Nitric oxide: a novel link between synaptic and nonsynaptic transmission

János P. Kiss and E. Sylvester Vizi

Accumulating evidence indicates that nitric oxide (NO) inhibits the function of monoamine transporters. Because the production of NO by neuronal NO synthase (nNOS) is closely related to the activation of NMDA receptors, the level of NO around nNOS-containing synapses reflects the activity of glutamate-mediated neurotransmission. Glutamate participates mainly in synaptic interactions, but with the help of NO, the strength of excitatory input might be nonsynaptically signaled to the surrounding monoaminergic neurons, which can adapt to the changes without receiving glutamatergic input and without synthesizing glutamate receptors. Thus, the effect of NO on transporters represents a new form of interneuronal communication, a nonsynaptic interaction without receptors.

Brain function is based primarily on chemical neurotransmission. For a long time it was believed that communication between neurons occurred exclusively via synaptic contacts. However, in the past few decades the idea has emerged that neurons can communicate without synaptic connections and that this crosstalk is of physiological importance. The inhibitory effect of NO on transporters has also been verified in functional release experiments. It has been shown that dimethylphenylpiperazinium is able to induce a carrier-mediated release of NA from rat hippocampal slices and that inhibition of neuronal nitric oxide synthase (nNOS) might potentiate this response, that is, in the absence of NO the reversed transport of NA is increased. The electrical stimulation-elicited release of DA from striatal slices is decreased by the NOS inhibitor l-nitro-arginine methyl ester (l-NAME). In vivo microdialysis experiments have shown that striatal release of DA in anesthetized rats is also decreased by

This type of interaction is qualitatively different from the previously known forms because it does not require specific receptors, although the monoaminergic neuron response to glutamate-receptor activation is very specific.

Inhibitory effect of nitric oxide on monoamine transporters

Accumulating data indicate that NO participates in the regulation of monoamine-mediated neurotransmission. Among other mechanisms, NO is able to inhibit the function of monoamine transporters. NO gas and NO generators inhibit the uptake of norepinephrine (DA), noradrenaline (NA) and 5-HT into striatal and hippocampal synaptosomes or PC12 cells. The effect is blocked by the NO scavenger hemoglobin. From kinetic analysis of data, it has been concluded that NO reversibly inhibits the monoamine uptake without affecting the recognition site of transporters.

‘...there is now convincing evidence for the existence of a completely new form of nonsynaptic communication in the CNS...’

The inhibitory effect of NO on transporters has also been verified in functional release experiments. It has been shown that dimethylphenylpiperazinium is able to induce a carrier-mediated release of NA from rat hippocampal slices and that inhibition of neuronal nitric oxide synthase (nNOS) might potentiate this response, that is, in the absence of NO the reversed transport of NA is increased. The electrical stimulation-elicited release of DA from striatal slices is decreased by the NOS inhibitor l-nitro-arginine methyl ester (l-NAME). In vivo microdialysis experiments have shown that striatal release of DA in anesthetized rats is also decreased by...
Box 1. Nonsynaptic interactions in the nervous system

Our knowledge of how information is transmitted chemically from one cell to another was heavily influenced by textbook data about the neuromuscular junction, where acetylcholine (ACh) is released in quanta. During the past 30 years, however, functional and morphological evidence has shown that some neurotransmitters might be released from both synaptic and nonsynaptic sites for diffusion to distant target cells without synaptic connections. In the gut\(^a,b\) and brain\(^c–d\), in response to activation of the noradrenergic neurons there was α-adrenergic receptor-mediated inhibition of ACh release without there being synaptic contact between noradrenergic and cholinergic terminals. These findings indicate a functional interaction (presynaptic inhibition) between neurons without morphological contacts\(^e–f\). This was supported by the fact that matches between release sites and receptors are exceptions rather than the rule\(^g\). The conclusion drawn from this ‘mismatch’ problem was that mismatches reflect an existence of high-affinity nonsynaptic receptors\(^h\) that are able to mediate ‘parasynaptic’\(^i\), volume\(^j\), paracrine\(^k\) or diffusion transmission\(^l\). Furthermore, it has been shown that the extracellular space is an important communication channel and that the nonsynaptic (or volume) transmission has a crucial role in the function of the nervous system\(^m–n\). Recent findings indicate that, in addition to monoamines, other transmitters (such as ACh; Refs \(^p,q\)) might also be involved in nonsynaptic interactions.

