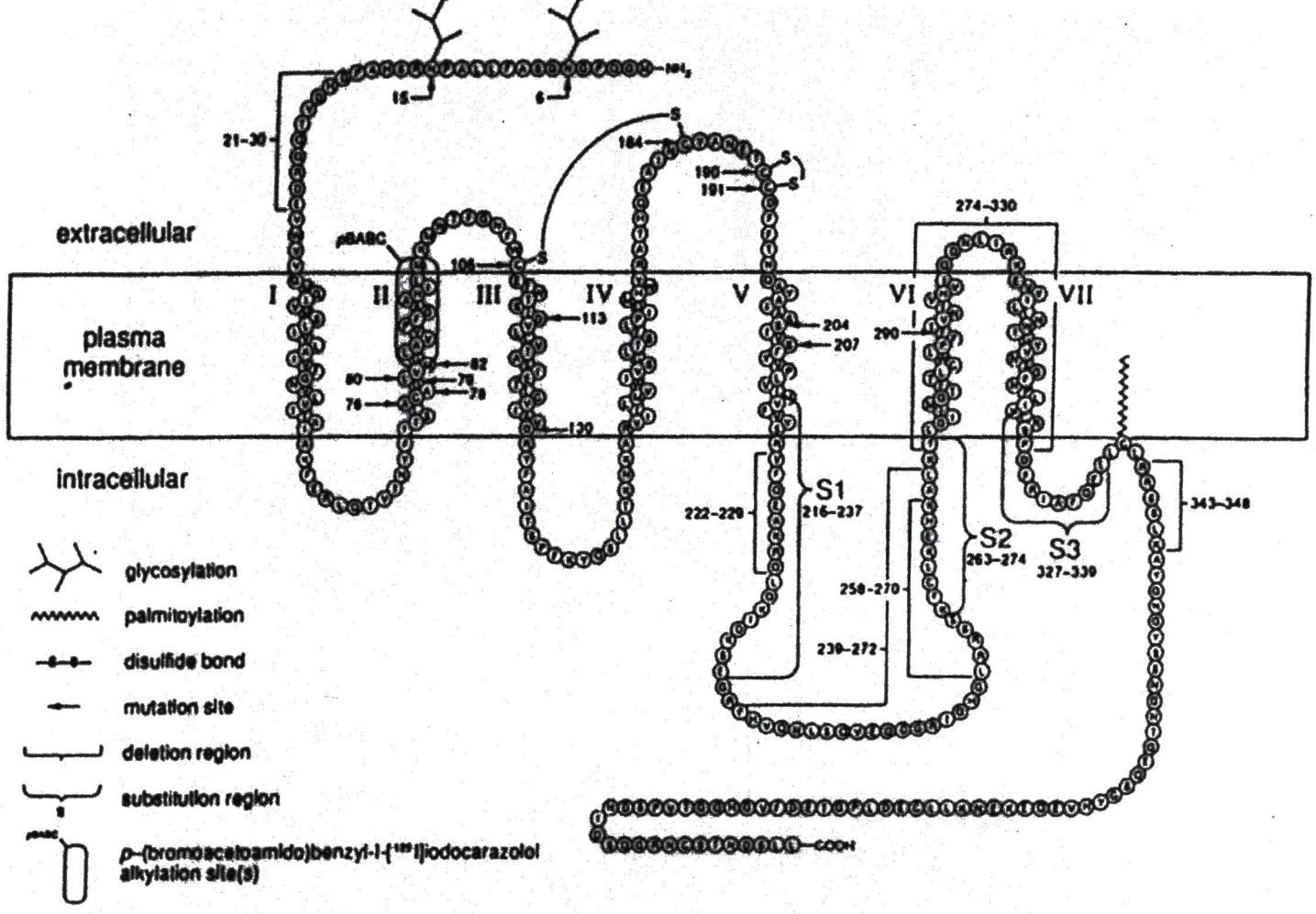


Figure 1. The Amino Acid Sequence of the β2-Adrenergic Receptor [From Ostrowski, L.J., et al., Mutagenesis of the β2-adrenergic receptor: How structure elucidates function, Annual Review of Pharmacology and Toxicology, 32: 167-183, 1992, pp169)]

Extracellular Domains of the β–AR

Upon treatment with specific proteases, it has been deduced that the N-terminus of the receptor faces the extracelluar side of the plasma membrane (17). It has been proposed that the N-terminal amino acid sequence of the β–AR is the site of specific N-linked glycosylation (17). For many cell surface proteins, glycosylation has been linked to the appropriate functioning and cellular distribution of the protein (18,19). Current data suggests that the carbohydrate chains on the N-terminus of the β–AR are needed for the transport of the receptor to the cell membrane. However, the glycosylated amino acid residues play no obvious role in ligand binding, coupling to the G-protein or activation of adenylate cyclase (20-22).

Hydrophobic Transmembrane Domains

Genetic and biochemical data through mutational analysis suggest that the amino acid residues found within the plasma membrane are crucial for ligand binding (17). The regions most likely involved in ligand binding are the second, third and seventh transmembranous segments of the human β–AR (24-27). Experiments focusing on the binding characteristics of the β–AR with the fluorescent antagonist carazolol have demonstrated that the ligand is bound to the receptor in a hydrophobic area that is buried deep within the core of transmembrane helices (28). Several studies involving the mutation of different areas of the hydrophobic transmembrane domain have revealed that the mutations lead to a substantial reduction in ligand binding (for review see ref. 16).

Hydrophilic Intracellular Domains

Three hydrophilic loops of the β–AR are present that extend into the extracellular space. These loops, containing disulfide bonds, are thought to be involved in the specific ligand-receptor interaction. Further, the rearrangement of disulfide bonds during posttranslational modification may induce receptor folding which would produce a thermodynamically stable form conducive for ligand binding (23).

Interaction of the β–AR with the Gs-protein occurs intracellularly subsequent to ligand binding (17). Data suggests that, of the three intracellular loops, the N- and C-terminal portions of the third loop are the primary regions responsible for the receptor-G protein interaction (16). Also, it is the third intracellular loop that must interact with other cytoplasmic domains, specifically the second intracellular loop, to obtain full efficiency and specificity in receptor-Gs protein coupling (16,27,29). Between the two major subtypes of β–AR, both the β_1- and β_2-AR require that the third intracellular loop act in concert with the second intracellular loop or the N-terminal portion of the cytoplasmic tail to obtain full efficiency and specificity in receptor-G protein coupling (16). Through mutational analysis it has been determined that a portion of the third cytoplasmic loop as well as numerous positions along the cytoplasmic tail of the β–AR are phosphorylated during the desensitization of the receptor (108). These intracellular sites must be phosphorylated by their appropriate kinases before the receptor can be desensitized, internalized and thus down-regulated. The mechanism of receptor desensitization and subsequent down-regulation will be discussed later in this review.

The cyclic AMP signal transduction pathway (Figure 2) is one of the most important mechanisms for transmembrane signaling in virtually all mammalian cells (30).

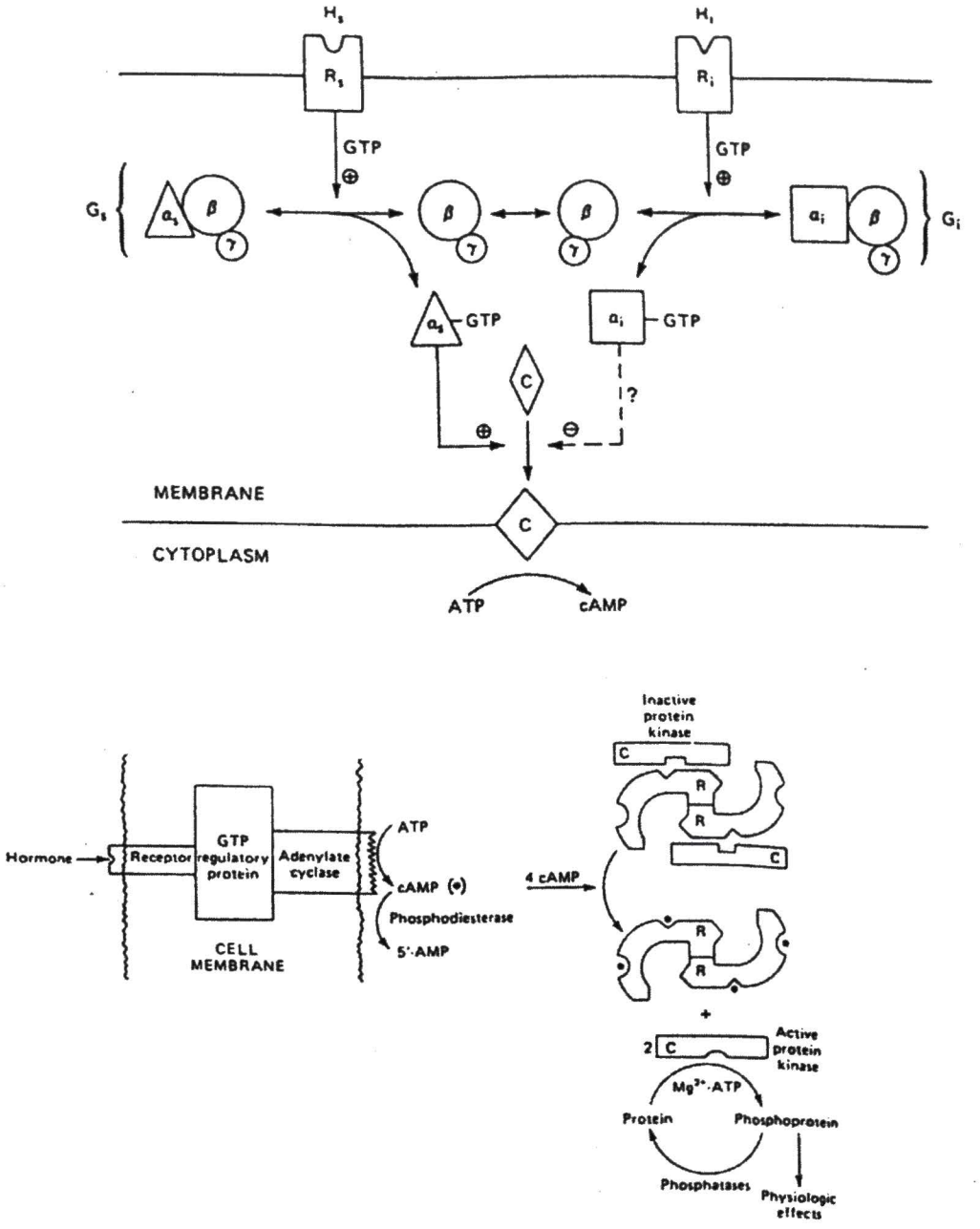


Figure 2. The G-Protein Mediated Second Messenger Intracellular Response Mechanism [From Harper's Review of Biochemistry, ed. 20, 1985, pps 508, 510. Modified from AG Gilman: G proteins and dual control of adenylate cyclase. Cell 1984; 36: 577. Copyright the Massachusetts Institute of Technology].

Catecholamines bind to the β–AR which causes the receptor to undergo a conformational change. This change allows for the interaction of the receptor with the stimulatory G protein, Gs. Beta-adrenergic stimulation promotes the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the alpha (α) subunit of the Gs protein and causes Gs protein activation and dissociation of the a subunit from the beta (β) and gamma (γ) subunits of the G protein trimeric complex. The α subunit-GTP complex then binds to and activates the enzyme adenylate cyclase (AC) that converts adenosine triphosphate (ATP) to cyclic adenosine 3',5'- monophosphate (cyclic AMP). The increase in cyclic AMP leads to the activation of the cyclic AMP-dependent protein kinase (PKA) which subsequently regulates cellular functioning through a cascade of phosphorylation events. In the heart, for example, cyclic AMP-dependent protein kinase phosphorylates proteins located in the sarcolemma, in the membrane of the sarcoplasmic reticulum and in the myofibrils of cardiomyocytes which mediate the effects of catecholamines on the heart. Whereas phosphorylation of the sarcoplasmic membrane proteins, specifically termed phospholambans, leads to an increased intracellular calcium (Ca^{2+}) level and sensitivity, phosphorylation of the thin filament regulatory protein, troponin I, leads to a decrease in sensitivity to Ca^{2+}. Together, these two phosphorylation events lead to the episodic contraction-relaxation action of catecholamines on the heart. This action is an important determinant of coronary perfusion and rapid diastolic filling of the ventricles and is also required for an increase in heart rate that is observed during AR stimulation. Effects of these phosphorylation events in other tissues include a β_2-AR mediated relaxation of vascular and bronchial smooth muscle, glucose and free fatty acid

mobilization, stimulation of norepinephrine release from sympathetic terminals, as well as many other tissue-specific physiologic responses (78). In any given cell type, including lymphocytes, the cyclic AMP-dependent protein kinase is thought to also phosphorylate the β–AR which leads to an attenuation of response termed heterologous desensitization.

