

4 Synaptic and Nonsynaptic Release of Transmitters

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Abstract: Nonclassical receptor functions represent revolutionary possibilities at the cellular level for some less-understood features of neural and cerebral activities. Although different forms of nonsynaptic communication often appear in different studies, their difference from synaptic actions is generally not recognized. The corner stones of interneuronal nonsynaptic communication include the release of transmitters into the extracellular space and the extrasynaptic receptors and transporters. Transmitters can be released from nonsynaptic varicosities without being coupled to frequency-coded neuronal activity and from synapses following high presynaptic activity via spillover. The released substances are able to diffuse over large distances to reach remote tissue. Extrasynaptic receptors may occur at all possible membrane surfaces in various systems. These receptors are of high affinity, providing targets for low-dose drugs in many instances of medical therapy.

List of Abbreviations: ACh, acetylcholine; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS, central nervous system; DA, dopamine; 5-HT, serotonin; GABA, gamma-aminobutyric acid; NA, noradrenaline; NMDA, *N*-methyl-D-aspartate; nAChR, nicotinic acetylcholine receptor; VTA, ventral tegmental area

1 Historical Background

Of all the cells in the body, only nerve cells are able to communicate regularly with one another. In the nineteenth century, it was believed that “nerve centers” were made up of a continuous intermediary network between the motor nerves and the sensitive and sensory nerves (Cajal, 1937). Ramon y Cajal in his experiments applied Golgi staining to discover that neurons are independent units, and not entities fused to each other. This was an important step that changed the way of thinking of many scientists. Ramon y Cajal received the Nobel Prize in 1906 for this discovery. The question arose of how neurons communicate, if they do not fuse to one another with anastomosis, forming large neural nets of an intermediary network.

Bernard demonstrated the dissociation of nerve and muscle activity by curarizing frogs (Bernard, 1857). This is regarded as one of the key experiments in the development of the concept of chemical transmission. The idea that nerves are able to release chemicals to communicate with other cells was first explicitly proposed for sympathetic nerves when Elliott, a young medical student in Cambridge, suggested in 1904, “adrenalin might... be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery.” This brilliant hypothesis was confirmed by Loewi (1921), who showed that the stimulation of sympathetic nerves in frog heart is mediated by *Acceleransstoff* (adrenaline). The observation that the action of adrenaline and sympathetic stimulation are similar (Elliot, 1905) was further supported by the finding that the action of acetylcholine (ACh) and parasympathetic stimulation are also similar (Loewi, 1922; Dale, 1956).

In spite of strong evidence suggesting otherwise, the alternative view that transmission is electrical enjoyed rather wide support during the first half of the twentieth century. This was mainly due to the fact that the electrical properties of what is now called conduction and transmission were seen to be similar (Erlanger, 1939; Gasser, 1939; Eccles, 1946). This belief gradually gained currency; a large number of neuroscientists believed that findings obtained in the neuromuscular junction were relevant not only to autonomic, but also to central synaptic transmission. Eccles in 1946 wrote “The original hypothesis was made as general as possible by applying it to the neuromuscular functions of skeletal muscle, and to the synapses of the sympathetic ganglia as well as of the central nervous system.” In fact, Eccles believed that the primary transmitter was electrical, but that chemical transmitters could be responsible only for slower and longer responses, detectable as a tail to the transmitter action on the postsynaptic site. This assumption was accepted almost universally by scientific society. Although different scientists (Dale, Feldberg, Kuffler, Uvnäs, etc.) provided rather strong evidence to support and confirm the hypothesis of chemical transmission, Eccles resisted until 1948, when he acknowledged that even the fast response is due to acetylcholine at the neuromuscular junction and accepted the idea of chemical transmission. After his Pauline conversion from electrical to chemical transmission, the community of neuroscientists generally accepted the theory of chemical transmission for example, the communication between nerves and between nerve endings and target cells is chemical. It means that chemicals are released from nerve terminals in response to electrical depolarization followed by Ca^{2+} -influx transmit messages between pre- and postsynaptic sites.

