ADRENOCEPTORS AS MODELS FOR G PROTEIN-COUPLED RECEPTORS: STRUCTURE, FUNCTION AND REGULATION

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Catecholamines, either endogenous hormones or a variety of exogenous synthetic pharmacological agents, bind to adrenoceptors to mediate their ultimate clinical effects [21]. Since adrenoceptors mediate many cardiovascular responses, pharmacological stimulation and inhibition of adrenoceptors may be useful to the anaesthetist. Ahlquist described originally two adrenoceptor subtypes (alpha and beta) in 1948 [1], but for many years four “classic” adrenoceptor subtypes (alpha, alpha2, beta1, and beta2) have guided our understanding of human physiological responses and clinical administration of catecholamine drugs. Recently, genes encoding nine distinct adrenoceptor subtypes (alpha1A, alpha1B, alpha1C, alpha2A, alpha2C, beta1, beta2, and beta3) have been discovered. The elucidation of new adrenoceptor subtypes has already facilitated the search for, and development of, adrenoceptor subtype selective agonists and antagonists.

Adrenoceptors are members of the larger guanine nucleotide binding protein (G protein)-coupled receptor super family [4, 27, 33, 36]. In general, G protein-coupled receptors are excitatory proteins, located in the cell membrane, coupled via intermediary G proteins to effector systems. Since activation of G protein-coupled receptors results in desired (or occasionally undesired) clinical effects, these receptor systems are extremely important in clinical medicine. However, not all clinically important physiological responses are mediated via G protein-coupled receptors. Thus in order to provide a framework in which to understand the importance of G protein-coupled receptors in clinical medicine and anaesthesia, this review begins with a basic overview of various methods of cell communication, with emphasis on excitatory transmembrane proteins.

Three basic types of excitatory transmembrane proteins can be described (fig. 2): voltage sensitive in order to facilitate a simplified approach while simultaneously providing in depth coverage of important concepts and topics.

EXCITABLE TRANSMEMBRANE PROTEINS

Cells communicate with their environment in many different ways. Three general methods of cell communication and effecting cellular (and ultimately clinical) responses are mediated via cytoplasmic receptors, stimulation or inhibition of enzyme systems, and excitatory transmembrane proteins (fig. 1). Cytoplasmic receptors may be represented by steroid hormone receptors. Since steroid ligands are lipophilic (non-charged, and hence soluble in lipid), they cross the lipid bilayer of the cell membrane easily and interact directly with steroid receptors located in the cytoplasm. In addition to cytoplasmic receptors, various enzyme systems are also located in the cytoplasm; these systems may be either associated closely with the plasma membrane or present in other intracellular locations. Inhibition or stimulation of enzymes may be important in medical therapeutics, as seen with drugs such as amrinone and milrinone, which inhibit type III (cAMP) phosphodiesterase. However, in spite of the above examples of pharmacological agents acting via cytoplasmic receptors and cellular enzyme systems, the vast majority of current clinically used drugs and endogenously secreted hormones mediate their effects via excitatory transmembrane proteins.

Three basic types of excitatory transmembrane proteins can be described (fig. 2): voltage sensitive

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Excitable transmembrane proteins

- Voltage sensitive channels
- Ligand-gated channels
- Transmembrane receptors

Fig. 2. Three types of excitable transmembrane proteins are important in mediating cell responses to endogenous hormones or exogenous drugs. These include voltage sensitive channels, ligand-gated channels and transmembrane receptors.

