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MINI-REVIEW

Presynaptic autoreceptors

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Presynaptic axon terminals of all known neuronal families possess on their external membrane release-regulating receptors sensitive to the neuron's own transmitter and which have been termed autoreceptors (Starke *et al*. 1989). Autoreceptors coexist with release-regulating heteroreceptors, which are activated by transmitters/modulators other than the neuron's own transmitter (Vizi and Kiss 1998). Particularly well studied are the α_2 adrenergic autoreceptors, generally viewed as part of a physiological mechanism mediating feedback inhibition of noradrenaline release (Langer 1974). Different subtypes of the α_2-adrenoceptor are present on noradrenergic terminals in the peripheral and central nervous system of different laboratory animals (see the following article by Starke and references therein). It should be added that inhibition of release by α_2-autoreceptors also occurs in noradrenergic terminals of the human cerebral cortex where the autoreceptors were characterized as α_{2A} subtype (Raiteri *et al*. 1992).

Over the last two decades the above 'presynaptic autoreceptor theory' has sometimes been questioned (see the following article by Kalsner and references therein; Laduron 1998). Based on the failure to demonstrate axonal transport of α_2 receptors to synaptic boutons, Laduron (1998) challenges the very existence of α_2-adrenoceptors on presynaptic noradrenergic terminals. However, α_2-adrenoceptors do exist on noradrenergic axon terminals in the CNS, as demonstrated by a technique that, using up–down superfused thin layers of synaptosomes, permits unequivocal functional demonstration that a given receptor type or subtype is localized on a given axon terminal family (Raiteri *et al*. 1974; Raiteri and Raiteri 2000). By exploiting superfused synaptosomes, not only the presence of release-inhibiting α_2-adrenoceptors on noradrenergic terminals could be demonstrated (Mulder *et al*. 1978), but also that of muscarinic M_2 receptors on cholinergic terminals (Marchi and Raiteri 1985), of $GABA_B$ receptors on GABAergic terminals (Bonanno and Raiteri 1993), of rat 5-HT_{1B} (Maura *et al*. 1986; Göthert *et al*. 1987) and human 5-HT_{1B} (Maura *et al*. 1993; Fink *et al*. 1995; Schlicker *et al*. 1997) receptors on rat and human serotonergic terminals.

In the present issue, Kalsner clearly accepts that noradrenergic axon terminals possess α_2-adrenoceptors and that these can be activated by exogenously administered noradrenaline or α_2 agonists; however, he does not believe that a local regulation of transmitter release by autoreceptors 'is routinely operative at axon terminals', thus questioning the physiological relevance of α_2-autoreceptors as well as of other autoreceptors. I have the impression that the arguments raised by Kalsner over the last decade and, in particular, in the following article are relevant and deserve to be carefully considered.

I must immediately recognize that our superfusion technique, although often considered as the preparation of choice to localize presynaptic receptors and characterize their pharmacological profile, can be of little help in solving the controversy on the physiological significance of autoreceptors because, in this system, the endogenously released transmitters are rapidly removed from the release sites. The question needs to be approached by using nervous tissue preparations in which the transmitter released in the synaptic space can reach concentrations sufficient to activate the receptors present on presynaptic terminals. Nevertheless, it has to be stressed that no experimental conditions can obviously be physiological: therefore, the debate on the physiological relevance of autoreceptors will possibly continue for several years.

In the meantime, I would like to make a few considerations, in part related to the above controversy and, in part, aimed to point out some general aspects of receptor-mediated release regulation.

One aspect that may deserve consideration is that presynaptic autoreceptors coexist with transporters for transmitter reuptake on axon terminals and that the affinities of autoreceptors and transporters for the released transmitter are very similar. As the physiological role of transporters has not been questioned, it seems difficult to explain why the transmitter present in the synapse should selectively bind to transporters versus autoreceptors. On the other hand, one could hypothesize that autoreceptors are extrasynaptic, i.e. out of the active zone: if this is the case, autoreceptors could not regulate routinely transmitter release but they would come into play only following transmitter 'spillover'. It might be interesting to use tissues from animals lacking the

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Abbreviation used: VSCCs, voltage sensitive Ca^{2+} channels.

noradrenaline transporter and to see if α_2-adrenoceptor antagonists are able to enhance the evoked release under stimulation conditions in which the compounds are ineffective in wild type animals (i.e. single-pulse or POP stimulation).