TRANSGENIC OVEREXPRESSION OF β–ARs

As stated previously, the contractility of the heart is increased in the trained individual resulting in an increased stroke volume. In a recent study, investigators generated transgenic mice with up to 200 times the normal number of β_2-adrenoceptors expressed on cardiomyocytes (97). Although the β_2-adrenergic receptor is less abundant than the b1-adrenergic receptor in the heart, it is much more sensitive towards the activation of the cyclic AMP transduction pathway than the β_1-subtype (106). The transgenic animals had faster heart rates and increased rates of pressure development in the left ventricle, as well as an increased contractility. The increased contractile performance was present under basal conditions, i.e., in the absence of any stimulation by exogenous catecholamines. Apparently, the increase in β–AR density alone led to an increase in intracellular events which in turn caused the increase in heart rate and contractility (97). The reasoning for utilizing the β_2-AR subtype in these types of studies is threefold. First, cardiac β_2-adrenergic receptors do not appear to undergo down-regulation, or loss of receptor number, with clinical heart failure (104,105). Secondly, the β_2-AR couples to adenylate cyclase with greater efficacy than the β_1-AR (106). Furthermore, expression of myocardial β_1-ARs in transgenic mice has resulted in only moderate gene expression with no changes in contractility (107).

As seen later in this review, homologous desensitization of the β–AR is due to the phosphorylation of the receptor by a β–AR kinase (βARK or GRK). Augmenting β–AR signaling through the overexpression of a βARK inhibitor enhanced β–AR-stimulated cardiac function (101). Additionally, overexpression of the α-subunit of the Gs protein also produced enhanced cardiac performance (102). These alterations, downstream of the β–AR, illustrate the importance of β–AR-Gs signaling efficiency in determining contractile function. Theoretically, genetic manipulation of the α subunit of Gs or GRK inhibitors could prove advantageous since signaling efficiency could be enhanced without altering the density of the β–AR.

The enhanced cardiac function described thus far consists of overexpression of the β–AR at only moderate levels (60-200 times the normal level). A recent study has shown that overexpressing the β–AR up to 350 times the normal can have deleterious effects on the heart (100). Transgenic animals that overexpressed the β_2-AR from 100 to 350-times the normal level of receptor exhibited progressive ventricular dysfunction directly related in severity of progression to the level of β_2-AR expression (100). At these high levels of expression, the β–AR exhibits ligand-independent signaling whereas at the low levels of expression, the β–AR requires binding from the appropriate ligand for stimulation. When β_2-AR blockade was attempted with the β-selective antagonist propranolol, contractile function was normalized in the cell lines that overexpressed the β–AR 60-times normal whereas blockade had no effect on the β–ARs overexpressed 350-times normal (100). Thus, expression of the β_2-AR at a level that enhances the in

vivo response to agonists but does not cause the in vivo intrinsic, ligand-independent signaling may improve cardiac functioning without negative effects.

Differences in Signaling Mediated by β_1- and β_2-ARs

The study cited above highlights several misconceptions and unfounded assumptions concerning the β–AR and its signaling properties in the heart, specifically the contrasting aspects between β_1- and β_2-ARs. The first erroneous assumption is that β_1-ARs and β_2-ARs are essentially equivalent in their signaling properties. Cardiomyopathy resulted from even low levels of transgenic overexpression of β_1-ARs (5-fold). In contrast, transgenic overexpression of β_2-ARs up to 100-fold significantly increased cardiac contractile force without any cardiomyopathic consequences (100). Only at even higher levels of expression (350-fold) were pathological changes observed.

The first study of transgenic overexpression of the β–AR in the mouse heart (97) reported high levels (200-fold) of expression of the β_2-AR. These animals had an elevated contractility that was unresponsive to further β–adrenergic stimulation. Like the animal lines expressing the highest levels of β–AR expression in this more recent study, the influencing of cardiac muscle contractility is due to the constitutive activity of the highly expanded pool of receptors and could not be reduced with the administration of β–blockers, such as propranolol (101). Even at much lower levels of expression (15-fold) of the β_2-AR, marked potentiation of catecholamine-stimulated contraction of the heart was observed with no pathological consequences (111).

Other observations also indicate that the signaling properties of the β_1- and β_2-ARs are quite different. Although both activate adenylate cyclase through the stimulatory G protein, Gs, β_2-ARs can also stimulate Gi, the inhibitory G protein. The consequences of this can be seen in the results above. For example, the cardiomyopathy seen in the overexpression of β_1-AR is not seen with the β_2-AR because its activation of Gi limits the extent of the contractile response to overexpressed β_2-ARs (101). Only when Gi proteins were inactivated by the administration of pertussis toxin did these overexpressed β_2-ARs models fully stimulate contractility (112).

As stated previously, β_1-ARs appear to undergo downregulation, a phenomenon that will be discussed in detail later. In contrast, β_2-ARs do not downregulate in the failing human heart (104,105). This differential regulation is paralleled by a reduction in the β_1-AR mRNA levels but no changes in the β_2-AR mRNA levels (105). The fact that only β_1-ARs mRNA levels are reduced in failing myocardial tissue is consistent with the concept of an agonist-dependent process leading to reduced messenger RNA levels (113), since in the failing human heart, β_1-ARs are probably stimulated to a greater extent by norepiniphrine released from sympathetic nerve endings than β_2-ARs stimulated only by circulating epinephrine (114).

Additionally, the efficacy of β_1-ARs in the generation of cyclic AMP through the activation of adenylate cyclase appears to be less than that of the β_2-AR subtype (106). This study indicates that the extent to which a receptor may activate the AC system (efficacy) depends on the inherent nature of the receptor not on the specific cell type

(106). By coexpressing the two subtypes of receptors in the same cell type, the study was able to compare the ability of the β_1- and β_2-AR to stimulate AC in a similar biochemical environment. When the numbers of the two subtypes of receptor were normalized, β_1-AR stimulation of AC was always partial and nonadditive to the response elicited by the β_2-AR (106).

Therapeutic Implications

Thus, increased β_2-AR activity or reduced GRK activity can improve myocardial performance without causing apparent negative effects on the heart. These observations challenge the popular notion that increasing β–adrenergic signaling in the heart will have deleterious consequences (101). Two general theoretical approaches have been proposed for the treatment of heart failure: the overexpression of the β_2-AR and the overexpression of an inhibitory peptide of the GRK enzyme. Overexpressing the β–AR at abnormally high levels produces a cardiomyopathic phenotype in mouse models. However, overexpression at moderate levels does promote an increase in cardiac performance through increased contractility. Thus, controlling the level of receptor expression is a critical variable in this hypothetical therapeutic approach. Experimentally, the issues concerning the use of the β_2-AR as a possible target for gene therapy can be approached in several ways. One is to cross animals overexpressing β_2-ARs in the heart with genetically engineered lines of mice that develop heart failure (115,116). Another approach is to transfer the β_2-AR with adenoviral vectors in cultured cardiac myocytes (117).

ALTERATIONS IN RECEPTOR DENSITY INDUCED BY EXERCISE

Epinephrine is released in significant amounts from the adrenal gland as an initial response to exercise. Acute physical exercise induces a 5 to 10-fold increase in plasma catecholamine levels (31-33). The increase in plasma catecholamine levels begins at the onset of exercise. During long-term aerobic exercise, concentrations of plasma catecholamines have been found to increase continuously until the exercise is stopped (Figure 3). Exercise, both long term and short term, influences membrane redistribution of β–ARs on human lymphocytes and myocardial tissue (6,34-44). Exposure to circulating epinephrine, as observed during exercise, or more practically, in vitro β–AR agonists, have been shown to effect the membrane density of β–ARs on human lymphocytes. Short-term, exhaustive exercise leads to an apparent initial increase in β–AR density on human lymphocytes (3,4,5,7,35,39,43,45-51) most likely due to the selective recruitment of lymphocytes high in β–AR density. Long-term exposure to β–AR agonists, analogous to chronic exercise, leads to a decreased density of β–ARs on human lymphocytes (46,52-70) due to down-regulation.

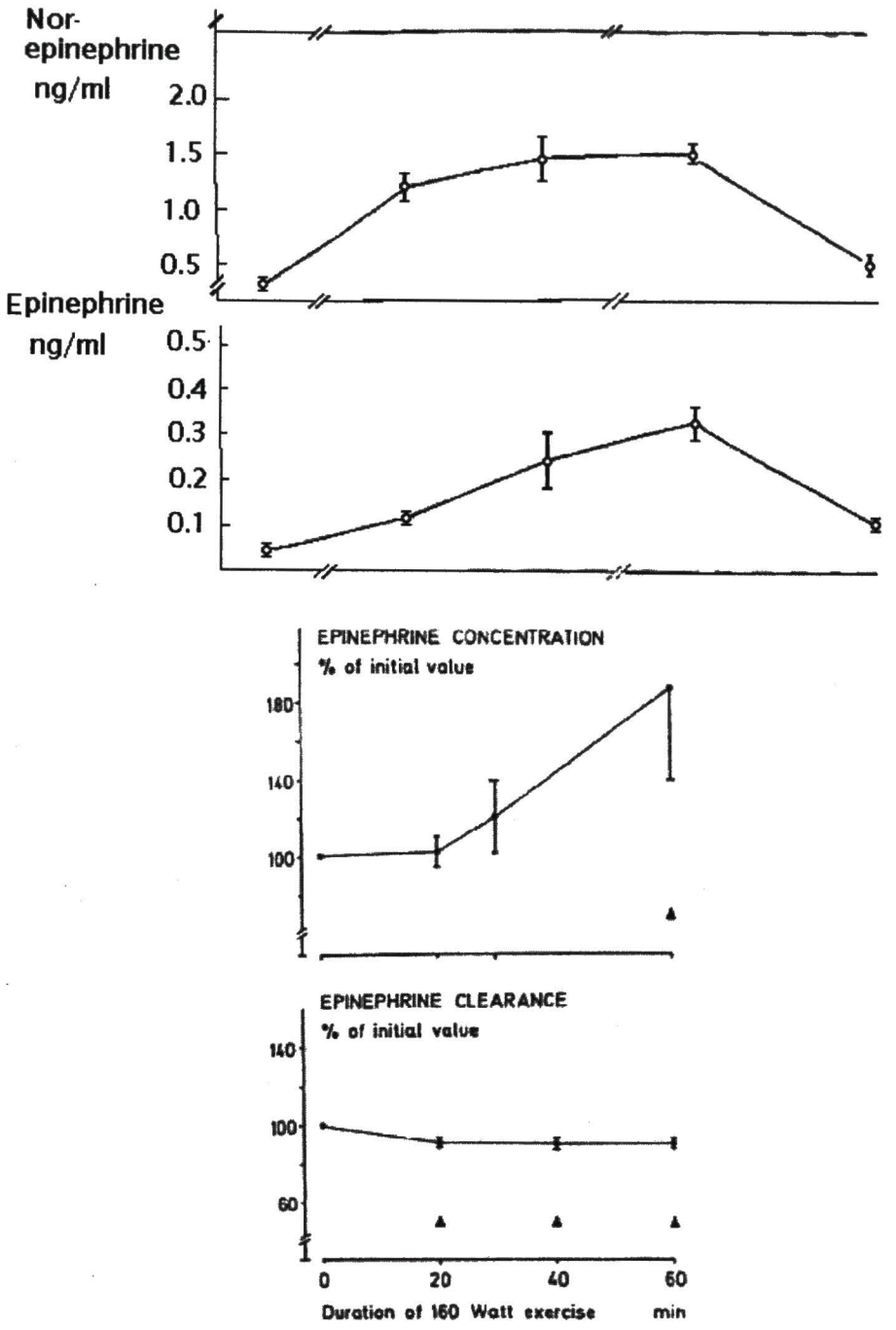


Figure 3: Time Course of Changes in Plasma Catecholamine Concentrations (% of Initial Value) During One Hour of Submaximal Bicycle Exercise [From Galbo, H., et al., Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. Journal of Applied Physiology, 1975, 38: pp73]

Short Term Up-Regulation

Acute, short-term activation of the sympathetic nervous system in response to dynamic exercise increases the apparent β–AR density on circulating human lymphocytes. Receptor translocation from the intracellular space to the cell surface is a possible mechanism responsible for the rapid increase in receptor number (35,76-79). Such an event would also enhance lymphocytic cell responsiveness during dynamic exercise. Another postulation is that receptors that were occupied with ligand become uncovered and able to bind free β–AR agonists (80). However, this explanation is not very plausible given the rapid dissociation kinetics of agonist ligands. Another hypothesis is that lymphocytic β–ARs can exist in several cellular compartments (81,82), and acute sympathetic stimulation may direct them to the cell surface from their previously sequestered state. A third possibility is based on data suggesting that circulating lymphocytes differ in their β–AR density (47,62,83,84). Acute sympathetic stimulation may somehow selectively recruit more of the lymphocytes with a higher density of β–AR thus allowing for an increased density, and subsequently, an increased responsiveness to catecholamines during short-term, dynamic exercise.