2 Communication Between Cells

2.1 Synaptic Interaction Between Cells

From Sherrington's classical work on "*Integrative action of the nervous system*," it has been generally accepted that the synapse, the "surface of separation" between neurons, is the primary site of neuronal information processing. Transmitter is released into the synaptic cleft in quantal packages. The average neuron forms about 1,000 synapses and it receives about 10,000 inputs. Since the human brain contains about 10^{11} nerve cells, it means that in the brain there are at least 10^{14} synapses for information processing.

The generally accepted form of chemical communication between nerves and between nerve endings and target cells is that the transmitter is released into the synaptic cleft in quantal packages as a result of action potentials arriving at the terminals. The transmitter acts on receptors located on the postsynaptic site and either opens or closes ion channels, thereby establishing chemical communication between pre- and postsynaptic sites. The effect of transmitters is terminated by either enzymatic degradation (e.g., in case of ACh) or by active reuptake into nerve terminals by transporters (cf. Amara and Kuhar, 1993; Raiteri et al., 2002). Nevertheless, our current knowledge of how information is conveyed chemically from one cell to another is derived from and heavily influenced by the textbook data regarding the neuromuscular junction (cf. Katz, 1969), where the transmitter is released in quanta. This system is adopted for very fast signaling; the information transfer occurs within millisecond time intervals and is able to transmit messages at a rate of several hundred impulses per second.

2.2 Nonsynaptic Interaction Between Neurons

In addition to transmitter substances acting at close range in chemical synaptic neurotransmission, chemical interaction exists and information processing occurs between neurons and between neurons and target cells without any close synaptic contact; there is a nonsynaptic communication system which operates over some distance in the extracellular spaces (cf. Vizi, 1974, 1979, 1980, 1984, 2000; Vizi et al., 1985; Agnati et al., 1986, 1995; Fuxe and Agnati, 1991; Bach-y-Rita, 1993; Vizi and Kiss, 1998). In the past few years, several neurochemical, anatomical, pharmacological, and neurophysiological observations have been made which suggest that chemical interaction between cells does not only take place across the synaptic gap between pre- and postsynaptic membranes but may also occur in the absence of such specialized contacts, i.e., nonsynaptically (🔗 [Figure 4-1](#)). Neurochemical evidence has been obtained suggesting that noradrenaline (NA) released from axon terminals, which do not make synaptic contact with cholinergic terminals in the gut (Furness and Costa, 1974; Gordon-Weeks, 1982), inhibits the release of acetylcholine from cholinergic varicosities of the Auerbach plexus (Vizi, 1968; Paton and Vizi, 1969; Knoll and Vizi, 1970; Vizi and Knoll, 1971). A very similar observation was first made in the cerebral cortex (Vizi, 1974, 1979, 1980) where the majority of noradrenergic varicosities do not make synaptic contacts (Descarries et al., 1977).

This new concept of information processing, now known as nonsynaptic chemical transmission (Vizi, 1980, 1984), has been shown to be a rule rather than an exemption in the CNS (central nervous system) and has gained widespread acceptance (since 1986, it has also been called volume transmission, Agnati et al., 1986; Fuxe and Agnati, 1991; Agnati et al., 1995, paracrine release, spillover, nonconventional release, etc). Compelling neurochemical, functional, and pharmacological evidence (cf. Vizi, 2000) has accumulated suggesting that transmitters released from axon terminals are able to diffuse far away from the release site and have an effect on receptors located nonsynaptically.

