Biochemical Pharmacology, Vol. 34, No. 4, pp. 467–470, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

COMMENTARY

PRESYNAPTIC HETERORECEPTORS IN REGULATION OF NEURONAL TRANSMISSION

PIERRE M. LADURON
Department of Biochemical Pharmacology, Janssen Pharmaceutica, B-2340 Beerse, Belgium

Among the numerous factors involved in the control of neurotransmitter release, the presynaptic receptors seem to play a prominent role; these receptors, located at the nerve terminals, may either activate or inhibit transmitter release; they are thus part of a positive or negative regulatory mechanism. For instance, adrenaline is believed to enhance noradrenaline release by acting on presynaptic β-adrenergic receptors in sympathetic nerve terminals [1], whereas presynaptic α_2-adrenoceptors [1, 2] and muscarinic [3] receptors are inhibitory at the same terminals. However, these two inhibitory receptors are quite different: the first one, the α_2-adrenoceptor, is an autoreceptor whereas the muscarinic one is not. An autoreceptor may be defined as a presynaptic receptor, which is located on terminals of a given neurone and is sensitive to the transmitter released by that neurone. In other words, the neurone is at once donor and recipient; it provides the signal and possesses the receptor site for the signal which can, thus, regulate its own release.

The purpose of this paper is to propose the concept of presynaptic heteroreceptors and to show to what extent the recent data on axonal transport of receptors have highlighted them; hitherto the regulation of neuronal transmission has only been proved to occur through presynaptic heteroreceptors rather than through autoreceptors.

THE CONCEPT OF PRESYNAPTIC HETERORECEPTORS

Presynaptic muscarinic receptors in sympathetic nerves are not autoreceptors because they are not sensitive to the transmitter released by those nerves, i.e. noradrenaline; the noradrenergic neurone is thus the recipient but not the donor. We propose that these receptors should be called presynaptic heteroreceptors (hetero: receptor for *another* transmitter thus originating from *another* neurone). Figure 1 illustrates schematically the concept: the neurone A provides the transmitter for presynaptic receptors of neurone B which, by its turn, is the recipient for the signal originating from neurone A and vice versa; for instance, acetylcholine from parasympathetic nerves may be considered as the signal for presynaptic muscarinic heteroreceptors of sympathetic nerves. However, this type of regulation can also occur within a single nerve trunk if it is formed from different types of neurones; in the vagus nerve which contains at least four different neuro-

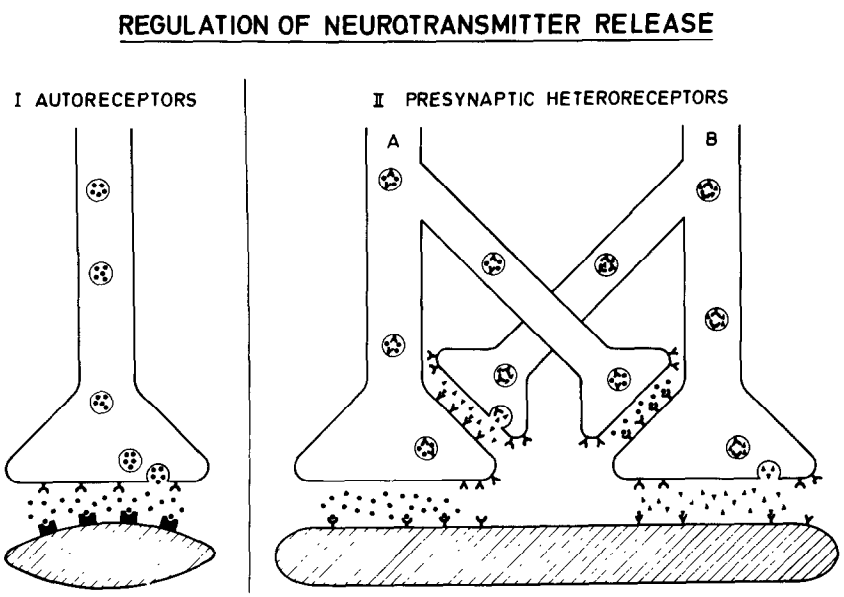

Fig. 1. Scheme illustrating the regulation of neurotransmitter release through autoreceptors and presynaptic heteroreceptors.