Long Term Desensitization and Down-Regulation

It is well documented that long-term exposure of in vitro β–AR agonists to the receptor leads to a decreased density of that receptor on human lymphocytes. More specifically, chronic exposure to sympathomimetic amines leads to decreased β–adrenoceptor densities in animal tissue models and in human peripheral blood

lymphocytes. This phenomenon, known as receptor down-regulation, involves receptor sequestration from the cell membrane and is preceded by a process termed desensitization which involves covalent modifications that affect the functioning of the receptor and its guanine nucleotide regulatory proteins (108).

Despite continuous stimulation of β–adrenoceptors by their appropriate agonists, the cellular responses that result soon begin to diminish. Termed desensitization, this phenomenon has been well documented in β–ARs and its mechanism involves an alteration in functioning of the receptor (108). Phosphorylation events acting on the β–AR by two distinct kinases (G protein-coupled receptor kinase or GRK and cyclic AMP dependent protein kinase) causes the receptor to uncouple from the stimulatory G protein Gs. Once uncoupled from the receptor, the Gs protein-receptor complex is much less efficient in activating adenylate cyclase resulting in a decreased amount of cyclic AMP production from intracellular ATP (Figure 4).

Phosphorylation of the β–AR by the GRK family of kinases is termed homologous desensitization and refers to the observation that the kinases can only phosphorylate receptors that are already occupied by the appropriate ligand (108,118). This strict agonist-binding requirement for GRK phosphorylation makes this mechanism strictly homologous in that only the stimulated receptor can be desensitized (125). Phosphorylation of receptors by kinases results in only minimal desensitization but this phosphorylation increases the affinity of the receptor for proteins called arrestins. Binding of the arrestin to the receptor then results in maximal homologous desensitization (125). This kinase rapidly ($t_{1/2}$~20 seconds) phosphorylates agonist-

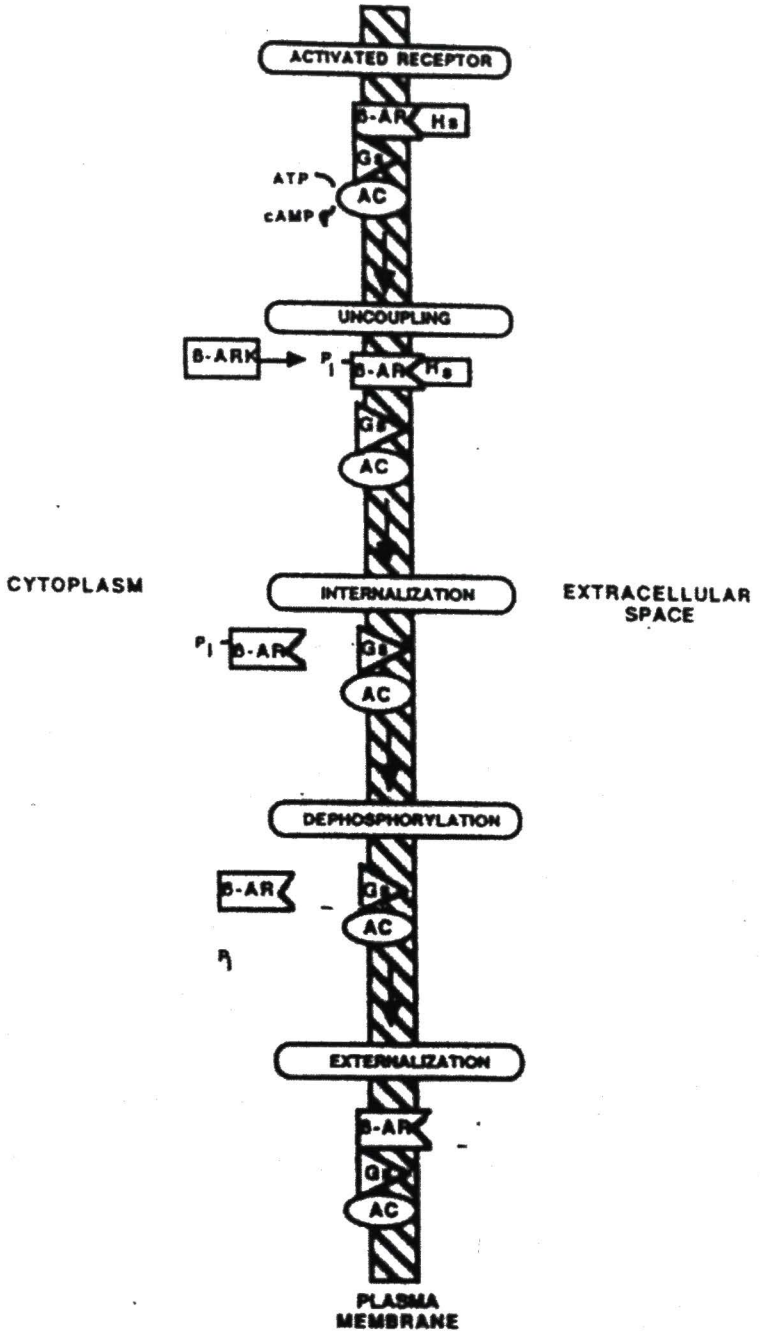


Figure 4: Sequence of Events Involved in Desensitization and Down-Regulation of the β-Adrenergic Receptor. Subsequent to the ligand binding to the receptor, adenylate cyclase is activated by the stimulatory Gs-protein. After agonist stimulation, the beta-adrenergic receptor kinase (β-ARK) phosphorylates the receptor, which becomes uncoupled from the adenylate cyclase. Desensitization is reversed by dephosphorylation. [From Maki, T., et al., The beta-adrenergic response system in man: Physiological and pathophysiological response. Scandinavian Journal of Clinical Laboratory Investigation 1990; 50, (Supp 201): pp30).]

occupied receptors in response to high levels of agonists and is independent of cyclic AMP levels (109,118). Phosphorylation by the cyclic AMP dependent protein kinase enzyme is responsible for heterologous desensitization which acts more slowly ($t_{1/2}$~3.5 minutes). Heterologous desensitization, a more generalized form of uncoupling, can take place regardless of receptor occupancy and the kinase can be activated through a number of different receptors upstream other than the β–AR i.e., any receptor that stimulates elevated cyclic AMP levels and PKA activation. Unlike homologous desensitization, phosphorylation of the β–AR by the cyclic AMP protein-dependent kinase can be initiated by rather low concentrations of β–receptor agonists (108,118). For example, prostaglandins acting on PG receptors activate the cyclic AMP second messenger pathway thus activating PKA, which can in turn phosphorylate the β–AR even though the β–AR was not responsible for the kinase activity and is not in the active conformation.

Studies have shown that homologous and heterologous desensitization appear to be completely independent mechanisms. However, some recent studies on GRKs and arrestins have revealed that these proteins are regulated by the second messenger-dependent kinases (cyclic AMP protein-dependent kinase) that mediate heterologous desensitization. This phenomenon, loosely termed "cross talk", is dictated by the second messenger-dependent kinase mediating changes in the cellular expression and activity of GRKs and arrestins, thus determining the efficacy of the homologous desensitization apparatus (125).

Down-regulation, distinct from desensitization, involves a decrease in total receptor number due to sequestration from the cell membrane into the intracellular compartment (108). Whereas receptor desensitization occurs within seconds to minutes of agonist stimulation, sequestration and down-regulation of β–ARs require a greater duration of stimulation. Bridging desensitization to down-regulation are a group of cytosolic proteins called arrestins which through homologous desensitization (GRK-mediated receptor phosphorylation) bind to and further uncouple the receptor from the G protein (Figure 5). Arrestins are not thought to be involved in heterologous desensitization suggesting that down-regulation does not occur through the action of the cyclic AMP protein-dependent kinase. The phosphorylation events of the βARK enzyme that lead to desensitization are required before the arrestin protein can bind to the receptor. Once bound to the receptor, the arrestin-phosphorylated receptor complex promotes the mobilization of the receptor to clathrin coated vesicle-mediated endocytosis (124). Recovery, or resensitization, of the β–AR occurs within minutes after the removal of the desensitizing agonist (109). However, recovery from profound, long-term desensitization induced by prolonged exposure to agonist takes days and requires new protein synthesis (109).

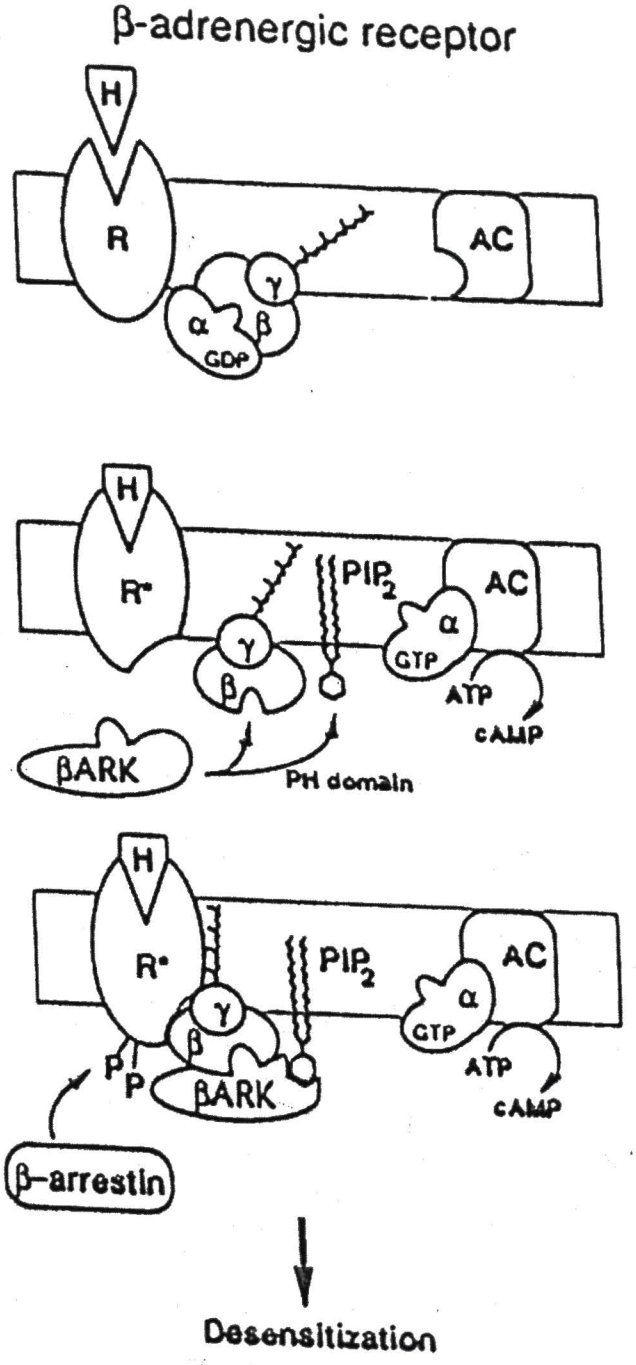


FIGURE 5: Proposed Role of β-Adrenergic Receptor Kinase (βARK) and β-Arrestin in Homologous Desensitization of the β-Adrenergic Receptor [Lefkowitz R.J., H.A. Rockman, W.J. Koch. Catecholamines, cardiac β-adrenergic receptors, and heart failure. *Circulation.* **101**: 1634-1637. 2000.]

