Mechanisms of homologous neurotransmitter receptor desensitization in the central nervous system

Stephen X. Arthur

Desensitization of neurotransmitter receptors is a reduced response to repeated agonist stimulation over time-courses ranging from msec to days, depending on the system. It has implications for neuronal homeostasis, information processing, tonic inhibition, synaptic plasticity, and epigenesis. In homologous desensitization the cell is desensitized to only one agonist, whereas in heterologous desensitization there is a diminished response to other agonists as well. Desensitization can also be modulated, that is, the rate of desensitization by one agonist can be changed by the actions of another receptor agonist or second messenger. By necessity this paper will focus on mechanisms of homologous desensitization only, with occational reference to heterologous desensitization and modulation where warranted.

Myriad examples of receptor desensitization have been found outside the CNS (Hollenberg, 1985a), such as with hormones, growth factors, and autonomic neurotransmission. Many mechanisms have been proposed, both homologous and heterologous, including receptor phosphorylation, disulphide-sulfhydral exchange, receptor proteolysis, membrane potential, microclustering, guanine-nucleotide mediated allosteric changes in receptor affinity, and changes in membrane lipids (Hollenberg, 1985b). As models for discussion of recent evidence about desensitization mechanisms in the CNS I will describe two well-studied and very different peripheral systems, the B-adrenergic receptor (B-AR) and the neuromuscular junction (NMJ).

The Beta-adrenergic model

In frog and turkey erythrocyte and in mamalian cells such as astrocytoma cell lines, desensitization with catecholamines shows a decrease in B-AR Bmax and a decrease in adenylate cyclase (AC) activity, but no decrease in AC activity initiated by other receptor agonists (Sibley et al, 1985). The most recent review of the sequential steps and mechanisms is by Fishman and Perkins (1988): (1) There is loss of agonist-induced activity without a change in Bmax, with an affinity change that is interpreted to be a functional uncoupling of B-AR from stimulatory G-protein (Gs). (2) With a decrease in Bmax, B-ARs are then sequestered into a lighter-density fraction that shows only low-affinity, GTP-insensitive binding, thus showing a physical separation from Gs, although direct morphological evidence for endocytosis is still lacking. (3) Finally, radioligands that cross the plasma membrane show an absolute decrease in receptor number, and in some cell types recovery can be blocked by blocking protein synthesis.

Desensitization correlates with phosphorylation of B-AR, and both phosphorylation and sequestering can occur in transplanted systems that lack G-proteins. Experiments with reconstituted lipid vesicles (Sibley et al, 1986) show that a cAMP-independent protein kinase is involved, phosphorylating only the agonist-bound form of B-AR after being translocated from the cytosol, suggesting that a conformational change of the receptor exposes phosphorylation sites for this B-AR kinase (BARK). Membrane-transplanted sequestered B-AR are not functionally impaired, implying de-phosphorylation in the sequestered compartment. Replacement of phosphorylation-site residues of B-AR delays desensitization (Bouvier et al, 1988). B-AR activity is reduced 80% by other kinases but only 20% by BARK alone, suggesting an additinal factor -- adding pure retinal arrestin (involved in rhodopsin phosphorylation) restores full desensitization (Benovic et al, 1987).

Extension of the B-AR homologous model includes the following observations in other systems: 1) the cloned mACh receptor has similar phosphorylation sites (Fishman & Perkins, 1988); 2) mACh desensitization in heart requires a receptor-specific protein kinase (Kwatra et al, 1987); 3) smooth muscle alpha-1 receptors coupled to IP3 show the same picture as the B-AR AC-mediated system (Leeb-Lundberg, 1987); 4) electron microscopic immunocytochemistry shows endocytosis during mACh desensitization in fibroblasts (Rapaso et al, 1987); and 5) neuron cell culture mACh desensitization shows receptor turnover and an effect on both inhibition of AC and of membrane phospholipid turnover (two pathways) (Nathanson, 1982).