transmitters (acetylcholine, vasoactive intestinal polypeptide, substance P, and somatostatin) [4], presumably in separate neurones, each transmitter can regulate the release of the others through presynaptic heteroreceptors. In this model, the presynaptic heteroreceptors and the postsynaptic receptors (in Fig. 1 located on effector cells) are the same molecular entity; this is not necessarily the case for autoreceptors. The reader will realize how confusing are the terms postsynaptic and presynaptic; indeed, except for the autoreceptors, all the receptors may be considered as being postsynaptic if the localization is determined from the viewpoint of the neurone which provides the signal, thus the donor. In Fig. 1 receptors on terminals of neurone A are postsynaptic for neurone B but presynaptic when looking from neurone A. For practical reasons, the term presynaptic heteroreceptor will be adopted because it is easier to localize receptor sites on a recipient neurone than to identify the donor neurone.

Finally the concept of presynaptic heteroreceptor also postulates that, within the neurone, heteroreceptors would coexist with a neurotransmitter in the same intracellular vesicles.

Such a model represents, therefore, a more unified concept than that involving the existence of hypothetical autoreceptors.

HOW DO RECEPTORS REACH NERVE TERMINALS?

It is well known that protein synthesis exclusively occurs in the cell body or the soma of neurones; therefore, all the macromolecules present in nerve terminals must come from the cell body by means of axonal transport mechanisms [5]. The rate of axonal flow can be fast or slow depending on whether or not the macromolecules are particle-bound [6, 7]. For instance, tyrosine hydroxylase and dopa-decarboxylase which are not associated with subcellular particles move much more slowly than dopamine-β-hydroxylase which is contained within synaptic vesicles [7]. Small molecules like neurotransmitters, including peptides, also are transported anterogradely at a fast rate along the axon as has been demonstrated through nerve ligation experiments [4, 6–13]. However, a local synthesis of biogenic amines also occurs in the nerve terminals where vesicles can, therefore, be reused. By contrast, peptide vesicles have to return to the perikaryon for reloading since peptide synthesis is restricted to this neuronal compartment.

The neurotransmitter receptors, as all the intrinsic proteins, also have to be assembled in the perikaryon of the neurone. The first biochemical evidence for an axoplasmic transport of receptors was found in noradrenergic nerves [13, 14]: presynaptic muscarinic receptors accumulated on both sides of a ligature placed on dog splenic nerves, thus suggesting a bidirectional axoplasmic flow. Since then, this finding has been confirmed in different nerves and in different species (cf. Table 1). The main feature of this axonal transport is its fast rate which was found to be identical to that of noradrenaline and dopamine-β-hydroxylase [13–16]. Interestingly, the accumulation of muscarinic receptors above a ligature was linear up to 24 hr in rat sciatic nerves [15] but up to 48 hr in dog splenic nerves [16]; this as well as other experiments using double ligature have been interpreted in terms of receptor recycling [15]. This means that receptors returning from nerve terminals to the cell body can be recycled in the perikaryon and then move again anterogradely to the periphery [15]. Recently, fractionation experiments provided evidence that presynaptic muscarinic receptors in dog splenic nerves are transported in synaptic vesicles containing noradrenaline and dopamine-β-hydroxylase [16], suggesting thus a possible coexistence of receptor and neurotransmitter in the same vesicles. The presynaptic muscarinic receptor in sympathetic nerves is, thus, a heteroreceptor also because it is associated in vesicles with a neurotransmitter noradrenaline, which does not bind on this receptor site.