CHRONIC EXERCISE AND RECEPTOR DENSITY

Rregulation by the sympathetic nervous system plays an important role in exercise training and in the development of cardiac fatigue as a result of overtraining. Receptor regulation and post-receptor mechanisms may be involved in the adaptation to exercise or responsible for the symptoms of overtraining. The hypothesis that alterations in the sympathetic nervous or sympathetic adrenal system may detrimentally affect cardiac function during prolonged exercise training is not new since it is one cornerstone of the pathophysiology of congestive heart failure (36). As seen below, comparing the trained heart with the diseased heart could provide useful information into the correlation between chronic exercise training and β–AR density

Although the up-regulation of β–ARs during short-term exhaustive exercise has been well documented, less is known about the effects of long-term endurance training on beta-receptor density. Additionally, the long-term exposure of the β–AR to exogenous agonists and its effect on receptor number has been well studied, but the data on endogenous catecholamine exposure is lacking. However, studying the diseased heart could help elucidate the relationship between chronic exercise and β–AR density. Circulating plasma catecholamines are elevated in patients with chronic congestive heart failure much the same as with dynamic exercise (59). One could conclude, in theory, that the decreased density of β–ARs seen in the failing heart could also be seen in the trained heart from agonist-induced desensitization followed by down-regulation of the receptor.

WHOLE LYMPHOCYTES AS MODEL CELLS

Circulating lymphocytes containing a homogeneous population of β–ARs coupled to the adenylate cyclase system and are a frequently used model to study alterations in β–AR function in man (85). Human lymphocytic cell membranes contain 1000-2000 β–ARs per cell (86,87), and the receptors are predominantly of the β_2-subtype (86). Whole lymphocytes not only possess exceptional durability, they are also very easily accessible from human whole blood by gradient centrifugation (88). Anatomically, the sympathetic nervous system and the immune system (lymphocytes) are linked by a dense innervation of the spleen and other immunological tissues (47). In these tissues, sympathetic nerve endings form extremely close contacts with lymphocytes containing receptors for sympathetic neurotransmitters making them susceptible to sympathetic stimulation (47). Additionally, studying lymphocytic β–AR responsiveness in humans allows correlation to cardiovascular (cardiac muscle cells) responses in humans due to the fact that the density and functionality of these two cell types are significantly related (3-6,61,89). Although possible to measure and extrapolate data on receptor density using biopsy samples from myocardial tissue during surgery, such a practice is of little practical use. Since there is such a close correlation between myocardial and lymphopcytic β_2-AR density and functionality, certain inferences concerning cardiac responses to exercise can be drawn by observing data from human lymphocytes. Although both β_1-and β_2-ARs

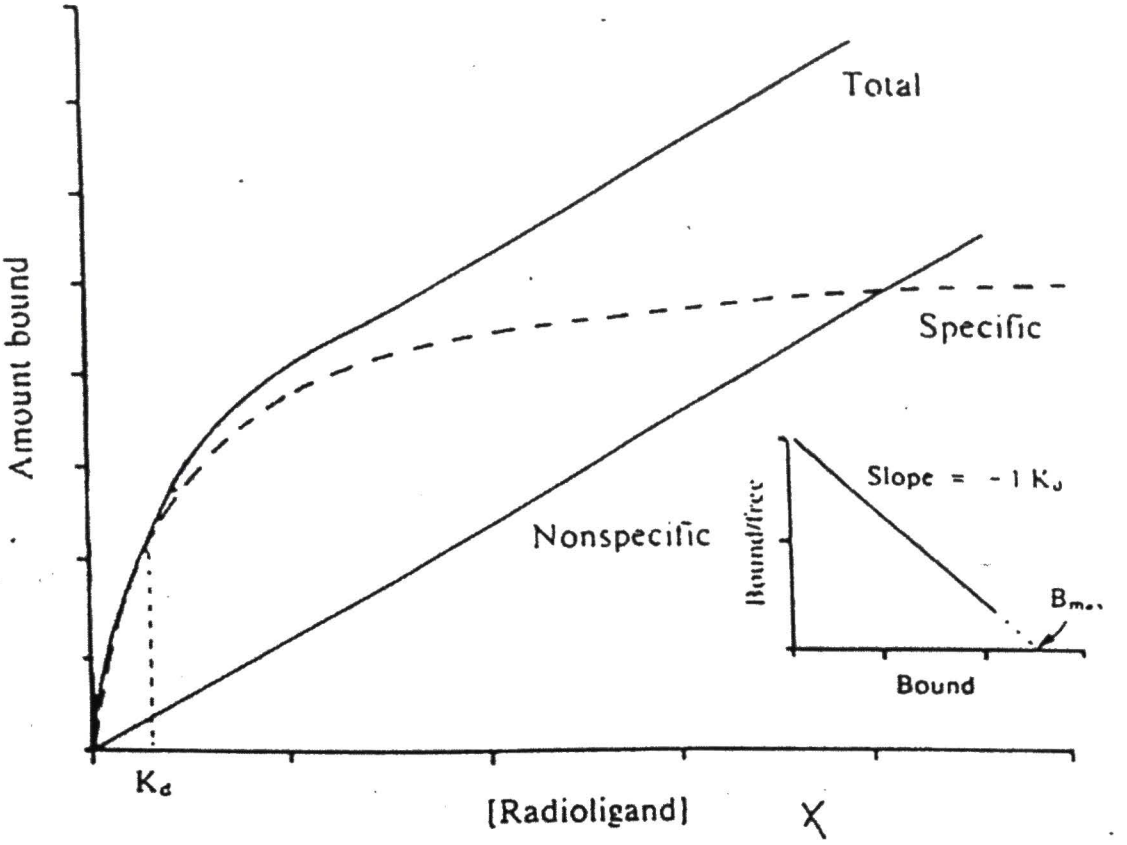
have been found to coexist in myocardial tissue, it has been suggested that changes in lymphocytic β_2-ARs mirror precisely β_2-ARs changes in the human heart (89).

It has been proposed that the initial increase in density of lymphocytic β–AR during short term exercise is due to the fact that circulating lymphocytes increase in number as a physiological response to exercise (39,90). Recent studies, however, have convincingly shown that this hypothesis is not valid. It has been shown that the up-regulation of β–ARs occurs even when there is no change in total lymphocyte count or in the distribution of different lymphocyte subpopulations (46,91). One particular study, through reverse transcription-PCR techniques, looked at β–AR mRNA levels after short-term, dynamic exercise. This study concluded that dynamic exercise induced an increase in β–AR number on human lymphocytes and this increase is accompanied with an increase in β–AR mRNA levels (110). This rapid increase in receptor protein synthesis could be an early adaptive response to dynamic exercise.

BINDING THEORY AND [^{125}IODO]CYANOPINDOLOL

Radioligands provide probes that allow for the specific examination of the ligand (drug)-receptor interaction (92) and the use of stereospecific, radioactive ligands in the measurement of receptor density and functionality has been a commonly used pharmacological assay for three decades (Figure 6). The number of ligands radiolabeled with either tritium (^{3}H) or iodide (^{125}I) ligands has increased tremendously resulting in a rapid expansion in the use of binding assays to characterize receptors and receptor subtypes (92). Before the widespread use of in vitro binding assays, the properties of receptors were studied from the measurement of certain biological responses (92). This approach has several shortcomings including varied tissue distribution of a drug, the metabolism of drugs before they interact with the receptor and the attenuation of response caused by feedback mechanisms.

The use of the β–AR antagonist [^{125}I]-iodocyanopindolol (^{125}I-CYP) as a radioligand was first described almost twenty years ago (86,93). The creation of this new radioligand proved advantageous to researchers due to the ease of which it could be labeled, its unequivocal structure (Figure 7) and much improved binding properties to β–ARs (93). Compared to previously used ligands, the specific activity of ^{125}I-CYP towards β–ARs was high, and the degree of non-specific activity was low and was reported to lack significant interaction with either a-adrenergic or serotonin-type receptors (94).



$$[B] = \frac{B_{max}[L]}{[L] + K_d} \qquad \frac{[B]}{[L]} = \frac{[B]}{K_d} - \frac{B_{max}}{K_d}$$

Figure 6: Graphical Representation of Radioligand Binding Data: "Total" represents total radioligand bound to receptor and non-receptor sites. "Nonspecific" represents radioligand bound only to non-receptor sites and is determined by competing for binding sites with excess nonlabeled ligand. The difference between total and nonspecific binding is defined as "Specific" binding and is assumed to reflect receptor-specific radioligand binding. The inset shows the data transformed as a Scatchard plot. The equations at the bottom describe specific binding (B) at any given ligand concentration (L) to a binding site with affinity (Kd) and a maximal number of binding sites (Bmax). [From Basic Neurochemistry: Molecular, Cellular and Medical Aspects, 4th ed., Quantitative aspects of drug-receptor interactions, Chapter 9, 1989, pp187].

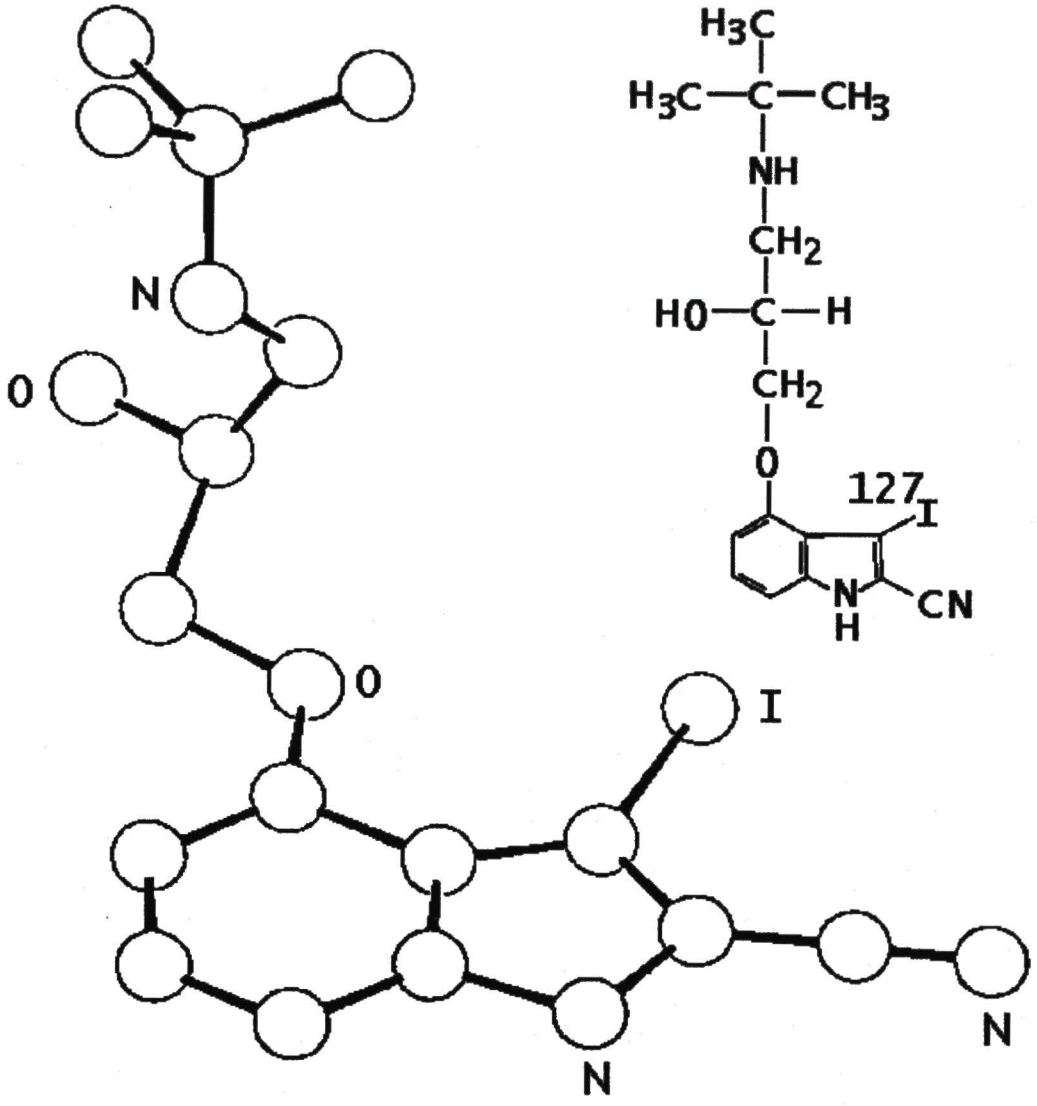


Figure 7: The molecular structure of ICYP [From [125Iodo]cyanopindolol, a new ligand for β-adrenoceptors: Identification and quantitation of subclasses of β-adrenoceptors in guinea pig. From Engel, G., et al., Naunyn-Schmiedeberg's Archive of Pharmacology, 1981, 317: pp280. X-ray crystallographic data from Dr. H.P. Weber, unpublished results]

Evidence presented a decade later (94), however, along with data from this laboratory, indicates that ^{125}I-CYP may not be selective to just β–ARs but also exhibits reactivity towards specific non-β–adrenergic binding sites. When ritancerin, a selective ligand towards serotonin-type receptors, competes for ^{125}I-CYP binding to myocardial membrane preparations. Thus, the apparent total β–AR binding is decreased significantly when these non-β–AR sites are taken into account.