Ion channels, ligand-gated ion channels and transmembrane receptors. Voltage sensitive ion channels open and close depending on cell membrane voltage and are represented by classic ion channels, such as sodium, chloride, potassium and calcium channels. Voltage sensitive ion channels are reviewed in detail elsewhere in this issue and so will not be described further in this review. The second type of clinically important excitable transmembrane proteins include ligand-gated ion channels, such as nicotinic cholinergic receptors and amino acid receptors (including GABA<sub>A</sub> and N-methyl-D-aspartate (NMDA) receptors). Ligand-gated ion channels are receptor-ion channel complexes in which the ion channel is an integral part of a larger and more complex transmembrane protein (fig. 3). Interestingly, ligand-gated ion channel complexes are modulated frequently by more than one ligand, ion, or both. For example, GABA<sub>A</sub> receptors bind GABA, benzodiazepines, barbiturates and ethanol, while NMDA receptors bind NMDA, phencyclidine (for which ketamine is an analogue), glycine and various ions. For further information regarding ligand-gated ion channels, the reader is referred to a recent review [4].

The third general type of excitable transmembrane proteins involved in cell communication and activity are transmembrane receptors. Cell membrane receptors may be defined as excitable transmembrane proteins which interact selectively with extracellular compounds (either endogenous hormones or exogenous drugs) to initiate a cascade of biochemical changes which lead to the ultimate physiological effect. Since transmembrane receptors are located in the lipid cell membrane, they are able to bind hydrophilic (water soluble, and therefore frequently charged) ligands located in the extracellular space. Thus many hydrophilic hormones and drugs do not have to cross the lipid bilayer to interact with the cell. However, this process then necessitates a mechanism by which transmembrane receptors notify the cell of receptor occupancy by ligands. Such a process is frequently referred to as signal transduction. Since signal transduction is reviewed separately in this symposium, only a brief overview will be given here.

Many receptors communicate agonist occupancy via guanine nucleotide proteins (G proteins). With the energy provided by hydrolysis of GTP to GDP, activated G proteins are then able to interact with effector systems (E), ultimately leading to clinical effects.

Fig. 3. Ligand-gated ion channels are receptor-ion channel complexes in which the ion channel is an integral part of a larger and more complex transmembrane protein. The ion channel is depicted as two thin rectangles traversing the cell membrane (shaded boxes). Black squares, triangles and half circles represent binding sites for ligands and ion modulators of the channel (see text for details and examples).

Fig. 4. Transmembrane receptors (R) are located in the cell membrane and bind drugs or hormones on the extracellular surface. Agonist bound receptors then interact with intermediary guanine nucleotide proteins (G proteins). Figure 4 shows a schematic diagram of a model G protein-coupled receptor system. An extracellular ligand (either an endogenous hormone or exogenous drug) couples to the transmembrane receptor. Thus activated, the receptor is able to interact with the intermediate G protein. Hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) provides the energy for the activated G protein to then interact with the effector molecule (either an enzyme system or ion channel) to mediate the final cascade of biological reactions within the cell which ultimately lead to the observed biological effects. Many different transmembrane receptors are part of the larger super family of G protein-coupled receptors. Examples of clinically important G protein-coupled receptor systems used currently and possibly in the future by the anaesthetist include adrenergic, muscarinic cholinergic, opioid, serotonin, dopamine, endothelin, atrial natriuretic, cannabinoid and cholecystokinin receptors. Since adrenoceptors have been studied intensively and
ADRENOCEPTORS AS MODELS FOR G PROTEIN-COUPLED RECEPTORS

nine distinct subtypes have now been cloned, adrenoceptors provide good models for G protein-coupled receptors. The rest of this review will be devoted to adrenoceptors, including their subtype classification, classical pharmacology and molecular pharmacology. It is important to keep in mind, however, that many of the structural features of adrenergic receptors can be generalized to other G protein-coupled receptors.