The heterologous type of desensitization of AC activity has been shown in many systems, for example, prolonged application of catecholamines or prostoglandins can attenuate each other's affect on cAMP levels. Cyclic AMP desensitizes B-AR-activated AC activity (suggesting a heterologous mechanism) and involves phosphorylation by cAMP-dependent protein kinase of both the receptor and Gs, producing an uncoupling but not sequestering (Sibley, 1985). Protein kinase C stimulation also desensitizes B-AR-induced AC activity and is additive with B-AR agonist desensitization, shown to work through different mechanisms, with no decrease in Bmax or affinity (Toews et al, 1987).

The neuromuscular junction model

There are no known mechanisms of heterologous desensitization of the nicotinic receptors (nAChR) at the NMJ, although modulation of homologous desensitization has been shown (by peptides and by phosphorylation). Mechanisms of nAChR homologous desensitization and its modulation are reviewed by Ochoa et al (1988). An entirely different picture is seen from that of the B-AR: desensitization is acute rather than chronic, characterized by faster rates of both development and reversability; desensitization is due solely to an inactivation of the receptor/channel by an allosteric conformational change, with no loss of receptor; and desensitization is accompanied by an increase in receptor affinity, which is attributed to the conformational change of the receptor.

There are four types of inactivation: ultrafast (less than a msec), fast (msec to seconds, with change in conformation and affinity), slow (seconds to minutes, with change in conformation and affinity), and ultraslow (minutes to hrs). The ultraslow inactivation is a modulatory phenomenon and will not be addressed here. Receptors reconstitued into lipid bilayers still show these phases of inactivation, indicating that these actions are intrinsic to the molecule itself. There appears to be an increased affinity of agonist for the receptor in its desensitized state. A spontaneous switching to the desensitized state occurs such that even without agonist about 20% of the receptors are desensitized. Binding of only one agonist molecule can promote inactivation. During full desensitization there are sometimes observed spontaneous bursts of single channel current, representing a transient return from the desensitized state (showing the same open time as non-desensitized receptors).

A host of kinetic studies suggest a four state model where the nAChR can switch between the following states: 1) active; 2) resting, with an ultralow affinity (Kd = 100 uM); 3) intermediate, with a low affinity and rapidly desensitizing (rate constant 2-7/s, Kd = 1 uM); and 4) desensitized, with a high affinity and slowly desensitizing (rate constant .1 - .01/s, Kd = 3 nM). The usual low affinity state observed is actually the intermediate state. Desensitization happens in two ways: in the fast, intermediate state, in 100 ms to 1 s; and in the slow, desensitized state, in seconds. (The ultrafast inactivation shows up as a voltage dependence of desensitization rate due to the binding to a separate voltage-dependent ACh binding site).

Desensitization of nAChR is accellerated by Calciium (this modulation is relevant when we compare the nAChR to the glutamate receptor). Calcium increases affinity, implying a stabilization of the desensitized state. It modulates only from the cytoplasmic side, and appears to be a direct effect on the channel/receptor.

The central nervous system

Recent investigations on the mechanisms of homologous receptor desensitization in the CNS indicate that the ligand-gated ion channels conform to the NMJ model while the second-messenger mediated receptors follow the B-AR model. There are, however, important differences.

Glutamate

Focal application of glutamate receptor agonists to voltage-clamped, cultured rat hippocampal neurons reveals acute desensitization on two timescales (Trussel et al, 1987): fast (in 10 to 100 ms) and slow (seconds). This is reminiscent of the NMJ, except for one striking difference: the two forms of desensitization are found on separate receptor subtypes. The fast form occurs with non-NMDA receptors (not blocked by APV), while the slow form occurs with NMDA receptors (i.e., showing Mg-dependent rectification). "Hot spots" found only for fast desensitization suggest that the non-NMDA receptors are at synapses while NMDA receptors are more extrasynaptic. (Oddly, kainate does not produce fast

desensitization, though application of glutamate does desensitize the kainate response.)