EXPERIMENTAL DATA

HYPOTHESIS

It is not known if the density of β-ARs expressed in various tissues is increased, decreased, or unchanged as one of the many adaptive changes that occur in response to long-term, chronic exercise. However, studies involving patients with congestive heart failure, a condition in which there is compensatory increased sympathetic tone and in vitro assays that expose receptor to β-AR agonists, specifically epinephrine, over time reveal a decreased density of β-AR. The mechanism of receptor desensitization and down regulation have been well documented for the β-AR (2, 57, 101,108). Desensitization and down regulation are distinct from each other. Desensitization, or lack of responsiveness, occurs within seconds to minutes after exposure to agonists and shows no change in receptor number. In contrast, down regulation requires a longer exposure time to agonist and receptor number is decreased. It is hypothesized that with repeated exposure to epinephrine, as seen with chronic exercise, the β-AR is down regulated and thus the density of receptor is decreased in the endurance-trained athlete compared to untrained, sedentary control individuals.

METHODOLOGY

In addition to a review of the literature, a limited number of experiments were performed as a part of this project. These experiments served to establish the

methodology necessary to conduct larger scale studies. The hypothesis is that there is a measurable difference in the density of β -AR expressed in human lymphocytes between untrained individuals and athletes that have a documented record of long-term, high intensity endurance training. Lymphocytes were used as the model tissue since they are a recognized tissue source for analysis of β-AR changes in humans (6).

Study Subjects

Six healthy, high fit individuals (four male and two female) and four average fit individuals (two male and two female) were recruited to voluntarily participate in the proposed study. The procedures, risks, and benefits involved in the study, and the rights of the research subjects were explained in an Informed Consent Document approved by the Institutional Review Board.

All subjects were given a physical exam and questioned about their fitness level, training history, and activity level. Inclusion into either the high fit or average fit groups was determined by asking the test subjects to perform a treadmill run until exhaustion using a customized protocol of maximum oxygen utilization (VO2max)(P.B. Raven, unpublished results). The criteria for inclusion in the high fit group was VO2max>60ml/kg/min. Average fit VO2max values were approximately 40ml/kg/min.

Isolation of Lymphocytes

Study subjects will be asked to fast from 8:00 pm until a blood sample is drawn the following morning at approximately 8:00 to 9:00 am. After lying supine for 30

minutes, a 40 ml sample of whole blood will be drawn into four EDTA (0.5 mM) coated 10 ml sterile syringes. An additional 3 ml blood sample will be drawn into an EDTA-coated vacuum specimen tube for standard Coulter mechanized complete blood count (CBC) analysis performed by the Department of Pathology.

Leukocytes were separated from the whole blood sample by the method of Boyum (88). Gently and briefly, 10 ml of whole blood is layered onto a 20 ml layer of HistopaqueTM 1077 and centrifuged at 400 x g for 20 minutes at 20°C. The plasma fraction is aspirated and discarded and the "buffy coat" fraction containing both monocytes and lymphocytes was collected. An aliquot of this buffy coat fraction was saved for determination of cell count and differential count of each mononuclear leukocyte fraction by Coulter counter. The mononuclear leukocyte fraction was diluted with 15-20 ml of homogenization buffer (10 mM Tris-HCl, 2 mM MgCl2, 0.5 mM DTT, 0.1 mM EGTA, pH 7.4) and homogenized with a type PT 10/35 Polytron at a setting of "7" for four separate 10 second bursts at 4°C. The resulting cell lysate was centrifuged at 40,000 x g at 4oC for 30 minutes. The pellet was resuspended in homogenization buffer to give approximately 6 x 106 cell equivalents/ml. Typical preparations yielded 96% lymphocytes by Coulter analysis.

Binding Assay

A modification of the methods of Engel, et al. (93) were used as follows. For determination of receptor density, washed crude pellet freactions from lysed lymphocytes (1.5-4.0 x 105 cell equivalents/ml) were incubated in a final volume of 0.25 ml of 10 mM

Tris-HCl, 0.154 M NaCl, pH 7.4 (TS Buffer) with increasing concentrations (5-200 pM) of [^{125}I]-(-)-Iodocyanopindolol (^{125}I-CYP) (2200 Ci/mmol; NEN Life Science Products) for 60 minutes at 37°C. A parallel set of tubes also contained 1μM S (-)-propranolol (RBI/Sigma) for the determination of nonspecific binding. Competitive inhibition experiments were conducted in a similar manner except using 50 pM ^{125}I-CYP in the presence or the absence of increasing concentrations of competing ligand. The binding reaction was terminated by rapid addition of 10 ml ice-cold TS Buffer followed by rapid filtration through glass fiber filters (Schleicher & Schuell, #30). The filters are washed with an additional 10 ml of ice-cold TS Buffer and then filter-bound radioactivity was determined using a Packard Instruments Cobra scintillation spectrometer. All assays were conducted in triplicate. Nonspecific binding was defined as filter-bound radioactivity in the presence of 1 μM S (-)-propranolol (93).

Data Analysis

Radioligand saturation binding isotherm data weree fit to a model of mass action binding to a single population of binding sites using nonlinear least-squares regression analysis (TableCurve^{TM}, Jandel Scientific). Competitive ligand binding data were fit to one or two site models using the same software. Differences in receptor density between groups were analyzed by a two-tailed Student's t-test assuming unequal variances.

RESULTS

Pilot experiments were conducted to establish the methodology necessary to measure the density of β-adrenergic receptors on human lymphocyte preparations. As

shown in Figure 8, specific ^{125}I-CYP binding was saturable and was best fit to a model of binding to a population of sites with a single affinity. Estimates of the total number of receptor sites (Bmax) ranged from 0.8 to 1.4 pM or 600 to 1200 binding sites per cell. Kd values, a measure of receptor affinity for ligand ranged from 30 to 91 pM. These values are generally in agreement with published values (51, 53, 60).

These preliminary experiments also established that so called "nonspecific" binding varied considerably from preparation to preparation. Based on published standard methods we chose to define nonspecific binding as the filter bound radioactivity measured in the presence of 1 μM propranolol. Additional experiments were performed to confirm that this was the appropriate definition of non-receptor or nonspecific binding. Competition binding experiments were conducted using the standard nonselective β-receptor antagonist propranolol (Figure 9). Surprisingly, propranolol binding was best fit to a two site model of binding with approximately 40 % of the total sites displaying a low apparent affinity (IC50 value) of 1.9×10^{-5} M and the remaining 60% of the sites displaying a high apparent affinity of 5.8×10^{-7} M.

Based other published reports of non-β-receptor binding of ^{125}I-CYP (94), we also examined the ability of the serotoninergic antagonist ritanserin to compete for ^{125}I-CYP binding sites. As shown in Figure 9, ritanserin effectively competed for ^{125}I-CYP bindng over the concentration range of 10nM to 1 mM and displaced the radioligand to same level as high concentrations (10μM) of propranolol. Ritanserin binding data, like that of propranolol, was best fit to a two site model with a high affinity component (IC50 = 8.5 x 1^{-8} M; 22% of the sites) and a low affinity component (IC50 = 1.0×10^{-4} M; 78% of the

sites). There was also a non-displaceable binding component that probably represents "true" nonspecific binding.

DISCUSSION

The use of the β-AR antagonist [^{125}I]-CYP as a radioligand was first described almost twenty years ago (86,93). The creation of this new radioligand proved advantageous to researchers due to the ease of which it could be labeled, its unequivocal structure (Figure 7) and much improved binding properties to β-ARs (93). Compared to previously used ligands, the specific activity of ^{125}I-CYP towards β-ARs was high, and the degree of non-specific activity was low and was initially reported not to interact with neither α-adrenergic or serotonin-type receptors (93).

Evidence presented a decade later, however, along with data from this laboratory (Figure 9), indicates that ^{125}I-CYP may not be as selective as once reported. This ligand exhibits reactivity towards non-β-adrenergic binding sites, including serotonin receptors (94). If human lymphocytes express significant levels of serotonin receptors, interpretation of ^{125}I-CYP binding data may be more difficult.

Another limitation in the exploration of this hypothesis is the discrepancy in the population between the two major subtypes of beta-adrenergic receptors on lymphocytes and cardiac tissue. Lymphocytes contain only the β2-subtype while the heart contains approximately three times more β1- than β2-ARs. It is not clear if any differences noted in the lymphocytic β-AR of trained and untrained individuals will truly reflect the same regulatory phenomena that occurs in cardiac tissue (5).

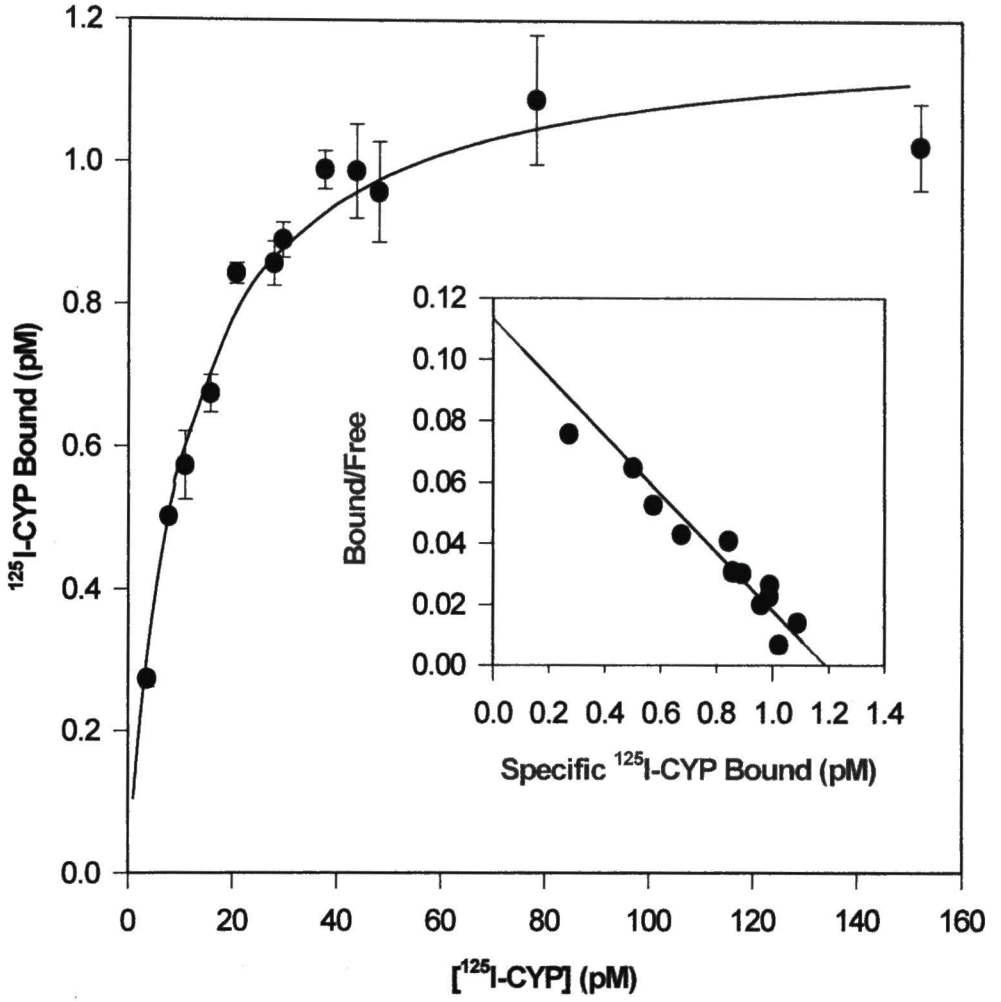


Figure 8: ^{125}I-CYP Saturation Binding to Human Lymphocytes. The data show specific ^{125}I-CYP binding to a purified human lymphoctye preparation (average fit individual). The inset shows the same data transformed as a Scatchard plot. Each point represent the mean ± S.D. of triplicate determinations.