ADRENOCEPTORS

In 1948, Ahlquist identified two types of receptors which he denoted alpha and beta [1]. In 1967, Lands and colleagues further distinguished two beta adrenoceptor subtypes: beta1 and beta2 [25, 26]. During the 1970s, four adrenoceptor subtypes became apparent: alpha1, alpha2a, beta1 and beta2. Each of these receptors couples to distinct signal transduction pathways and will be referred to as the “classic” adrenoceptor subtypes in this review (as most of the actions of clinically available catecholamine agonists and antagonists are thought of in terms of these four subtypes). During the late 1980s and early 1990s, genes encoding nine distinct adrenoceptor subtypes were cloned, so it is known that at least nine distinct adrenoceptor subtypes exist: these include alpha1A, alpha1B, alpha2C, alpha2D, alpha2E, beta1, beta2, alpha2C1, beta2C, and beta2D adrenoceptors [12, 13, 16, 17, 22, 24, 29, 30, 35, 37, 38, 46]. In terms of physiological cardiovascualr responses, alpha1 adrenoceptors are located on postsynaptic membranes and mediate vasoconstriction. Alpha2 adrenoceptors are located both presynaptic where they inhibit the release of noradrenaline at the nerve terminal, and postsynaptic where they also mediate vasoconstriction. Beta1 adrenoceptors are thought classically to mediate cardiac effects, such as increased heart rate and myocardial inotropy, while beta2 adrenoceptors mediate smooth muscle dilation such as bronchodilation in the lungs and vasodilation in peripheral vessels. These classic physiological descriptions are now known to be incomplete. For example, it is clear that beta2 adrenoceptors are also located in human myocardial tissue where they mediate increased myocardial inotropy as well as possibly mediating increases in heart rate. In spite of these limitations, classic descriptions of adrenoceptor subtype-mediated physiological effects have been useful in that they provide an effective framework in which to use therapeutic agents currently available clinically.

In addition to describing adrenoceptors in terms of end-organ physiology, each subtype can be characterized in terms of classical pharmacology experiments, specifically agonist potency series and selective agonists and antagonists. The agonist potency series for alpha1 adrenoceptors is as follows: adrenaline > noradrenaline > phenylephrine > isoprenaline. Interestingly, the agonist potency series for alpha2 adrenoceptors is identical to that for alpha1 adrenoceptors. This is one of the reasons that identification of alpha adrenoceptor subtypes (specifically alpha1 and alpha2) was difficult historically. Instead, definitive characterization of alpha1 and alpha2 adrenoceptors came with the discovery of selective antagonists such as prazosin (alpha1 adrenoceptor), also idazoxan and yohimbine (alpha2, adrenoceptor). Selective agonists such as phenylephrine (alpha1), clonidine and dexametomidine (alpha2) have since been discovered. Beta adrenoceptors have the following agonist potency series: beta1, isoprenaline > adrenaline > noradrenaline > phenylephrine; beta2, isoprenaline > adrenaline > noradrenaline > phenylephrine. In general, subtle differences between adrenaline and noradrenaline affinity discriminate beta1 from beta2 adrenoceptors. Relatively selective antagonists have been found for beta adrenoceptors, for example metoprolol and betaxolol (beta1) and pindolol (beta2). A selective agonist for beta2 adrenoceptors is not yet available, but salbutamol is a clinically available selective beta2 adrenoceptor agonist.

In addition to physiological end-organ response and ligand binding, adrenoceptor subtypes also utilize different G proteins and second messenger pathways (fig. 5) [4, 15, 19, 21, 27, 33, 36]. In general, beta adrenoceptors (once bound with agonist) interact with the stimulatory G protein, Gs, to activate the enzyme adenylyl cyclase [21]. Stimulation of adenylyl cyclase results in the production of cAMP which ultimately activates various protein kinases within the cell to cause phosphorylation of a cascade of proteins which result in the ultimate physiological response [9, 39]. Alpha1 adrenoceptor subtypes couple to an opposing pathway. Once bound by agonists, alpha1 adrenoceptors couple to the inhibitory G protein, Gi, to inhibit the activity of adenylyl cyclase [34]. This decreases production of cAMP in the cell. In fact, in order to study alpha1 adrenoceptor activation, it is necessary frequently to stimulate the adenylyl cyclase moiety directly with forskolin and monitor inhibition of forskolin stimulation of adenylyl cyclase activity. Interestingly, alpha1 adrenoceptors activate a completely different pathway. Agonist activation of alpha1 adrenoceptors enables receptor coupling to the newly described G protein Gia [6, 11], which then activates the enzyme phospholipase C. Activation of phospholipase C results in the hydrolysis of membrane phospholipids to major products, inositol triphosphate (IP3) and diacylglycerol (DAG) [5]. IP3 then binds to its own receptor located on intracellular membranes to cause the release of intracellular calcium from non-mitochondrial stores, particularly the sarcoplasmic reticulum. DAG activates the enzyme protein kinase C, which then modulates this process [32].