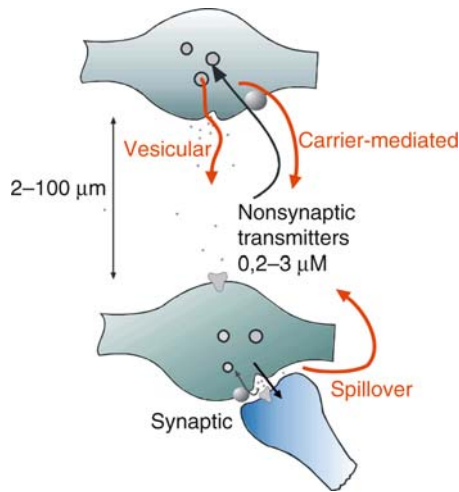
3 Release of Transmitters

3.1 Nonsynaptic Release of Transmitter

The idea that transmitters can be released from nonsynaptic areas was first suggested for transmitters in the autonomic nervous system, where the axon terminals rarely make synaptic contact with the target cells.

■ Figure 4-1

Nonsynaptic chemical transmission. A proportional diagram of two synaptic clefts: a typical synapse with a gap of 20 nm and a free axon terminal with its remote target cell (e.g., the vegetative nervous system). Note the difference in volume. Let us assume that both transmission sites are cholinergic. The average diameter of a vesicle containing acetylcholine (ACh) is 50 nm, therefore its volume is about $65,000 \text{ nm}^3$ and its ACh concentration is about 0.1 M (cf. Marchbanks, 1979). If we assume that its content is completely discharged into a small synaptic cleft whose volume is about $200,000 \text{ nm}^3$ ($20 \times 100 \times 100 \text{ nm}$), the final concentration of ACh in the cleft is 30 mM ($0.1 / (200,000 / 65,000)$), which is an extremely high concentration. Let us suppose that, for example, in Auerbach's plexus, where the target smooth muscle cell is far from the varicose axon terminals (100–1,000 nm), only one vesicle is released. The volume in which the ACh released is 10^9 nm^3 ($1,000 \times 1,000 \times 1,000$), i.e., a volume 10^4 times larger than that of a vesicle. Therefore, the ACh released from the vesicle is diluted by a factor of 10,000. If the cholinesterase is not active, the final concentration of ACh which reaches the muscarinic receptors of the smooth muscle is about 10^{-4} M . In this calculation, the cytoplasmic release has not been taken into account. Note the concentration of ACh in the cleft



Since then, a large body of evidence has shown that transmitters/modulators can be released from regions other than the nerve-ending.

Electron microscope and histochemical studies of the relationship between nerve terminals and target cells have shown that there are wide varieties of normal distances. The minimum width of the cleft between nerve varicosities and effector cells varies considerably in different tissues. In the vas deferens and sphincter pupillae, the separation is about 15–20 nm. In blood vessels, the smallest space between varicosities in the perivascular plexus at the advential-medial border and smooth muscle cells varies from about 50 nm to 2 μm (small muscular arteries, large arterioles, large elastic arteries) (see Burnstock, 1979). In these cases, no postjunctional specializations have been found with any consistency for wider neuromuscular junctions.

Release from axonal varicosities devoid of synaptic membrane specialization has recently been suggested to be the function of the central monoamine terminals (Descarries et al., 1977; Beaudet and Descarries, 1978). Beaudet and Descarries (1978) claimed that the release of biogenic amines solely from varicosities making synaptic contact could hardly account for the total amount released from axon terminals. As the number of locus coeruleus cells in the rat is only about 1,400 (Descarries and Saucier, 1972) and the density of noradrenergic varicosities is 2 million/mm³, each cell body has an average of about 140,000 varicosities in the hippocampus. It means that the excitatory inputs to a noradrenergic cell body might activate a neuron whose transmitter, noradrenaline, released from varicosities might control a rather large field. It also means that a transmitter or modulator released from nonsynaptic varicosities could affect very large neuronal assemblies.