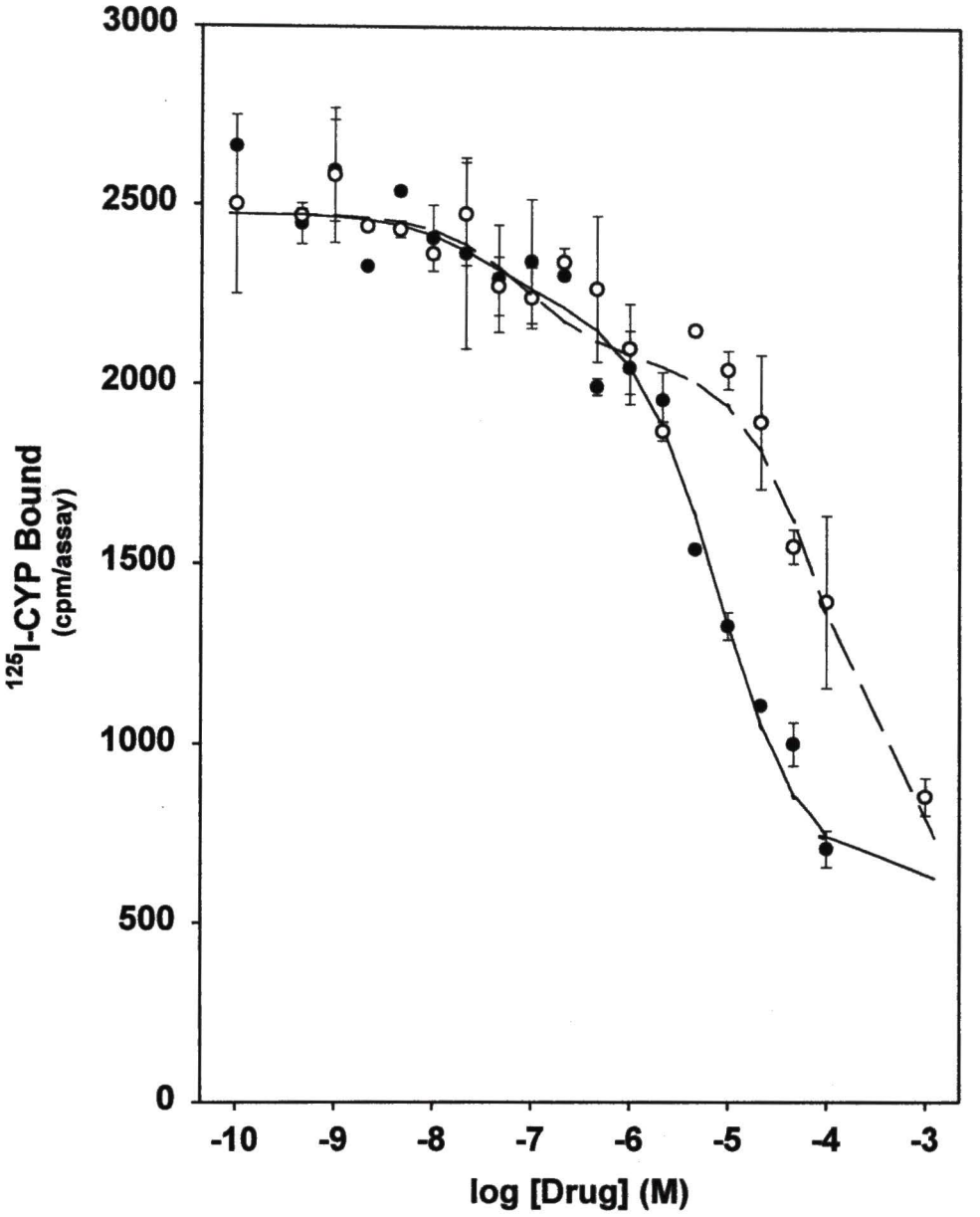


Figure 9: Competitive Inhibition of ^{125}I-CYP Binding to Human Lymphocytes by Propranolol (O) and Ritanserin (O). Lymphocytes were incubated with a fixed concentration of ^{125}I-CYP (14 pM) with or without the indicated concentration of competing drugs. Each point is the mean ± S.D. of triplicate determinations.

CONCLUSION

Important cardiovascular responses to exercise are mediated through the beta-adrenergic receptor. Certain cardiovascular adaptations to chronic exercise, namely a decreased resting heart rate, could be due to the proposed down-regulation of β–AR density as seen with chronic exercise. Although the trained heart and the diseased heart exemplify a large discrepancy in their efficiency, the β–ARs in both are exposed to elevated levels of catecholamines. Although the decreased density and functionality of cardiac β–ARs in human heart failure is well documented (101), studies on the effect of long-term chronic exercise on β–AR density is lacking. It is well documented that long-term exposure of β–ARs to exogenous beta-agonists leads to a decrease in receptor density on a number of different tissues including human lymphocytes. It has also been demonstrated that acute, short-term exercise leads to an increase in not only endogenous catecholamines released and circulating lymphocytes, but also in β–adrenergic receptor density. These changes in β–AR density have shown to be reciprocally related to the total amount or duration of exposure to circulating catecholamines. The alterations in β–AR density with short-term exposure to catecholamines (analogous to short-term, exhaustive exercise), is proposed to take place through both intracellular translocation to the cell membrane along with selective recruitment of lymphocytes with higher densities of β–ARs. Alterations in β–AR density resulting from long-term exposure to agonist

(analogous to chronic exercise) is believed to take place through receptor desensitization and subsequent down-regulation.

Human lymphocytes have shown to be a suitable model system for studying β–adrenergic receptors and in relating this information to cardiac responsiveness to exercise. Numerous studies have shown the correlation between the lymphocytic and myocardial β–adrenergic receptor density and functionality. As stated previously, the majority of beta-receptors in the myocardium are of the β_1-subtype whereas lymphocytic beta-receptors are primarily of the β_2-subtype. However, changes in the composition of β_2-ARs in the myocardium have been shown to mirror changes in lymphocytic β_2-ARs.

Future research needs to focus on lymphocytic β-ARs and their alterations, if any, in response to chronic long-term exercise. Until then, correlations can be made by exposing β-ARs to exogenous agonists in vitro but conclusions can not be made until alterations are seen in a living system.

REFERENCES

1. MAKI, T. Density and functioning of human lymphocytic β–adrenergic receptors during prolonged physical exercise. *Acta Physiol Scand.* **136**: 569-574, 1989.
2. LEFKOWITZ, R.J., CARON, M.G, STILES, G.L. Mechanisms of membrane-receptor regulation. Biochemical, physiological and clinical insights derived from studies f the adrenergic receptors. *N Eng J Med.* **310**: 1570-1579, 1984.
3. BRODDE, O.E., A. DAUL, N. O'HARA. β-adrenoreceptor changes in human lymphocytes, induced by dynamic exercise. *Naun Schmd Arch Pharmacol.* **325**:190-192, 1984.
4. BRODDE, O.E., A.E. DAUL, X.L. WANG, M.C. MICHEL, O. GALAL. Dynamic exercise-induced increase in lymphocyte beta-2-adrenoreceptors: Abnormality in essential hypertension and its correction in antihypertensives. *Clin Pharmacol Ther.* **41**: 371-379, 1987.
5. BRODDE, O.E., A. DAUL, A. WELLSTEIN, D.PALM, M.C. MICHEL, J.J. BRECKENRIGH. Differentiation of β1-and-β2 adrenoreceptor-mediated effects in humans. *Am J Physiol.* **254**: H199-H206, 1988.
6. BRODDE, O.E., R. KRETSCH, K. IKEZONO, H.R. ZERKOWSKI, J.C. REIDMEISTER. Human β–adrenoreceptors: Relation of mycardial and lymphocyte β-adrenoreceptor density. *Science.* **231**: 1584-1585, 1986.
7. BUTLER, J., J.G. KELLY, K. O'MALLEY, F. PIDGEON. β-adrenergic adaptation to acute exercise. *J Physiol.* **344**: 113-117, 1983.
8. FRASER, J., J. NADAU, D. ROBERTSON, A.J.J. WOOD. Regulation of human leukocyte beta receptors by endogenous catecholamines: relationship of leukocyte beta receptor density to the cardiac sensitivity to isoproterenol. *J Clin Invest.* **67**: 1777-1784, 1981.
9. DIXON, R.A.F., F. KOBILKA, B.K. STRADER, D.J. BENOVIC, H.G. DOHLMAN. Cloning of the gene and cDNA for mammalian β-adrenergic receptor and homology with rhodopsin. *Nature.* **321**: 75-79, 1986.
10. GOCAYNE, J., D.A. ROBINSON, M.G. FITZGERALD, F.Z. CHUNG, A.R. KERLAVAGE. Primary structure of rat β–adrenergic and muscarinic cholinergic receptors obtained by automated DNA sequence analysis: Further evidence of a multigene family. *Proc Natl Acad Sci.* **84**: 8296-8300. 1987.
11. BUCKLAND, P.R., R.M. HILL, S.F. TIDMARSH, P. McGUFFIN. Primary structure of the rat beta-2-adrenergic receptor gene. *Nucl Acid Res.* **18**: 682, 1990.

12. ALLEN, J.M., E.E. BAETGE, I.B. ABRASS, R.D. PALMITER. Isoproterenol response following transfection of the mouse β2-adrenergic receptor gene into Y1 cells. *EMBO J.* **7**: 133-138. 1988.
13. KOBILKA, B.K., R.A.F. DIXON, T. FRIELLE, H.G. DOHLMAN, M.A. BOLANOWSKI. .cDNA for the human β-adrenergic receptor: A protein with multiple-membrane spanning domains and a chromosomal location shared with the PDGF receptor gene. *Proc Natl Acad Sci.* **84**:46-50. 1987.
14. KOBILKA, B.K., T. FRIELLE, H.G. DOHLMAN, M.A. BOLANOWSKI, R.A.F. DIXON. Delineation of the intronless nature of the genes for the human and hamster β2-adrenergic receptor and their putative promoter region. *J Biol Chem.* **262**: 7321- 7327. 1987.
15. EMORINE, L.J., S. MAURULLO, C. DELAVIER-KLUTCHKO, S.V. KAVERI, O. DURIEU-TRAUTMANN, A.D. STROSBERG. Structure of the gene for human β2-adrenergic receptor: Expression and promoter characterization. *Proc Natl Acad Sci.* **84**: 6995-6999. 1987.
16. OSTROWSKI, J., M.A. KJELSBERG, M.G. CARON, R.J. LEFKOWITZ. Mutagenesis of the β2-adrenergic receptor: How structure elucidates fuction. *Annu Rev Pharmacol Toxicol.* **32**: 167-183. 1992.
17. DOHLMAN, H.G., M. BOUVIER, J.L. BENOVIC, M.G. CARON, R.J. LEFKOWITZ. The multiple membrane spanning topography of the β2-adrenergic receptor. Localization of the sites of binding, glycosylation and regulatory phosphorylation by limited proteolysis. *J Biol Chem.* **262**: 14282-14288. 1987.
18. PFEFFER, S.R., J.E. ROTHMAN. Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. *Annu Rev Biochem.* **56**: 829-852. 1987.
19. ELBEIN, A.D. Inhibitors of the biosynthesis and processing of N-linked oligosaccharide chains. *Annu Rev Biochem.* **56**: 497-534. 1987.
20. BENOVIC, J.L., C. STANISZEWSKI, R.A. CERIONE, J. CODINA, R.J. LEFKOWITZ, M.G. CARON. The mammalian β–adrenergic receptor: Structural and functional characterization of the carbohydrate moiety. *J Receptor Res.* **7**: 257-281. 1987.
21. RANDS, R., M.R. CANDELORE, A.H. CHEUNG, W.S. HILL, C.D. STRADER, R.A.F. DIXON. Mutational analysis of β–adrenergic receptor glycosylation. *J Biol Chem.* **265**: 10759-10764. 1990.
22. GEORGE, S.T., A.E. RUOHO, C.C. MALBON. N-Glycosylation in expression and function of β–adrenergic receptors. *J Biol Chem.* **261**: 16559-16564. 1986.
23. OLSON, T.S., M.D. LANE. A common mechanism for posttranslational activation of plasma membrane receptors. *FASEB J.* **3**: 1618-1624. 1989.
24. DIXON, R.A.F, I.S. SIGAL, M.R. CADELORE, et al. Structural features required for ligand binding to the β–adrenergic receptor. *EMBO J.* **6**: 3269-3275. 1987.