It is important to understand signal transduction pathways for each major adrenoceptor subtype in order to understand the mechanism of action of clinically important drugs. This becomes particularly important when two drugs affecting a given signal transduction pathway at different effector points are administered simultaneously. For example, in acute heart failure during cardiac surgery, administration of a beta adrenoceptor agonist, such as adrenaline, is clinically efficacious. Since activation of myocardial beta adrenoceptors by adrenaline leads to increased intracellular cAMP,
addition of a phosphodiesterase inhibitor, such as amrinone (which inhibits the breakdown of cAMP), will augment cAMP concentrations in myocardial cells. This ultimately leads to additive (and potentially synergistic) effects of increased myocardial inotropy. Hence, by understanding receptor systems and their modulation in disease states, clinical effects of drug administration may often be predicted for a given patient.

MOLECULAR PHARMACOLOGY OF ADRENOCEPTORS

Molecular pharmacology focuses on understanding receptor structure as it relates to pharmacological properties and ultimate function and regulation. Included are concepts such as general receptor structure, elucidation of regions of the receptor important for ligand binding, second messenger coupling and desensitization or regulation. However, in order to understand the molecular pharmacology of a given receptor, it is important to isolate the receptor from others. This process is made difficult by the fact that many receptors (and receptor subtypes) are present simultaneously in a given human cell or tissue. Therefore, in order to study the properties of a single adrenoceptor subtype, it is important to isolate the receptor from others. This process is made difficult by the fact that many receptors (and receptor subtypes) are present simultaneously in a given human cell or tissue. Therefore, in order to study the properties of a single adrenoceptor subtype, it is important to isolate the receptor from others. This process is made difficult by the fact that many receptors (and receptor subtypes) are present simultaneously in a given human cell or tissue. Therefore, in order to study the properties of a single adrenoceptor subtype, it is important to isolate the receptor from others.

The first step in purifying a single transmembrane receptor is to remove the lipid cell membrane; this is frequently accomplished using a detergent such as digitonin. The next steps relate to purification of the desired receptor from other receptors present in the tissue. A major advance in receptor purification over the past 15 years is the concept of affinity chromatography. An affinity chromatography column is a solid support attached to long side chain molecules ending with a ligand congener which binds the receptor. Therefore, once solubilized, receptors may be separated by affinity chromatography. Only the receptor which binds the ligand attached to the column should stick, while other receptors remain free. After repeated washes, isolated receptor can be eluted from the column with a greater concentration of ligand or salt, resulting in a receptor which is highly purified. Frequently, if complete homogeneity is required, then several columns can be used.

Receptor purification to homogeneity has been accomplished over the past decade in many receptor systems, enabling rapid advances in the field of molecular pharmacology. However, while receptor purification has provided important steps forward in studying receptors, this process may be long and tedious, sometimes taking days to weeks to purify enough receptor for a single experiment. Introduction of molecular biology techniques has greatly simplified this process and therefore is presented briefly below.