The most important confirmation of a NMJ model for glutamate receptors would be demonstration of an increase in affinity, conformational change, and stability of Bmax, but binding studies have not yet been done. Desensitized glutamate channels do, however, show spontaneous bursts, where the mean channel open time is independent of agonist concentration (Zorumski, 1989). Additionally, voltage-clamp studies in chick spinal cord culture (Zorumski, 1989) show that the slow NMDA desensitization is accellerated by Ca. The Ca has its largest effect in zero magnesium, suggesting channel entry and action on the internal face, much like the nAChR. Studies in isolated membranes also suggest a direct action of Ca on the channel.

The NMJ cyclic state-change model implies that a fraction of desensitized receptors should exist even in the absense of agonist. Binding studies may determine whether this is true for the glutamate receptor (i.e., calculate the number of functional channels from single channel currents and compare to total binding).

GABA-A

Studies of chloride ion flux using radiolabled chloride ions in rat brain membrane vesicles (Cash & Subbarao, 1987) show a picture very much like the glutamate receptors. There are two phases of desensitization, on the order of 100 ms and of seconds. Upon removing the fast phase by GABA preincubation, the slow phase is found to be described by processes that are first order, and the authors thus conclude that the two phases are attributable to two separate receptor subtypes on the same membrane.

Nicotinic acetylcholine

There has been little direct investigation of mechanisms of homologous desensitization of neuronal nAChR, although modulation of homologous desensitization has been studied (see later). Neuronal nAChRs are composed of different subunits than those of the NMJ (reviewed by Berg et al, 1989), however they show similar permeation, kinetics, and modulation. Additionally, the number of functional receptors can be altered by membrane potential, as at the NMJ, but it is in the opposite direction (Smith et al, 1986). Depolarization (elevated external potassium) decreases agonist response without affecting conductance, mean open time, probability of opening, affinity, Bmax, or protein synthesis, thus implying an allosteric state change from active to desensitized. This reduction in functional receptors by depolarization could represent a mechanism of either homologous or heterologous desensitization, although it is not referred to as such in the papers reviewed. It may be a very significant factor since the total number of nAChRs is ten times the number of receptors normally detected electrophysiologically.

As an aside -- the issue of nicotinic "silent receptors" in brain may represent another form of heterologous desensitization. In chick ciliary ganglion in culture, cAMP increases the number of functional receptors (Berg et al, 1989; Margiotta

et al, 1987). Thus a decrease in cAMP leading to a change of channel state may be a mechanism for a kind of heterologous desensitization.

Beta-adrenergic adenylate cyclase

In-vivo rat cortex chronic (days) application of the reuptake blocker desipramine shows an early stage of desensitization that cannot be accounted for solely by decrease in Bmax, and there is no impairment of Gs or AC (Okada et al, 1986). Gpp(NH)p-induced activation of membrane AC has a time lag of a few minutes in control and drug-treated rats. This lag is shortened by addition of agonist, indicating that B-ARs are coupled to Gs in such a manner as to facilitate the exchange of added Gpp(NH)p with GTP on Gs. This effect of agonist rapidly decreased during desipramine treatment, thus supporting the B-AR model's attribution of the early phase to a functional uncoupling from Gs. Recently, desensitization has been shown in brain microdialysis of extracellular cAMP, demonstrating that homologous B-AR desensitization can now be studied directly in vivo (Stone et al, 1989).

Receptors coupled to phospholipase C

In cultured cerebellar granule cells with lithium to assay muscarinic [3H]phosphoinositide turnover (Xu & Chuang, 1987), one hour of agonist application reduced turnover but not Bmax, whereas after 18 hours desensitization was associated with a drop in Bmax, thus conforming to the B-AR model.

Recently, in the same preparation, Dillon-Carter and Chuang (1989) applied carbachol, histamine, norepinephrine, and serotonin (all with receptors known to be coupled to the phospholipase C pathway) and looked for both homologous and heterologous desensitization. Heterologous desensitization was expected because phorbol esters (mimicking DAG) reduce agonist-induced phospholipase C activity and responses to the above agonists. After 30 minutes, only homologous desensitization was found. They conclude the following: 1) desensitization to these agonists may use separate receptor-specific kinases (as in the B-AR model); 2) and/or each receptor type may use distinct pools of IP3; 3) they cannot rule out the possibility that each receptor type is on a distinct, separate cell population; 4) phorbol-ester induced desensitization may not reflect a naturally occurring process -- it is resistant to degradation whereas DAG is transient due to rapid enzymatic conversion, or phorbol esters may act directly on phospholipase C rather than on the receptor via PKC phosphorylation.