25. STRADER, C.D., I.S. SIGAL, R.B. REGISTER, M.R. CANDELORE, E. RANDS R.A.F. DIXON. Identification of residues required for ligand binding to the β–adrenergic receptor. *Proc Natl Acad Sci.* **84**: 4384-4388. 1987.
26. DOHLMAN, H.G., M.G. CARON, C.D. STRADER, N. AMLAIKY, R.J. LEFKOWITZ. Identification and sequence of a binding site peptide of the β2-adrenergic receptor. *Biochemistry.* **27**: 1813-1817. 1988.
27. KOBILKA, B.K., T.S. KOBILKA, K. DANIEL, J.W. REGAN, M.G. CARON, R.J. LEFKOWITZ. Chimeric β2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. *Science.* **240**: 1310-1316. 1988.
28. TOTA, M.R., C.D. STRADER. Characterization of the binding domain of the β–adrenergic receptor with the fluorescent antagonist carazolol. *J Biol Chem.* **265**: 16891-16897. 1990.
29. STRADER, C.D., I.S. SIGAL, A.D. BLAKE, et al. The carboxyl terminus of the hamster β–adrenergic receptor expressed in mouse L cells is not required for receptor sequestration. *Cell.* **49**: 855-863. 1987.
30. DOHLMAN, H.G., J. THORNER, M.G. CARON, R.J. LEFKOWITZ. Model systems for the study of seven-transmembrane-segment receptors. *Annu Rev Biochem.* **60**: 653-688. 1991.
31. GALBO, H., J.J. HOLST, N.J. CHRISTENSEN. Glucagon and plasma catecholamines responses tograded and prolonged exercise in man. *J Appl Physiol.* **38**: 70-76. 1975.
32. GALBO, H., J.J. HOLST, N.J. CHISTENSEN, J. HILSTED. Glucagon and plasma chatecholamines during β–receptor blockade in exercising man. *J Appl Physiol.* **40**: 855-863. 1976.
33. NAVERI, H. Blood hormone and metabolite levels during graded cycle ergometer exercise. *Scand J Clin Lab Invest.* **45**: 599-603. 1985.
34. BARNARD, R.J., H.W. DUNCAN, K.M. BALDWIN, G. GRIMDITCH G.D. BUCKBERG. Effects of intense exercise training on myocardial performance and coronary blood flow. *J Appl Physiol.* **49**: 444-449. 1980.
35. DEBLASI, A., A.S MAISEL, R.D. FELDMAN, M.G. ZEIGLER, M. FRATELLI, M. DILALLO, D.A. SMITH, C.C. LAI, H.J. MOLTUSKY. In vivo regulation of β–adrenergic receptors on human mononuclear leukocytes: Assessment of receptor number, location and function after posture change, exercise and isoproterenol infusion. *J Clin Endoc Metabol.* **63**: 847-853. 1986.
36. EYSEMANN, S.B., E. GERVINO, D.E. VATNER, S.E. KATZ, L. DECKER, P.S. DOUGLAS. Prolonged exercise alter β–adrenergic responsiveness in healthy sedentary humans. *J Appl Physiol.* **80** (2): 616-622. 1996.
37. HOPKINS, M.G., R.J. SPINA, A.A. EHSANI. Enhanced β–adrenergic-mediated cardiovascular responses in endurance athletes. *J Appl Physiol.* **80** (2): 516-521. 1996.

38. LANDMANN, R.M.A , E. BURGISSER, F.R. BUHLER. Human lymphocytes as a model for beta-adrenergic receptors in clinical investigation. *J Rec Res.* **3**(1 and 2): 71-88. 1983.

39. LANDMANN, R.M.A., M. PORTENIER, M. STAEHELIN, M. WESP, R. BOX. Changes in β–adrenoceptors and leukocyte subpopulations after physical exercise in normal subjects. *Arch Pharmacol.* **337**: 261-266. 1988.

40. MAISEL, A.S., T. HARRIS, C.A. REARDON, M.C. MICHEL. β–adrenergic receptors in lymphocyte subsets after exercise. *Circulation.* **82**: 2003-2010. 1990.

41. TIBBITS, G.F., T. NAGATOMO, M. SASAKI, R.J. BARNARD. Cardiac sarcolemma: Compositional adaptation to exercise. *Science.* **213**: 1271-1273. 1981.

42. MAKI, T., H. NAVERI, M. HARKONEN, K. KONTULA. Propranolol attenuates attenuates exercise-induced increment in human lymphocytic β–adrenergic receptors. *Scand J Clin Lab Invest.* **48**: 357-363. 1988.

43. BURMAN,K.D., E.W. FERGUSON, Y.Y. DJUH, L. WARTOFSKY, K. LATHAM. Beta receptors in peripheral mononuclear cells increase acutely during exercise. *Acta Endocrinol.* **109**: 563-568. 1985.

44. MAKI, T., H. LEINONEN, H. NAVERI, A. SOVIJARVI, A. KONTUAL, M. HARKONEN. Response of the β–adrenergic system to maximal dynamic exercise in congestive heart failure secondary to idiopathic dilated cardiomyopathy. *Amer J Cardiol.* **63**: 1348-1353. 1989.

45. MAKI, T., H. NAVERI, H. LEINONEN, A. SOVIJARVI, B. LEWKO, M HARKONEN, K. KONTULA. Effect of propranolol and pindolol on the up-regulation of lymphocytic β–adrenoceptors during acute submaximal physical exercise. *J Cardio Pharmacol* (in press).

46. TOHMEH, J.F., P.E. CRYER. Biphasic adrenergic modulation of β–adrenergic receptors in man: agonist-induced early increment and late decrement in β–adrenergic receptor number. *J Clin Invest.* **65**: 836-840. 1980.

47. vanTITS, L.J.H, M.C. MICHEL, H. GROSSE-WILDE, M. HAPPEL, F.W. EIGLER, A. SOLIMAN, O.E. BRODDE. Catecholamine increase β2-adrenergic receptors via a β2-adrenergic, spleen-dependent process. *Am J Physiol.* **258**: E191-E202. 1990.

48. BRODDE, O.E., A. DAUL, M. MICHEL-REHER, F. BOOMSMA, A.J. VELD, P. SCHLIEPER, M.C. MICHEL. Agonist-induced desensitiztion of β–adrenoceptor function in man: Subtype-selective reduction in β1- or β2-adrenoceptor-mediated hysiological effects by xamoterol or procaterol. *Circulation.* **18**: 914-921. 1990.

49. MAISEL, A.S., P. LEE, C.M. WRIGHT, S. CARTER. Exercise-induced abnormalities in cAMP responses in congestive heart failure (abstract). *J Amer Coll Cardiol.* **11**: 97A. 1988.

50. MIDDEKE, M., M. REMIEN, H. HOLZGREVE. The influence of sex, age, blood pressure and physical stress on β2-adrenoceptor density of mononuclear cells. *J Hypertens.* **2**: 261-264. 1984.
51. MANCINI, D.M., M.J. FREY, D. FISCHBERG, P.B. MOLINOFF, J.R. WILSON. Characterization of lymphocyte beta-adrenergic receptors at rest and during exercise in ambulatory patients with chronic congestive heart failure. *Amer J Cardiol.* **63**: 307-312. 1989.
52. AARONS, R.D., A.S. NIES, J.G. GERBER, P.B. MOLINOFF. Decreased beta-adrenergic receptor density on human lymphocytes after chronic treatment with agonists. *J Pharmacol Exp Ther.* **224**: 1-6. 1983.
53. BRODDE, O.E., M. BRINKMANN, R. SCHEMUTH, N, O'HARA, A. DAUL. Terbutaline-induced desensitization of human lymphocyte β2-adrenoceptors, accelerated restoration of β–adrenoceptor responsiveness by prednisone and ketotifen. *J Clin Invest.* **76**: 1096-1101. 1985.
54. MICKEY, J., R. TATE, J. LEFKOWITZ. Subsensitivity of adenylate cyclase and decreased β–adrenergic receptor binding after chronic exposure to (-)isoproterenol in vitro. *J Biol Chem.* **250**: 5727-5729. 1975.
55. KEBABIAN, J.W., M. ZATZ, J.A. ROMERO, J. AXELROD. Rapid changes in rat pineal β–adrenergic receptor; alterations in L-[3H]alprenolol binding and adenylate cyclase. *Proc Natln Acad Sci.* **72**: 3735-3739. 1975.
56. BUTLER J., M. O'BRIEN, K. O'MALLEY, J.G. KELLY. Relationship of β–adrenoceptor density to fitness in athletes. *Nature, London.* **298**: 60-62. 1982.
57. HARDEN, T.K. Agonist-induced desesitization of the β–adrenergic receptor-linked adenylate cyclase. *Pharmacol Rev.* **35**: 5-32. 1983.
58. MOTULSKY, H.J., P.A. INSEL. Adrenergic receptors in man: Direct identification, physiologic regulation and clinincal alterations. *N Engl J Med.* **307**: 18-29. 1982.
59. BRODDE, O.E. β–Adrenoceptors, in Williams M, Glennon RA, Timmermans PBMWM (eds): Receptor Parmacology and Function. New York, Marcel Dekker, 1989, pp 207-255.
60. MAISEL, A.S., MOTULSKY, H.J. Receptor distribution does not accompany terbutaline-induced down regulation of beta-adrenergic receptors on human mononuclear leukocytes. *Clin Pharmacol Ther.* **42**: 100-106. 1987.
61. MICHEL M.C., A. PINGSMANN, M. NOHLEN, U. SIEKMANN, O.E. BRODDE. Decreased myometrial beta-adrenoceptors in women receiving beta-2-adrenergic tocolytic therapy: Correlation with lymphocyte beta-adrenoceptors. *Clin Pharmacol Ther.* **45**: 1-8. 1989.
62. MAISEL, A.S., P. FOWLER, A. REARDON, H.J. MOTULSKY, M.C. MICHEL. A new method for isolation of human lymphocyte subsets receals differential regulation of β–adrenergic receptors by terbutaline treatment. *Clin Pharmacol Ther.* **46**: 429-439. 1989.

63. BECKENRINGH, J.J., O.E. BRODDE, A. DAUL, M.C. MICHEL, X.L. WANG. Haemodynamic and lymphocyte β2-adrenoceptor changes during procaterol treatment in man (Abstract). *Br J Pharmacol.* **90**, Suppl: 43P. 1987.

64. KRALL, J.F., M. CONNELLY, M.L. TUCK. Acute regulation of beta-adrenergic catecholamine sensitivity in human lymphocytes. *J Pharmacol Exp Ther.* **214**: 554-560. 1980.

65. CONOLLY, M.E., J.K. GREENACRE. The lymphocyte beta-adrenoceptor in normal subjects and patients with bronchial asthma. The effect of different forms of treatment on receptor function. *J Clin Invest.* **58**: 1307-1316. 1976.

66. MORRIS, H.G., S.A. RUSNAKE, J.C. SELNER, K. BARZENS, J. BARNES. Adrenergic desensitization in leukocytes of normal and asthmatic subjects. *J Cyclic Nucleotide Res.* **3**: 439-446. 1977.

67. GREENACRE, J.K., P. SCHOFIELD, M.E. CONOLLY. Desensitization of the β–adrenoceptor of lymphocytes from normal subjects and asthmatic patients. *Br J Clin Pharmacol.* **5**: 199-206. 1978.

68. GALANT, S.P., L. DURISETI, S. UNDERWOOD, P.A. INSEL. Decreased beta-adrenergic receptors on polymorphonuclear leukocytes after adrenergic therapy. *N Engl J Med.* **299**: 933-936. 1978.

69. LEE, T.P. Regulation of beta-adrenergic response in human lymphocytes: agonist induced subsensitivity. *Res Commun Chem Pathol Pharmacol.* **22**: 233-242. 1978.

70. GALANT, S.P., L. DURISETI, S. UNDERWOOD, S. ALLRED, P.A. INSEL. Beta-adrenergic receptors of polymorphonuclear particulates in bronchial asthma. *J Clin Invest.* **65**: 577-585. 1980.

