

Adrenergic Receptors
Paul J. Mills, Ph.D.
University of California, San Diego

The catecholamines norepinephrine and epinephrine initiate biochemical and physiological events by binding to the three subclasses of β -adrenergic and the six subclasses of α -adrenergic receptors (Hall, 2004; Piascik & Perez, 2001; Taylor & Bristow, 2004). These adrenergic receptors provide the functional link between catecholamines and the numerous end organ responses they generate. In addition to modulating catecholamine release and re-uptake, they mediate end organ responses such as blood pressure, heart rate, myocardial contractility, vascular constriction and relaxation, and renin release and inhibition, as well as a host of immune functions such as immune cell trafficking, adhesion, and cytokine responses, all of relevance to mind-body medicine (Brodde, 1990; Sanders, 1995).

Since the sensitivity and density of agonist receptors are dynamically regulated in response to changing concentrations of adrenergic agonists (e.g., desensitization and down-regulation of receptors), it can be important in certain research models to be able to measure them directly. There are both *in vivo* and *in vitro* techniques to determine the functionality of adrenergic receptors. *In vivo* techniques involve infusing adrenergic agonists and assessing a specific end organ response. These methods are typically carried out in a clinical research setting and require medical oversight. *In vitro* techniques typically involve isolating peripheral cells or specific organ tissue and quantifying either the number or sensitivity or both of the adrenergic receptors expressed in that tissue. We briefly review the methodologies of these techniques.

In vivo Techniques to Assess Adrenergic Receptors

An *in vivo* technique for assessing β -adrenergic receptor sensitivity involves infusing the β adrenergic agonist isoproterenol and then measuring the heart rate response. An *in vivo* technique for assessing α -adrenergic receptor sensitivity involves infusing the α -adrenergic agonist phenylephrine and then measuring the blood pressure response. The general approach to both methods is similar. We will present details of the method to assess β -adrenergic receptor sensitivity. This technique is called the “chronotropic 25 dose”, or CD_{25} for short. The method involves intravenously infusing a series of bolus doses of isoproterenol and then measuring the heart rate response (Mills et al., 1998; Dimsdale and Mills, 2002). Doses are typically 0, 0.10, 0.25, 0.50, 1.0, 2.0 and 4.0 μg . Heart rate is charted continuously by ECG and the maximum heart rate response to each dose is recorded. CD_{25} is calculated using the following formula: CD_{25} (μg isoproterenol) = [(basal heart rate + 25) - intercept] / slope. The slope and intercept for each individual's heart rate response to isoproterenol is calculated by linear regression. The CD_{25} value can then be tested for differences between groups by t-test or ANOVA.

In vitro Techniques to Assess Adrenergic Receptors

Whereas the *in vivo* techniques can only provide information on the functional sensitivity of adrenergic receptors, *in vitro* techniques can provide information on both sensitivity and density of adrenergic receptors. In addition to conducting the assays on tissues of interest, such as cardiac or lung tissue for the β -adrenergic receptor or adipose tissue for the α -adrenergic receptor, there are less invasive ways to access these receptors by using peripheral blood cells. Lymphocytes, for example, express β_2 -adrenergic receptors which can serve as a model for β -adrenergic receptors on the heart and lung. Platelets contain α_2 -adrenergic receptors which

have been used in psychiatry and behavioral medicine research as a model of human α -adrenergic receptors and drug responsiveness.

For these *in vitro* experiments using peripheral cells, lymphocytes and platelets are isolated from whole blood using a variety of techniques and then washed in preparation for the sensitivity and/or density assays. Such techniques have been used widely in studies on stress, hypertension, antihypertensive drug therapy, sleep apnea, and spaceflight (Bao et al., 2005; Meck et al., 2004; Mills et al., 2002). There are limitations to using peripheral blood cells as models of adrenergic receptors that researchers should be aware of (Mills & Dimsdale, 1993).

As with the *in vivo* techniques, the *in vitro* techniques for assessing adrenergic receptor sensitivity involve stimulating the receptor of interest with an agonist and then measuring the response. Since many of the adrenergic receptors act through the activation of the membrane bound enzyme adenylate cyclase, which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), determining the amount of cAMP following receptor stimulation can be used as an index of receptor sensitivity. Typically, the greater the sensitivity and density of β -adrenergic receptors, the greater the amount of cAMP that is generated in the cell in response to stimulation. In the case of the β -adrenergic receptor, stimulation with a maximal dose of isoproterenol [10^{-5} mol/L] results in a 3 to 5-fold increase in lymphocyte intracellular cAMP levels.