It has been observed that agonists at presynaptic metabotropic release-inhibiting receptors, when exogenously added to superfused synaptosomes, can inhibit transmitter release only when this occurs by exocytosis and not by transporter reversal (Levi and Raiteri 1993). Furthermore, the release modulation occurs when vesicular exocytosis is 'quasi-physiological', i.e. it is consequent to mild depolarization and opening of voltage-sensitive Ca^{2+} channels (VSCCs), not when it is triggered by Ca^{2+} ionophores, which bypass VSCCs (Fassio *et al*. 1999) or by hypertonic sucrose (Rosenmund and Stevens 1996). Could such selectivity of modulation be suggestive of a physiological function of autoreceptors? Could the proposed strict relations between autoreceptors and VSCCs (thought to be present in the active zone) suggest that autoreceptors may not be extrasynaptic?

The above results are compatible with the idea that some presynaptic auto- and heteroreceptors can interact with the complex machinery of exocytosis (for a review see Parnas *et al*. 2000). Due to the multiplicity of the proteins that seem to be involved in the exocytotic process, understanding their interactions with presynaptic receptors will be a formidable task. In this context it must be stressed that the autoreceptors constitute a numerous family whose components work through quite diverse, though poorly understood, mechanisms. Although we know enough about inhibitory α_2-autoreceptors, it is clear that release-stimulating ionotropic nicotinic autoreceptors work differently. Again, the mechanisms underlying the stimulation or the inhibition of glutamate release brought about by different subtypes of metabotropic glutamate autoreceptors must of course be quite dissimilar. In light of such ignorance, one should be very cautious in extrapolating from α_2 noradrenergic autoreceptors to autoreceptors in general.

Transmitter coexistence in a same neuron is a frequent phenomenon. In general a classical transmitter coexists with one or more neuropeptides. Sometimes two classical transmitters are costored in the same neuron, for instance GABA and glycine in spinal cord interneurons that co-release both transmitters onto motoneurons. Terminals that co-release GABA and glycine possess two transporters (Raiteri *et al*. 2001). It is unknown if these terminals also possess two release-regulating autoreceptors, one for each cotransmitter and if, in the positive, activation of one autoreceptor selectively regulates the release of the corresponding transmitter or that of both.

It is well known that, in electrically stimulated slices, the potency of exogenous autoreceptor agonists decreases with increasing frequencies of stimulation. The usual (and seemingly obvious) explanation is that the exogenous agonist has to compete for the autoreceptor with increasing concentrations of endogenously released transmitter. This may not be the only reason, however: in superfused synaptosomes, where endogenously released transmitters are rapidly removed before they can accumulate in the vicinity of the autoreceptor, exogenously added agonists gradually loose their potency as the extent of depolarization increases. Why this occurs is unclear at present.

As mentioned above, α_2-adrenoceptors exist as multiple subtypes. The relative function of $\alpha_{2A/D}$- and α_{2C}-adrenoceptors as release-regulating autoreceptors remains unclear, even after the exploitation of knock-out mice (Hein *et al*. 1999; Scheibner *et al*. 2001). The use of knock-out animals may in general help studies of autoreceptors and heteroreceptors. One needs to be cautious, however, in selecting the experimental model, due to the well known compensatory mechanisms occurring in these genetically modified animals. Moreover, animals knocked-out for the receptor subtype presumed to be the terminal autoreceptor, also lack the same receptors sited elsewhere, including other axon terminal families. As several transmitters are released when a slice is stimulated electrically, the effects of knocking out all these regulatory receptors are unpredictable. The use of superfused synaptosomes may be particularly useful in determining what receptor subtype among pharmacologically similar receptors is the autoreceptor candidate (L'hirondel *et al*. 1998) or if, as a compensatory process, a different subtype takes the place of the original autoreceptor in the knock-out animal.

Autoreceptors coexist with heteroreceptors on the same axon terminals. If autoreceptors are in general inhibitory, heteroreceptors can either inhibit or enhance transmitter exocytosis. A relatively unexplored aspect concerns the cross-talks between coexisting auto- and heteroreceptors or between heteroreceptors.

In the following two articles, arguments in favour (Klaus Starke) and against (Stanley Kalsner) the physiological relevance of α_2-autoreceptors are provided. Some readers will have a good opportunity to strengthen their own opinions by removing their doubts; some may become more doubtful; some may even start doubting. Personally, I feel that all presynaptic auto- and heteroreceptors, even if physiologically irrelevant, are gifts made by nature to pharmacologists.

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