71. SCARPACE, P.J., M.R. LITTNER, D.P. TASHKIN, I.B. ABRASS. Lymphocyte beta-adrenergic refractoriness induced by theophylline or metaproterenol in healthy and asthmatic subjects. *Life Sci.* **31**: 1567-1573. 1982.

72. TASHKIN, D.P., M.E. CONOLLY, R.I. DEUTSCH, K.P.P. HUI, M. LITTNER, P. SCARPACE, I. ABRASS. Subsensitization of beta-adrenoceptors in airways and lymphocytes of healthy and asthmatic subjects. *Am Rev Respir Dis.* **125**: 185-193. 1982.

73. VAN DEN BERG, W., J.G. LEFERINK, J.K. FOKKENS, J. KREUKNIET, R.A.A. MAES, P.L.B. BRUYNZEEL. Clinical implications of drug-induced desensitization of the beta-receptor after continuous oral use of terbutaline. *J Allergy Clin Immunol.* **69**: 410-417. 1982.

74. SANO, Y., G. WATT, R.G. TOWNLEY. Decreased mononuclear cell beta-adrenergic receptors in bronchial asthma: parallel studies of lymphocyte and granulocyte desensitization. *J Allergy Clin Immunol.* **72**: 495-503. 1983.

75. FELDMAN, R.D., L.E. LIMBIRD, J. NADEAU, D. ROBERTSON, A.J.J. WOOD. Leukocyte beta-receptor alterations in hypertensive subjects. *J Clin Invest.* **73**: 648-653. 1984.

76. FREY, M.J., D. MANCINI, D. FISCHBERG, J.R. WILSON, P.B. MOLINOFF. Effect of exercise duration on density and coupling of β–adrenergic receptor on human mononuclear cells. *J Appl Physiol.* **66**: 1494-1500. 1989.
77. IZAWA, T., T. KOMABAYASHI, K. SUDA, Y. KUNISADA, S. SHINODA, M. TUBOI. An acute exercise-induced translocation of β–adrenergic receptors in rat myocardium. *J Biochem.* **105**: 110-113. 1989.
78. MAKI, T., K. KONTULA, M. HARKONEN. The beta-adrenergic system in man: Physiological and pathophysiological response. Regulation of receptor density and functioning. *Scand J Clin Invest.* **50** (Suppl 201): 25-43. 1990.
79. FUGII, N., H. MIYAZAKI, S. HOMMA, H. IKEGAMI. Dynamic exercise induces translocation of β–adrenergic receptors in human lymphocytes. *Acta Physiol Scand.* **148**: 463-464. 1993.
80. STITTMATTER, W.J., F. HIRATA, J. AXELROD. Regulation of the β–adrenergic receptor by methylation of membrane phospholipids. In: Advances in Cyclic Nucleotide and Protein Phosphorylation Research, edited by J.E. Dumont, P. Greengard and G.A. Robsion. New York: Raven, vol. 14, pp 83-91, 1981.
81. DEBLASI, A., M. LIPARTITI, H.J. MOTULSKY, P.A. INSEL, M. FRATELLI. Agonist-induced redistribution of β–adrenergic receptors on intact human mononuclear leukocytes. Redistributed receptors are nonfunctional. *J Clin Endocrinol Metab.* **61**: 1081-1088. 1985.
82. MOTULSKY, H.J., E.M.S. CUNNINGHAM, A. DEBLASI, P.A. INSEL. Desensitization and redistribution of β–adrenergic receptors on human mononuclear leukocytes. *Am J Physiol.* **250** (Endocrinol Metab 13): E583-E590. 1986.
83. KAHN, M.M., P. SANSONI, E.D. SILVERMAN, E.G. ENGLEMAN, K.L. MELMON. Beta-adrenergic receptors on human suppressor, helper and cytolytic lymphocytes. *Biochem Pharmacol.* **35**: 1137-1142. 1986.
84. LANDMANN, R.M.A., E. BURGISSER, M. WESP, F.R. BUHLER. Beta-adrenergic receptors are different in subpopulations of human circulating lymphocytes. *J Recept Res.* **4**: 37-50. 1984.
85. BRODDE, O.E. Molecular Pharmacology of β–adrenoceptors. *J Cardiovasc Pharmacol.* **8** (suppl 4): S16-S20. 1986.
86. BRODDE, O.E., G. ENGEL, D. HOYER, K.D. BOCK, F. WEBER. The β–adrenergic receptor in human lymphocytes: subclassification by the use of a new radioligand (+,-)-125iodocyanopindolol. *Life Sci.* **29**: 2189-2198. 1981.
87. BRODDE, O.E., X.L. WANG, N. O'HARA, A. DAUL, W. SCHIESS. Effect of propranolol, alprenolol, pindolol and bopindolol on β2-adrenoceptor density in human lymphocytes. *J Cardiovasc Pharmacol.* **8** (suppl 6): S70-S73. 1986.
88. BOYUM, A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest.* **21** (suppl 97): 77-89. 1968.

89. MICHEL M.C., J.J. BRECKERINGH, I. KATSUMI, R. KRETSCH, O.E. BRODDE. Lymphocyte beta2-adrenoceptors mirror precisely beta2-adrenoceptor, but poorly beta1-adrenoceptor changes in the human heart. *J Hypertens.* **4** (suppl 6): S215-S218. 1986.
90. BRODDE, O.E., X.L. WANG. Beta-adenoceptor changes in blood lymphocytes and altered drug responsiveness. *Ann Clin Res.* **20**: 311-323. 1988.
91. MICHEL, M.C., T. JOHNSTON, M. NOHLEN. Acute regulation of human lymphocyte β2-adrenoceptor density by β–agonists. *Naunyn-Schmiedeberg's Arch Pharmacol.* **335** (suppl): R87. 1987.
92. McGONIGLE, P., P.B. MOLINOFF. Quantitative aspects of drug-receptor interactions, (Chapter 9), from Basic Neurochemistry: Molecular, Cellular and Medical Aspects, 4th ed., edited by G.J. Siegel et al. Raven Press, Ltd., New York 1989. pp 183-200.
93. ENGEL, G., D. HOYER, R. BERTHOLD, H. WAGNER. (+,-)[125Iodo]-cyanopindolol a new ligand for β–adrenoceptors: identification and quantification of subclasses of β–adrenoceptors in guinea pig. *Naunyn-Schmiedeberg's Arch Pharmacol.* **317**: 277-285. 1981.
94. BJORNERHEIM, R., S. GOLF, V. HANSSON. Specific non-β–adrenergic binding sites for 125I-iodocyanopindolol in myocardial membrane preparations: a comparative study between human, rat and porcine hearts. *Cardiovasc Res.* **25**: 764-773. 1991.
95. LEHMANN, M., J. KEUL, G. HUBER, M. Da PRADA. Plasma catecholamines in trained and untrained volunteers during graded exercise. *Int J Sports Med.* **2**: 143-147. 1981.
96. LEHMANN, M., H.H. DICKHUTH, P. SCHMIDT, H. PORZIG, J. KEUL. Plasma catecholamines, β–adrenergic receptors, and isoproterenol sensitivity in endurance trained and non-endurance trained volunteers. *Eur J Appl Physiol.* **52**: 362-369. 1984.
97. MILANO, C.A., L.E. ALLEN, H.A. ROCKMAN, et al. Enhanced myocardial function in transgenic mice overexpressing the β2-adrenergic receptor. *Science.* **264**: 582-586. 1994.
98. KLAUSEN, K., N. SECHER, J. CLAUSEN, O. HARTLING, J. TRAP-JENSEN. Central and regional circulatory adaptations to one-leg training. *J Appl Physiol.* **52**: 976-983. 1982.
99. ROWELL, L.B. Cardiovascular adaptations to chronic physical activity and inactivity. In: Human Circulation. New York: Oxford University Press; 257-286. 1986.
100. LIGGETT, S.B., et al. Early and delayed consequences of β2-adrenergic receptor overexpression in mouse hearts. *Circulation.* **101**: 1707-1714. 2000.
101. LEFKOWITZ, R.J., H.A. ROCKMAN, W.J. KOCH. Catecholamines, cardiac β–adrenergic receptors, and heart failure. *Circulation.* **101**: 1634-1637. 2000.
102. VATNER, D.E., et al. Adverse effects of chronic endogenous sympathetic drive induced by cardiac Gas overexpression. *Circ Res.* **78**: 517-524. 1996.

103. ENGELHARDT, S., I. HEIN, F. WIESMANN, M.J. LOHSE. Progressive hypertrophy and heart failure in β–adrenergic receptor transgenic mice. *Proc Natl Acad Sci.* **96**: 7059-7064. 1999.
104. BRISTOW, M.R., et al. N Eng J Med. 307: 205. 1982. M.R. BRISTOW et al. *Circulation.* **82**: (suppl 1), I 12. 1990. M.R. BRISTOW et al. J Mol Cell Cardiol. 17: (suppl 2), 41. 1985.
105. UNGERER, M., M. BOHM, J.S. ELCE, E. ERDMANN, M.J. LOHSE. *Circulation.* **87**: 454. 1993.
106. LEVY, F.O., X. ZHU, A.J. KAUMANN, L. BIRNBAUMER. *Proc Natl Acad Sci.* **90**: .10798. 1993.
107. BERTIN, B., et al. *Cardiovasc Res.* **27**: 1606. 1993.
108. LEFKOWITZ, R.J., N.J. FREEDMAN. Desensitization of G Protein-coupled receptors. *Rec Prog Hormone Res.* **51**: 319-352. 1996.
109. LEFKOWITZ, R.J., M.G. CARON, W.P. HAUSDORF. Turning off the signal: desensitization of β–adrenergic function. *FASEB J.* **4**: 2881-2889. 1990.
110. FUJII, N., et al. Exercise induced changes in β–adrenergic receptor mRNA level measured by competitive RT-PCR. *J Appl Physiol.* **82** (6): 1926-1931. 1997.
111. LIGGETT, S.B. et al. *Proc Natl Acad Sci.* **93**: 10483-10488. 1996.
112. LEFKOWITZ, R.J., et al. Coupling of β2-adrenoceptor to Gi proteins and its physiological relevance in murine cardiac myocytes. *Circ Res.* **84**: 43-52. 1999.
113. HADCOCK, J.R., M. ROS, C.C. MALBON. Agonist regulation of β–adrenergic receptor mRNA: Analysis in S49 mouse lymphoma mutants. *J Biol Chem.* **264**: 13956-13961. 1989.
114. WILHELMSEN, L., et al. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. *Circulation.* **82**: 1730-1736. 1990.
115. LEFKOWITZ, R.J., et al. Expression of a β–adrenergic receptor kinase I inhibitor prevents the development of mycardial failure in gene-targeted mice. *Proc Natl Acad Sci.* **95**: 7000-7005. 1998.
116. LIGGETT, S.B., et al. Low- and high-level transgenic expression of β–adrenergic receptors differentially affect cardiac hypertrophy and function in Gaq-overexpressing mice. *Proc Natl Acad Sci.* **96**: 6400-6405. 1999.
117. KOCH, W.J., et al. Restoration of β–adrenergic signaling in failing cardiac ventricular myocytes via adenoviral-mediated gene transfer. *Proc Natl Acad Sci.* **94**: 12100-12105. 1997.
118. LOHSE, M.J., J.L. BENOVIC, M.G. CARON, R.J. LEFKOWITZ. Multiple pathways of rapid β2-adrenergic receptor desensitization. *J Biol Chem.* **265**: 3202-3209. 1990.
119. TUREK, D., et al. The influence of exercise training on mechanical catecholamine responsiveness, β–adrenoceptor density and myosin isozyme pattern of rat ventricular myocardium. *Basic Res Cardiol.* **80**: 88-99. 1985.
120. ZAK, R. Cell proliferation during cardiac growth. *Am J Cardiol.* **31**: 211-219. 1973.

121. 121. YELAMARTY, R.V., et al. Chronic exercise alter contractility and morphology of isolated rat cardiac myocytes. *Am J Physiol.* **264**: C1180-1189. 1993.
122. MOORE, R.L. Cellular adaptations of the heart muscle to exercise training. *Ann Med.* Suppl 1: 46-53. 1998.
123. CHEUNG, J.Y. et al. Endurance training does not affect intrinsic calcium current characteristics in rat myocardium. *Am J Physiol.* **273**: H1193-1197. 1997.
124. FERGUSON, S.S.G., et al. *Science.* **271**: 363-366. 1996.
125. De BLASI, A., et al. G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. *TIPS.* **17**: 416-421. 1996.