There is an interesting difference in the localization of receptors on effector cells where the transmitter is released into a small synaptic gap compared with those where it is released into a large extraneuronal space. In the former case, there is a small area of the cell where the receptors are concentrated. At the neuromuscular junction, for example the extrasynaptic area is relatively insensitive to the transmitter, because only a few receptors are there. However, when the transmitter release site and the target cells (e.g., Auerbach's plexus varicose axon terminals and smooth muscle cells) are widely separated from each other (100–1,000 nm), there is no specific subsynaptic arrangement, and the receptors are evenly distributed along the whole surface of the smooth muscle cell. This morphological arrangement accommodates any type of diffusion-mediated transmission, where the advantages of quantal release cannot be used. In the CNS, there are large amount of receptors located extrasynaptically and they are of high affinity.

Even the cholinergic neurons fail to make synaptic contact in the hippocampus. Jones and Wonnacott (2004) provided evidence that in the ventral tegmental area (VTA), 27% of presynaptic α_7 nAChRs (nicotinic acetylcholine receptors) are located extrasynaptically. The absence of axo-axonic synapses (Descarries et al., 1997), i.e., direct cholinergic synaptic input to presynaptic α_7 nAChRs, indicates that these receptors are likely to be activated by choline or ACh released from cholinergic varicosities (boutons) that are far away. Electron microscopy studies revealed that cholinergic varicosities in the hippocampal CA1 region are largely (93%) nonsynaptic compared with another transmitter system (Umbriaco et al., 1995), e.g., GABAergic and glutamatergic neurons, which make exclusively synaptic contacts.

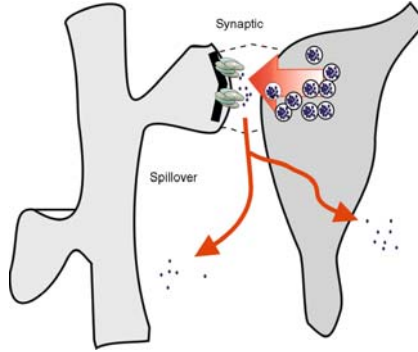
The nonsynaptic control of chemical neurotransmission by different modulators released from axonal varicosities lacking junctions might play a physiological role both in the CNS and in the neurovegetative system in shaping emotion, behavior, or learning processes, or in controlling the balance between the parasympathetic and the sympathetic nervous systems.

3.2 Spillover of Transmitters

Though glutamate is the major excitatory transmitter, GABA is the most important inhibitory transmitter in the brain and spinal cord. Both glutamatergic and GABAergic terminals make exclusively synaptic contacts with other neurons. In this respect, they are different from monoaminergic nerve terminals which in the majority do not make synaptic contact (cf. Vizi, 2000). In recent years, it has become increasingly clear that receptors sensitive to glutamate and GABA besides their subsynaptic localization, they are also expressed extrasynaptically. The question that arises is where glutamate and GABA come from to signal these extrasynaptic receptors if they are only released into the synaptic cleft. The plausibility of the spillover (▶ [Figure 4-2](#)) depends on how much glutamate or GABA is released and how easily it can diffuse out of the synaptic cleft and how transporters terminate it to diffuse away. Glutamate, for example, the major excitatory transmitter of the brain, participates mainly in synaptic interactions between glutamatergic release sites predominantly located within synapses (Umbriaco et al., 1995) and AMPA and NMDA (*N*-methyl-*D*-aspartate) receptors. Some synaptic spillover of glutamate has been observed and its effect on extrasynaptic NMDA (but not on AMPA) receptors was shown (Asztely et al., 1997; Kullmann and Asztely, 1998; Semyanov and Kullmann, 2000). The probability of spillover depends on how much glutamate is released, how easily it can diffuse out of the synaptic cleft, and how intra- and extrasynaptic transporters terminate it to diffuse away. The diffusion of glutamate away from synapses is therefore very limited because of effective neuronal and glial uptake processes. As far as the functional role of spillover is concerned, it has been shown (Mitchell and Silver, 2000) that spillover of glutamate released from excitatory mossy fibers is able to inhibit GABA release from neighboring Golgi cell terminals by activating presynaptic mGluRs. This heteroreceptor-mediated inhibition of inhibitory fibers, in fact, boosts the efficacy of excitatory fibers. Kaneda and colleagues (1995) showed in cerebellar granule cells using voltage-clamp experiments that in response to application of GABA_A receptor antagonists there is a reduction in the “holding current.” A similar observation was made by Nusser and Mody (2002) on granule cells of the dentate gyrus. The inhibitory role of ambient GABA concentration was also shown (Semyanov et al., 2003, 2004) in hippocampal interneurons of CA1 region of the hippocampus, but interestingly not in adult pyramidal cells of hippocampus (Demarque et al., 2002). Nevertheless, there is a significant tonic GABA_A