Molecular biology is based on the fact that every protein in the body, albeit an enzyme, structural element or transmembrane receptor, is encoded in deoxyribonucleic acid (DNA). The nucleotide sequence present in DNA which encodes an entire protein is called a gene. Since DNA is found in the cell nucleus and the building blocks of proteins (i.e. amino acids) are assembled in the cytoplasm, an intermediate molecule is necessary to transmit information from the nucleus to the cytoplasm. Messenger ribonucleic acid (mRNA) is such an intermediary molecule; once information is transcribed from DNA to mRNA, mRNA then moves to the cytoplasm where it interacts with
ADRENOCEPTORS AS MODELS FOR G PROTEIN-COUPLED RECEPTORS

Ribosomal RNA and transfer RNA which make up the "factories" where proteins are built. The process of taking encoded information from mRNA and making a protein is called translation. Thus information is transcribed from DNA to RNA and then translated into proteins. The protein is then mobilized to its appropriate location. Interestingly, the human genome frequently contains non-translated sequences surrounding each gene which are thought to modulate transcription rate, ultimately controlling the concentration of final encoded protein.

Using classical molecular biology techniques [31, 45], genes encoding nine adrenoceptor subtypes have been discovered [12, 13, 16, 17, 22, 24, 29, 30, 35, 37, 38, 46]. While the first adrenoceptor gene to be isolated and sequenced encoded the beta2 subtype, we now know that at least three alpha1, three alpha2, and three beta adrenoceptor subtypes exist. Incorporation of adrenoceptor genes into cells not normally expressing these receptors results in cell lines containing individual adrenoceptor subtypes. Purification (both partial and completely to homogeneity) may be accomplished more easily from cell lines expressing large adrenoceptor subtype concentrations (i.e. 2–5 pmol receptor/mg total protein), while tests of physiological function may be performed in cells expressing smaller, more physiological concentrations of receptor protein (i.e. 100–250 fmol/mg total protein). Because of these molecular biology techniques, a great deal of progress has been made in understanding how receptor structure relates to physiological function. Therefore, the rest of this review will summarize general structural properties of adrenoceptors, specifically regions of adrenoceptors involved in ligand binding, G protein-coupling and regulation. More in depth analysis of these topics may be found in more detailed general reviews [27, 33, 36], as well as specific reviews cited in each section below.

**General structure**

One of the most striking features of adrenoceptors and G protein-coupled receptors in general is the seven transmembrane motif (fig. 6A). The presence of seven stretches of 20–25 lipophilic amino acids suggests that the receptor protein winds in and out of the cell membrane seven times, creating an extracellular amino terminus, intracellular carboxyl terminus, three small extracellular loops and three intracellular loops [27]. Of note, the third intracellular loop is larger and potentially more important in signal transduction than other intracellular loops. It is important to note that this overall structure has

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**Fig. 6.** Overall structure of adrenergic receptors in the cell membrane (A). This general seven transmembrane motif is common to all G protein-coupled receptors known to date. An extracellular amino terminus, three extracellular loops, three intracellular loops and an intracellular carboxyl terminus are apparent. Since the initial portion of the carboxyl terminus is anchored to the lipid membrane via palmitylation at a critical cysteine residue, a fourth intracellular loop is actually formed. Regions of the receptor important in ligand binding, G protein coupling and desensitization are shown in B, C, and D, respectively (see text for details).
protonated amine group. In contrast, serotonin moiety, hydroxyl groups at both the catecholamine and noradrenaline contain a catecholamine ring and beta hydroxy positions, as well as a protonated amine group. In contrast, serotonin and acetylcholine are different overall structures. In order to bind ligand to a receptor protein, it is imperative to have charged counterbalancing ions located in the receptor. Hence, how can ligand binding (which requires charged counter ions) involve transmembrane amino acids when these are known to be lipophilic (i.e. non-polar, non-charged)?

The answer appears to be that while transmembrane amino acids are lipophilic overall, side chain groups in critical amino acids are charged and thus provide the required counter ions [14].