The nonsynaptic interactions are specialized to function on a timescale of seconds/minutes and a distance scale of hundreds of micrometers. This type of transmission assumes that the transmitter released diffuses in the extracellular space (12–25% of the total brain volume\(^o\)) and reaches its target at a distance. The conditions under which this system is efficient imply that the affinity of targeted receptors for the neurotransmitter is very high\(^p\), as the concentration of molecules decreases as a cubic function of distance\(^q–r\). The existence of receptors that have affinities in the nanomolar range agrees well with such organization. Moreover, the fact that the released transmitter diffuses in the three dimensions of space multiplies its capacity to reach target cells: the affected receptors and transporters might be located on any part (soma, dendrite, varicosity) of the surrounding neurons.

These structures, which have no synaptic arrangements, are promiscuous and accessible to chemicals released from numerous synapses or nonsynaptic boutons\(^s\), or both. In addition, the nonsynaptic receptors and transporters are certainly accessible to medicines that reach micromolar concentrations in the brain, therefore drugs applied in neuropsychiatric diseases might exert their effects via these structures after diffusion through the extracellular space\(^t\).

This nonsynaptic chemical communication system has a similar degree of selectivity to that of synaptic circuitry but has, in addition, a domain of versatility and plasticity in ‘hardwired’ circuitry. The brain is a wired instrument, but its neurons, besides cabled information processing (through synapses), are capable of communicating without synaptic contact. The nonsynaptic tonic modulation of chemical transmission might play a physiological role in the brain (by shaping emotion, behavior or learning processes), in the periphery (by controlling the balance between the sympathetic and parasympathetic nervous systems) and in neuroimmune communication (by a local fine tuning of cytokine production\(^u\), steroid secretion\(^v\) and possibly many other functions not yet discovered).

References

d Vizi, E.S. (1980) Modulation of cortical release of acetylcholine by noradrenaline released from nerves arising from the rat locus coeruleus. Neurosci. 5, 239–244
f Rothman, R.B. et al. (1984) Visualization of rat brain receptors for the neuropeptide substance P. Brain Res. 309, 47–54
o Vizi, E.S. (2000) Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the central nervous system. Pharmacol. Rev. 52, 63–90
v Vizi, E.S. et al. (1992) Catecholamines released from local adrenergic axon terminals are possibly involved in the fine tuning of steroid secretion from zona glomerulosa cells: functional and morphological evidence. J. Endocrinol. 135, 551–561

http://tins.trends.com
DA, dopamine; NA, noradrenaline.

Glutamatergic synapse, even if the amount of released monoamines is unchanged. Abbreviations: L-NAME, an effect that disappears in the presence of uptake inhibitors15, suggesting that in the absence of NO the DA uptake is more efficient.

Two important conclusions can be drawn from these data. First, the inhibitory action of NO can be revealed not only by NO donors in synaptosomal preparations but also by NO inhibitors in functional in vitro and in vivo release experiments, indicating that endogenous NO exerts a tonic inhibitory effect on transporters. Thus, the level of NO might be a physiological regulatory factor of extracellular monoamine concentration. Second, the inhibitory action is independent of the actual direction of transport, that is, it can be observed during both the normal uptake process and reverse transport.

A recent paper has clarified the mechanism of this inhibition, at least in case of the NA transporter (NET)16. Kay et al. found that the NO donor S-nitroso-penicillamine (SNAP) significantly decreased the uptake of [3H]NA into Chinese hamster ovary cells transfected with cDNA for human NET; however, a site-directed mutagenesis of the transporter protein at Cys351 (to Ser351) produced a functional NET that was resistant to the inhibitory effect of NO donors. Further experiments revealed that S-nitrosylation of Cys351 mediated the inhibitory effect of NO. Although Kay et al. reported no effect on human DA transporters (DAT), other studies have suggested the existence of a similar mechanism in case of DA uptake. The only difference is that, according to these reports, the inhibitory effect is not exerted by NO itself but rather by reactive oxygen species (ROS), mainly peroxynitrite produced from reactions of NO and other free radicals17. The most probable targets of ROS are some cysteine groups in the DAT protein18.