Figure 4-2

Spillover of synaptic transmitters in the central nervous system. In this scheme, a presynaptic axon terminal (dark gray) makes a synapse on a dendritic spine (light gray). Transmitters (black dots) in the vesicles are released into the synaptic cleft where it reaches the synaptic receptors (pentameric structures in this case). Arrows indicate the spillover of the released transmitters from the synapse



receptor mediated current in pyramidal cells when the GABA uptake is inhibited (Bai et al., 2001). During development, the tonic activation of GABA_A receptors is induced by GABA released in a $[Ca^{2+}]_o$ -dependent way, but in adult rats the tonic activation of GABA_A receptors is produced by nonvesicular transmitter release (Rossi et al., 2003). This fact indicates that high-affinity GABA_A receptors are activated by extrasynaptic ambient GABA concentrations (Figure 4-2).

4 What Influences the Concentration of Transmitter in the Extracellular Space?

4.1 Amount of Transmitter Released

The amount of transmitter released is determined by the amplitude of the depolarization of the nerve terminals, which, in turn, is determined by the number and frequency of the action potentials in the axons. Transmitters are released in the form of packets, in quanta, that correspond to synaptic vesicles. There seems to be no evidence that quantal release can occur solely at varicosities with synaptic contact. In the varicosities of nerves, transmitter release occurs intermittently following the stimulation of the parent axon (Cunnane and Stjärne, 1982; Blakely et al., 1986) and is facilitated by high-frequency train stimulation (Cunnane and Stjärne, 1984). There is a low probability of release in any varicosity invaded by a nerve action potential (Cunnane and Stjärne, 1982). The intermittency might be due to a failure of conduction of nerve action potentials within the varicose terminals (Stjärne, 1978; Morita and North, 1981) so that large parts of the distal region of arborization could be intermittently excluded from transmitter secretion.

In places where the gap is large, where there is no synaptic specialization, or where the transmitter must cross distances of micrometers to reach the target cell, the transmitter released from the cytoplasm also plays a critical role in chemical transmission (Vizi et al., 1982). In fact, there is a large body of available evidence showing that the release of transmitter of cytoplasmic origin is also involved (carrier-mediated release).

4.2 Uptake of Transmitters by Plasma Membrane Transporters

Once a transmitter is released into extrasynaptic space, its effect on receptors is terminated by its reuptake into the surrounding nerve terminals and glia, a process mediated by plasma membrane transporters. These nonsynaptic transporters also terminate the overspill of the synaptically released transmitter, and thereby they play an important role in influencing the concentration of transmitters in the extraneuronal space. Monoamine uptake carriers belong to the family of Na^+/Cl^- -dependent membrane transporters containing

12 transmembrane domains (Amara and Arriza, 1993). The cloning and sequencing of monoamine transporters in the early 1990s (Blakely et al., 1991; Giros et al., 1991, 1992; Pacholczyk et al., 1991; Ramamoorthy et al., 1993) revealed that these proteins show a very high degree of structural homology. Extrasynaptic glutamate spillover was shown in the hippocampus (Asztely et al., 1997). It was also shown that glutamate transporters play a critical role in terminating the nonsynaptic diffusion of glutamate, thereby limiting cross talk between neighboring excitatory synapses. The activity of transporters is temperature-dependent (Amara and Arriza, 1993).