Dopamine

DA receptor desensitization has been studied in rat striatum slices (reviewed by Hanbauer & Sanna, 1986). D1 receptors activate AC via Gs, whereas D2 receptors inhibit AC via Gi. Only D1 receptors show DA desensitization of AC: D1 agonists decrease AC activity while D1 antagonists prevent desensitization; D2 agonists and antagonists have no desensitizing affect on AC inhibition. In DA desensitization, Bmax is not affected while affinity and Gs coupling efficiency is decreased, and desensitization is associated with phosphorylation by a cAMP-

dependent protein kinase. This matches the B-AR model for heterologous, not homologous, desensitization.

Hanbauer and Sanna (1986) extend this model by observing that DA desensitization involves phosphorylation of three different molecular weight proteins (a pattern identical to cholera toxin activation of AC). Although it is not clear from their evidence, they speculate that phosphorylation may include as substrates the D1 receptor, the AC regulatory subunit, or a subunit of the voltage operated calcium channel. They suggest that cAMP production leads to Ca influx and a role played by calcium-dependent kinase in desensitization (an enzyme that could also be stimulated by way of IP3-induced Ca release). The bottom line as far as this paper is concerned is that DA receptors do not seem to desensitize to DA by a homologous mechanism. However, the authors note that Gi, which is activated by D2 receptors, appears to affect the calcium mobilizing system, and this may be relevant to the D2 homologous desensitization by down-regulation of Bmax that occurs with a D2 antagonist used clinically, as follows.

Most chronic antipsychotic D2 antagonists increase D2 Bmax. Pimozide is exceptional in three ways: 1) it is especially effective on the "negative symptoms" of schizophrenia, on trigeminal neuralgia, on Tourette's syndrome, and on tardive diskinesia; 2) it decreases D2 Bmax; and 3) it is a calcium channel antagonist (Tecott et al, 1986). In their paper, Tecott et al proposed to investigate further the combined effect of D2 and calcium channel blockers on this form of homologous desensitization, but there have been no further papers on this topic by these authors and their paper has never been cited, which provokes the suspicion that this effect of pimozide could not be replicated.

A novel mechanism involved in muscarinic desensitization

Van Huizen et al (1989) have recently shown downregulation of mAChR Bmax on a timescale of one hour in living slices of rat cortex by the direct action of agents affecting potassium conductance. Depolarization by veratridine (on Na channels), high external potassium ion, picrotoxin, or glutamate all produce this desensitization. The downregulating effect of muscarinic agonists is additive with that of veratradine. Potassium channel blockers prevent veratradine- and carbachol-induced downregulation. TTX or calcium channel blockade do not attenuate the effect. This mechanism may be involved in homologous desensitization, since mAChR open potassium channels, and the ions may interact with the proposed receptor-specific kinase or with later stages of internalization and degradation.

A further implication of these experiments is that ligand-gated channels may exhibit a more chronic form of desensitization by downregulation that has not yet been examined. The authors note that in muscle myotubes nAChR downregulation is found and appears to be linked to the sodium influx (Bloch, 1986).

Modulation of homologous desensitization -- brief examples

In neuronal nAChR, phorbol esters enhance desensitization (Berg et al, 1989) (and in Torpedo, tyrosine kinase increases the fast phase only [Hopfield et al, 1988]). In rat brain, pentobarbital enhances desensitization of the slow and fast

forms of GABA-A differently (Cash & Subbarao, 1988). For rat brain slice B-AR, various peptides modulate affinity and attenuate downregulation (Nukina & LaBella, 1989). In mouse hippocampus, glycine decreases NMDA desensitization by direct binding to the receptor and by presumably promoting a conformational change (Mayer et al, 1989).

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