The *in vitro* assay for determining adrenergic receptor density is called radioligand binding, which can be performed on intact whole cells or membranes from fractionated cells. Radioligand binding involves incubating a radioligand with the cell of interest under highly controlled conditions. When using peripheral cells, typical radioligands for β -adrenergic receptors are [125 I]-iodocyanopindolol and [125 I]iodopindolol. [3 H]prazosin, [(3)H]rauwolscine, and [3 H]yohimbine are ligands for α -adrenergic receptors. Upon termination of the incubation by, usually by dilution, the unbound radioligand is removed by filtration. The remaining radioligand that is bound to the cell surface receptors is then measured and used to calculate the density of the receptor. The basic underlying principles of radioligand receptor binding are similar to physiologically linked receptor binding, although the inherent complexity of the physiologic receptor environment noted above is absent in the laboratory environment. There are a number of important issues that need to be addressed to ensure that optimal experimental conditions have been met and that the binding experiments measure the specific receptors of interest. Although radioligands are designed to bind specifically to the receptor of interest, there is always some nonspecific binding to other membrane proteins. This amount of nonspecific binding is determined by incubating the radioligand and tissue in the presence of a non-radioactive competing ligand which will bind to nearly all of the specific receptors of interest and leave the radioligand binding to only the nonspecific sites. The non-radioactive ligand is usually propranolol for β -adrenergic receptors and phentolamine for α -adrenergic receptors. By subtracting the radioactivity observed in the presence of the unlabeled drug (nonspecific binding) from that obtained in the absence of the unlabeled drug (total binding), the amount of specific binding is obtained. Specific binding represents the binding of interest. To determine the receptor density and binding affinity, radioligand binding isotherms are used. B_{max} , or the maximum amount of radioligand bound to the receptors, is the number of receptors expressed on the whole cell or the density expressed on cellular membranes. K_d , or the dissociation constant or binding affinity of the radioligand for the receptor, is the concentration of radioligand that binds to half of the specific receptors. Radioligand binding isotherms involve incubating six to eight concentrations of the radioligand with a constant number of cells or membranes. The data derived is then mathematically transformed and analyzed by nonlinear regression to yield B_{max} and K_d (Motulsky, 2001). Depending on factors such as age, fitness, hypertension, use of adrenergic receptor

antagonists, etc., receptor binding typically yields a Bmax of 600-2000 β 2-adrenergic receptors per lymphocyte and a Bmax of 240-600 α 2-adrenergic receptors per platelet.

References

Bao X, Mills PJ, Rana BK, Dimsdale JE, Schork NJ, Smith DW, Rao F, Milic M, O'Connor DT, Ziegler MG: Interactive effects of common beta2-adrenoceptor haplotypes and age on susceptibility to hypertension and receptor function. *Hypertension* 46(2):301-307, 2005.

Brodde OE: Physiology and pharmacology of cardiovascular catecholamine receptors: implications for treatment of chronic heart failure. *Am Heart J* 120(6 Pt 2):1565-1572, 1990.

Dimsdale JE, Mills PJ: An unanticipated effect of meditation on cardiovascular pharmacology and physiology. *Am J Cardiol* 90(8):908-909, 2002.

Hall RA: Beta-adrenergic receptors and their interacting proteins. *Semin Cell Dev Biol* 15(3):281-288, 2004.

Meck JV, Waters WW, Ziegler MG, deBlock HF, Mills PJ, Robertson D, Huang PL: Mechanisms of postspaceflight orthostatic hypotension: low alpha1-adrenergic receptor responses before flight and central autonomic dysregulation postflight. *Am J Physiol Heart Circ Physiol* 286(4):H1486-495, 2004.

Mills PJ, Dimsdale JE: The promise of adrenergic receptor studies in psychophysiological research II: Applications, limitations, and progress. *Psychosom Med* 55(5):448-457, 1993.

Mills PJ, Dimsdale JE, Ancoli-Israel S, Clausen J, Loreda JS: The effects of hypoxia and sleep apnea on isoproterenol sensitivity. *Sleep* 21(7):731-735, 1998.

Mills PJ, Perez CJ, Adler KA, Ziegler MG: The effects of spaceflight on adrenergic receptors and agonists and cell adhesion molecule expression. *J Neuroimmunol* 132(1-2):173-179. 2002.

Motulsky H: *The GraphPad Guide to Analyzing Radioligand Binding Data*, GraphPad, San Diego, CA, 2001. [<http://www.graphpad.com/www/radiolig/radiolig.htm>].

Piasecki MT, Perez DM: Alpha1-adrenergic receptors: new insights and directions. *J Pharmacol Exp Ther* 298(2):403-410, 2001.

Sanders VM: The role of adrenoceptor-mediated signals in the modulation of lymphocyte function. *Adv Neuroimmunol* 5(3):283-298, 1995

Taylor MR, Bristow MR: The emerging pharmacogenomics of the beta-adrenergic receptors. *Congest Heart Fail* 10(6):281-288, 2004.