When considering which regions in a receptor protein are important for ligand binding, it is important to remember that adrenoceptors are three-dimensional. Transmembrane domains in G protein-coupled receptors coalesce to form a binding pocket, permitting access from the extracellular space for binding of charged catecholamines [14, 42, 44]. In order to test which transmembrane regions are important in ligand binding, it is possible to take advantage of molecular biology techniques such as DNA “cutting and pasting” to form novel genes which encode synthetic receptors, specifically with alterations in the area of interest. For example, if regions of the beta2 adrenoceptor are gradually replaced with beta1 adrenoceptor starting from the amino terminus, ligand binding properties remain as would be expected for the beta1 adrenoceptor, until the third and fourth transmembrane regions are replaced with beta2 adrenoceptor [18]. At this point, agonist ligand binding properties change to those expected for the beta2 adrenoceptor even though the remainder of the receptor is still beta1 [18]. This suggests that the third and fourth transmembrane domains may be important in ligand binding [18, 42, 44]. Similar studies have determined that antagonist binding also involves the sixth and seventh transmembrane domains [23]. It is important to remember, however, that the seventh transmembrane may be close to the third and fourth transmembrane when the three-dimensional structure is taken into account. While synthetic chimeric receptors give an initial idea of which general regions are involved in ligand binding, it is important to know also which individual amino acids act as counter ions for ligands and are therefore critical in ligand binding. Mutation (or changing) of individual amino acids has also been performed using standard molecular biology techniques [31, 45], and distinct amino acids acting as counter ions for either agonists and antagonists have been identified for the beta1 adrenoceptor [42, 44]. These studies implicate distinct charged amino acids present in hydrophobic transmembrane areas as providing counter ions for the ligand cationic amine group, hydrogen bond interactions with catecholamine hydroxyl groups and aromatic interactions with the catecholamine ring.

Ligand binding

In order to understand the structural requirements for ligand binding to receptors, it is important to consider the overall structure of catecholamine ligands. Figure 7 shows the chemical structure of the catecholamines adrenaline and noradrenaline compared with serotonin and acetylcholine. Adrenaline and noradrenaline contain a catecholamine ring moiety, hydroxyl groups at both the catecholamine ring and beta hydroxy positions, as well as a protonated amine group. In contrast, serotonin contains a catecholamine ring but no hydroxyl group in the beta hydroxy position, and the protonated amine group is further from the catechol ring. Acetylcholine has a different overall structure. In order to bind ligand to a receptor protein, it is imperative to have charged counterbalancing ions located in the receptor. Hence, how can ligand binding (which requires charged counter ions) involve transmembrane amino acids when these are known to be lipophilic (i.e. non-polar, non-charged)?

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G protein coupling

Transmembrane receptors interact with G proteins on or near the cytoplasmic surface of the cell

FIG. 7. Chemical structure of catecholamine ligands (adrenaline (A) and noradrenaline (NA)) compared with serotonin (5-HT) and acetylcholine (ACh).

been confirmed using various biochemical experiments and more recently with antipeptide antibodies to every major region of the beta2 adrenoceptor receptor [27, 33, 36]. In comparing the overall structure of individual adrenoceptor subtypes, it is important to note that while the seven transmembrane motif holds, some overall structural differences do occur. For example, alpha1 adrenoceptors contain a very long intracellular carboxyl terminus, alpha2 adrenoceptors a very short intracellular carboxyl terminus but extremely long third intracellular loop, while beta adrenoceptors are intermediate between these two extremes.

When comparing individual amino acid sequences between adrenoceptor subtypes, another general feature is observed. If the identity of amino acids between two adrenoceptor subtypes (i.e. alpha2 and beta2) is considered, matching amino acids are found to be concentrated in the transmembrane regions. Interestingly, when comparing transmembrane amino acid identity between two adrenoceptor subtypes within the same subfamily (i.e. alpha2a and alpha2c adrenoceptors), transmembrane amino acid identity is as great as 72–75% [37]. Comparing amino acids between adrenoceptor subtypes in different subfamilies (i.e. alpha2 vs alpha1a, alpha1a vs beta), transmembrane amino acid identity decreases to approximately 42–45% [27, 33, 36]. Transmembrane amino acid identity between adrenoceptors and another G protein-coupled receptor system (i.e. muscarinic cholinergic receptors) is approximately 35%. Since ligand binding properties are related most closely within a given subfamily of adrenoceptors, this information suggests that the transmembrane region may be important in ligand binding.