Voltammetry and microdialysis techniques provided temporally resolved information concerning the concentration of transmitters in the extrasynaptic space. It turns out that in clinical practice, the chemicals are able to reach 0.1–23 μM in the extraneuronal space of the brain (🔗 [Table 4-1](#)).

■ **Table 4-1**

A few examples of presynaptic autoreceptors and heteroreceptors able to inhibit or facilitate the release of neurotransmitters

Neurotransmitter	Inhibitory autoreceptor	Facilitatory autoreceptor	Inhibitory heteroreceptors	Facilitatory heteroreceptors
Acetylcholine	M ₂	nAChR	α_2 , D ₂ /D ₃ , 5-HT _{1B}	NMDA, nAChR
Noradrenaline	$\alpha_{2A/D}$	β_2	Opiate, H ₃ , M ₂ , D ₂ , PGE ₂ , GABA _B	Angiotensin II, nAChR, NMDA, GABA _A , P2 _{x7}
Dopamine	D ₂ /D ₃	–	M ₂	nAChR, NMDA
5-HT	5-HT _{1D}	5-HT	α_2	–
GABA	GABA _B	–	GABA _B , CB ₁ , M ₂	–
Glutamate	Metabotropic, CB ₁	–	–	nAChR

Abbreviations: M₂, muscarinic acetylcholine receptor; nAChR, nicotinic acetylcholine receptor; NMDA, *N*-methyl-*D*-aspartate; PG, Prostaglandin. For literature, see Vizi and Kiss (1998), Starke (2001), Göbel et al. (2000)

The high degree of homology may explain the accumulating observations that functional segregation of monoaminergic pathways is not as marked as it was assumed previously. Accumulating evidence indicates the promiscuity of nonsynaptically located monoamine transporters. Using selective uptake blockers and specific pathway lesions, it was proved that (i) [³H]DA could be taken up by noradrenergic and serotonergic neurons (Descarries et al., 1987), (ii) dopaminergic terminals take up and release [³H]5-HT in the striatum, (iii) serotonergic varicosities take up and release [³H]DA in the hippocampus of rabbit (Feuerstein et al., 1986), (iv) serotonergic transporters take up NA, and (v) serotonergic varicosities can release NA (Vizi et al., 2004). These findings may provide a better understanding of the functional properties of monoaminergic systems and the mechanism of action of antidepressant drugs.

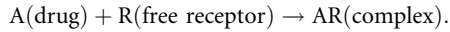
4.3 Volume of Extracellular Space

It has been shown that all the transmitters in the CNS are present in the extracellular space, which is about 12–25% of the brain volume (Nicholson, 1985). This space has been called “communication channel” (Nicholson et al., 1979), because the migration of chemical signals by diffusion plays a very important role in nonsynaptic transmission. Tortuosity and volume fraction of the extracellular space modifies the diffusion (Nicholson, 2005).

4.4 Effect of Drugs on Targets Located Intrasynaptically and Extrasynaptically: Law of Mass Action

According to pharmacological textbooks, most drugs produce their effects by binding to protein molecules (receptors, transporter molecules, enzymes, and ion channels). It is generally accepted that the magnitude of the biological response produced by an endogenous ligand is related to the number of receptors (target proteins)

occupied (Rang, 2006); the receptor can bind only one drug molecule at a time. The first step in the action of drugs on target proteins (receptors) is the formation of a reversible drug-target protein (receptor) complex.



This step is governed by the Law of Mass Action; therefore, the actual concentrations of agonist (endogenous ligand, e.g., noradrenaline) and antagonist (e.g., drug applied) and the affinity of target proteins (receptors) play very important roles in the effect. Suppose that the intrasynaptic concentration of transmitter released into the synaptic cleft is between 1 and 10 mM, and that the orally administered drug (e.g., 10 mg/70 kg) can reach a concentration of about 0.05–5 μM in the extracellular space (Table 4-2).