Close link between glutamate-mediated neurotransmission and NO production

NO is produced from L-arginine by NOS. There are three different forms of this enzyme19: the endothelial form, which is responsible for cardiovascular actions; the inducible form, found originally in macrophages and involved mainly in immunological processes; and the neuronal form (nNOS), which is located in nerve cells. Although all forms can be found in the CNS, because of the temporal and spatial properties (timing of activation, regional distribution) the specific actions on neurotransmission can be attributed primarily to NO produced by nNOS. This enzyme is constitutive and synthesized by only a few percent of neurons. The production of NO is a calmodulin-dependent process; therefore, it must be preceded by the elevation of intracellular Ca2+ concentration, [Ca2+]i (Ref. 19).

Interestingly, nNOS produces NO almost exclusively after activation of NMDA receptors20. As a wide range of receptors might mediate effects that result in increased [Ca2+]i, the close relationship between NMDA receptors and NO synthesis seems puzzling; however, clarification of the molecular organization of glutamatergic synapses provides explanation for this phenomenon. Neuronal NOS is connected to the NMDA receptors via a postsynaptic density protein (PSD95), thus the enzyme is directly exposed to the flux of Ca2+ entering the ion channel of activated NMDA receptors21. Ca2+ transients that arise from the activation of other receptors are presumably too diluted by the time they reach the vicinity of the enzyme – nNOS can therefore only be ‘switched on’ by NMDA receptors. Consequently, the level of endogenously produced NO around the NMDA receptor–nNOS-containing synapses reflects the activity of glutamate-mediated neurotransmission (Fig. 1).

NO as a nonsynaptic ‘extension’ of glutamate

Glutamate is the major excitatory transmitter of the brain. In contrast to monoamines, glutamate participates mainly in synaptic interactions, because glutamatergic sites are located predominantly within synapses, whereas the majority of monoaminergic varicosities release transmitters without synaptic contact with the extrasynaptic space22. Although some synaptic spillover of glutamate has been shown at room temperature23,24, this spillover almost completely disappears at 37°C.
owing to the very efficient neuronal and glial uptake mechanisms. These findings emphasize the crucial role of temperature in the regulation of uptake processes. Mitchell and Silver, nevertheless, observed spillover at 34–39°C, thus the in vivo significance of this phenomenon remains to be clarified. But even if spillover exists at body temperature, the diffusion of escaped glutamate is very limited because of the neuronal and glial uptake processes; therefore, under physiological conditions only a small amount of released glutamate actually leaves the synaptic cleft and might reach only those glutamate receptors located perisynaptically or in neighboring synapses.

However, because of its physicochemical properties, NO is an ideal mediator of nonsynaptic interactions. It is a highly diffusible gas that easily penetrates biological membranes. Although its half-life is only a few seconds, even during this short period it can diffuse a few hundred micrometers. Comparing this distance with the width of a synaptic cleft (20 nm) or the size of a cell body (a few micrometers), it is evident that NO produced postsynaptically by nNOS might influence the function of a large number of neurons in a sphere around the synapse. The effect of NO on monoamine uptake allows NO to signal glutamate-mediated activity to the environment through the change of inhibitory tone on transporters (Fig. 1). This effect could increase the concentration of monoamines in the extracellular space, and this rise represents the specific response of monoaminergic systems to the activation of glutamate-mediated neurotransmission. Thus, with the help of NO, glutamate might participate in long-range nonsynaptic interactions.

Significance of the effect of NO: nonsynaptic interaction without receptors

The interaction between glutamatergic and monoaminergic pathways is very important in the function of specific brain regions (for example, movement coordination in the striatum, learning and memory in the hippocampus). A common feature of monoaminergic systems is that the cell bodies are located in small subcortical nuclei, but the varicose axon arborization diffusely innervates large target areas. The release of monoamines within a brain area can be affected by glutamate in different ways. First, glutamatergic projections can form axo-axonic synapses on monoaminergic varicosities (Fig. 2a). In this case the glutamatergic synapse alters the release of monoamines at a
References
30 Vizi, E.S. et al. (2000) Role of high-affinity receptors and membrane transporters in non-synaptic communication and drug action in the central nervous system. Pharmacol. Rev. 52, 63–90
38 Calabresi, P. et al. (1996) The corticostriatal projection from synaptic plasticity to dysfunctions of the basal ganglia. Trends Neurosci. 19, 19–24