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G protein coupling

Transmembrane receptors interact with G proteins on or near the cytoplasmic surface of the cell
ADRENOCEPTORS AS MODELS FOR G PROTEIN-COUPLED RECEPTORS

Mechanisms of desensitization

- Uncoupling
  - (Phosphorylation and β-arrestin)
- Sequestration
  - (Internalization)
- Down-regulation
  - (Receptor destruction)

Fig. 8. Three basic mechanisms of desensitization have been discovered. The first involves receptor (black rectangle)–G protein (white half-circle) uncoupling. This includes receptor phosphorylation (triangles) and activation of other proteins such as beta arrestin (oval) which prevent receptor interaction with the G protein. The second mechanism is receptor internalization, or sequestration, and involves mobilization of the receptor to intracellular vesicles. Sequestered receptor can be recycled back to the surface once agonist stimulation terminates. The third mechanism of desensitization is receptor down-regulation, a process where sequestered receptors are destroyed (see text for details).

Regulation of adrenoceptors

Regulation of adrenoceptors becomes important when considering disease states involving the adrenergic nervous system, such as hypertension, congestive heart failure and acute tolerance to catecholamine drug therapy. One of the most common and intensively studied methods of regulating adrenoceptors is desensitization. Desensitization may be defined as a general biological phenomenon characterized by waning of a physiological response over time, despite the presence of a constant stimulus. It is important to remember that desensitization is therefore a functional definition describing a physiological end-point and does not imply in itself a specific mechanism. Desensitization may be thought of as an important response required to dampen extremes of physiology. For example, stimulation with adrenaline may increase heart rate significantly. Rapid heart rate is not well tolerated in the long term; desensitization therefore provides a protective mechanism to dampen heart rate response to adrenaline, gradually returning heart rate to normal.

Two general types of desensitization have been described, homologous and heterologous [36]. Homologous desensitization occurs when only the receptor which binds the stimulating agonist is desensitized (i.e. only beta adrenoceptors are desensitized to the beta adrenoceptor agonist isoprenaline and not other receptors). In contrast, heterologous desensitization occurs when stimulation of a distinct receptor results in the production of a second messenger which ultimately causes the desensitization of other receptor systems coupled to the same second messenger pathway (i.e. stimulation of the prostaglandin PGE, receptor which causes the production of cAMP, results in both PGE, receptor and beta adrenoceptor desensitization) [36].

While desensitization is a functional definition, three distinct mechanisms have been discovered to mediate this phenomenon (fig. 8). The first involves uncoupling of the receptor from the intermediary G protein. Receptor–G protein uncoupling occurs rapidly (within minutes to hours) following agonist exposure. Receptor phosphorylation is thought to result in inhibition of effective receptor–G protein interactions. Using the beta, adrenoceptor as a model, at small agonist concentrations (nmol litre⁻¹ range), cAMP-dependent protein kinase (protein kinase A) phosphorylates a site in the initial carboxyl terminus and also in the third intracellular loop [20]. At large agonist concentrations (i.e. μmol litre⁻¹), phosphorylation occurs via protein kinase A but also by another kinase called beta adrenoceptor kinase which phosphorylates agonist occupied beta, adrenoceptors on serine and threonine residues in the carboxyl terminus [2, 3, 20]. Phosphorylation via beta adrenoceptor kinase causes another protein molecule, beta arrestin [28] to bind to the receptor and effectively inhibit receptor–G protein binding [20]. Over a decade of intense research has led to detailed understanding of receptor sites of phosphorylation, enzymes involved and timing of events during receptor uncoupling in desensitization.
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ADRENOCEPTORS AS MODELS FOR G PROTEIN-COUPLED RECEPTORS