Table 4-2

Concentration of drugs in the extracellular space

Drug	Concentration (μM)		References
	Plasma	Cerebrospinal fluid	
Nicotine	0.4–4.5		Zevin et al. (1998)
Imipramine	1–2		Besret et al. (1996)
Citalopram	1		Hyttel (1982)
Desmethyylimipramine	2	0.1	Muscettola et al. (1978)
Fluoxetine	1		Pato et al. (1991)

It is expected that a similar concentration would be found in the synapse. Therefore, taking into account the Law of Mass Action, the drug effect is marginal, if any. Therefore, the site of action of most drugs is those binding proteins (receptors, transporters, ion channels, and enzymes), which are located nonsynaptically, i.e., outside the synapse, in the extracellular space where the receptors and transporters are of high affinity (Vizi, 2000).

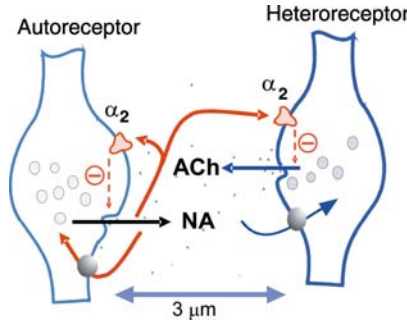
5 Conclusions

The nonsynaptic control of chemical neurotransmission by different modulators released from axonal varicosities lacking junctions might play a physiological role both in the CNS and in the neurovegetative system in shaping emotion, behavior, or learning processes, or in controlling the balance between the parasympathetic and sympathetic nervous system.

Are the receptors located outside the postsynaptic density of the synapse functionally a part of chemical transmission, or are they promiscuous and accessible to chemicals released from different boutons with (in case of spillover) and without synaptic arrangements? The answer is yes. Presynaptic release modulating receptors represent suitable targets for pharmacological intervention by exogenous compounds acting as agonists, partial agonists, or antagonists. It is thus possible that presynaptic release modulating autoreceptors and heteroreceptors (Figure 4-3) of high affinity may become the target of action for a new generation of drugs which can produce the desired therapeutic actions through the modulation or fine tuning of the release of neurotransmitters or cotransmitters. This novel mechanism differs from the well-established approach of using agonists or antagonists to directly stimulate or block postsynaptic receptors. An important way to control the activation of nonsynaptic receptors is to regulate the levels of transmitters in the extracellular space. Plasma membrane transporters play a very important role in terminating the levels of the transmitters released into the extraneuronal space. Clinically applied antidepressants reaching concentrations of 0.5–13 μM in the extraneuronal space may exert their effects on high-affinity nonsynaptic transporters (Vizi, 2000). Because of the major impact of tonic inhibition or stimulation by endogenous transmitters on neuronal activity, this form of influence, besides its physiological importance, could be an

■ **Figure 4-3**

Role of autoreceptors and heteroreceptors in modulation of transmitter release evoked by neuronal activity. This type of release is $[Ca^{2+}]$ -dependent. Heteroreceptor is sensitive to a transmitter which is not produced by the neuron on which the receptor is expressed. Autoreceptors are the receptors sensitive to the neurons' own transmitter substance. Scheme shows an example: noradrenaline (NA) inhibits its own release via the stimulation of presynaptic α_2 -autoreceptors. Noradrenaline inhibits the release of acetylcholine (ACh) via the activation of presynaptic α_2 -heteroreceptors. The effect of NA is terminated by its reuptake



important novel pharmacological target for the treatment of a wide range of disorders. Nonsynaptic and high-affinity GABA_A receptors responsible for the tonic inhibitory conductance may be of clinical importance as targets for anesthetics and sedative drugs (Bai et al., 2001). It seems very likely that GABA and glutamate receptors need not be restricted to synapses to serve physiological functions.

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