Opiate receptors also were found to undergo axonal flow in rat vagus nerves [21]; a large build-up of opiate receptors was found proximal to the ligature when light microscopic autoradiographic methods were used to identify the receptor sites. However, recent biochemical studies revealed a bidirectional transport of opiate receptors in the vagus using *in vitro* and *in vivo* binding techniques [22]. The *in vivo* binding consisted in injecting [^{3}H]lofentanil intravenously into rats; radioactivity accumulated mostly in the distal part of the nerve, thus below the ligature [22]. More recent experiments also showed an accumulation of labelling in the absence of ligature in the nodose ganglion 16 hr after the injection

Table 1. Evidence for axoplasmic transport of receptor sites in peripheral nerves

Receptor	Nerve	Species	Reference
Muscarinic	Splenic	Dog	13–16
	Sciatic	Rat	15, 17, 18
	Vagus	Rat	19
	Hypogastric	Cat	20
Opiate	Vagus	Rat	21–23
CCK	Vagus	Rat	24
Nicotinic	Dorsal root	Rat	25
	Sciatic	Cat	
		Monkey	
		Human	
β-Adrenergic	Sciatic	Rat	26

(unpublished results). Naloxone, vagotomy and chronic treatment with capsaicin markedly reduced the retention of [^{3}H]lofentanil in the nodose ganglion, indicating that labelling was transported from the vagus terminals to the ganglion. Interestingly, the accumulation of opiate receptors on both sides of ligatures in rat vagus nerves practically disappeared after chronic treatment with capsaicin, indicating that the receptor sites are transported in sensory neurones of the vagus namely those containing substance P [15, 23]. Here again, the axonal transport of opiate receptors occurs at a fast rate, and a possible coexistence of opiate receptors with substance P in the same vesicles might be expected.

Axonal transport was similarly demonstrated for other receptor sites like β-adrenergic [26], cholecystokinin [24] and nicotinic [25]. Hence, the origin of presynaptic receptors at the nerve terminals is quite obvious: they have to come from the cell body through axonal transport mechanisms. If the receptors are associated with a neurotransmitter in the synaptic vesicles—but this point needs further experimental evidence—one may expect that the receptor sites will become externalized during exocytosis of neurotransmitter.

PRESYNAPTIC HETERORECEPTORS RATHER THAN AUTORECEPTORS

The recent data on the axonal transport of receptors provide new insights into the role of presynaptic sites in neuronal transmission. During the last decade, numerous autoreceptors have been generated on the basis of pharmacological or biochemical experiments which were believed to be the consequence of specific effects occurring at the nerve terminals [1, 2, 27–30]. Now that the intraneuronal pathway of presynaptic receptors is demonstrated, other criteria are necessary to assess the presynaptic nature of a receptor site. This can only be proven if three criteria are fulfilled: (1) identification of the receptor in the cell body, the axon and the terminals of a given neurone; (2) axoplasmic transport of this receptor along the axon; and (3) physiological response at the nerve terminals. Up to now, this has been clearly demonstrated for heteroreceptors (cf. Table 1) but not for autoreceptors. In this respect, muscarinic and opiate receptors are certainly good examples; they were identified in the cell body and the axon, the superior coeliac ganglion and splenic nerves for muscarinic receptors [13, 14] and the nodose ganglion and vagus nerves for opiate receptors [15, 21, 22]; they were found to move anterogradely and retrogradely in axons [13–16, 21, 22]. Furthermore, chemical denervation with 6-hydroxydopamine and capsaicin led to a marked reduction of muscarinic sites in the rat spleen [15, 31] and of opiate sites in the rat vagus [23] respectively. Finally, acetylcholine is known to control noradrenaline release in the spleen [32], whereas morphine elicits presynaptic responses in the ileum [33] and the heart [34].

Such a demonstration could never have been obtained for autoreceptors not even for the presynaptic α_2-adrenoceptor (cf. Ref. 20 and unpublished results). Although the α_2-receptor site was originally proposed as a presynaptic site [35], the data reported in the literature during the last years tend to make them more and more postsynaptic or postjunctional (cf. Refs. 36 and 37). [^{3}H]Clonidine was found to label α_2-adrenoceptors on cholinergic neurones [38] but not on noradrenergic terminals; moreover, these sites did not decrease but often increased after denervation with 6-hydroxydopamine [1, 39]. Last but not least, a number of studies have seriously disputed the existence of presynaptic α_2-receptors [40–43]. As pointed out by Kalsner [43], the concept of feedback regulation of neurotransmitter release has been prematurely integrated in the corpus of accepted working hypotheses. The same process led to the hypotheses on the occurrence of dopamine [27, 29] and serotonin autoreceptors [28, 30]; here again direct evidence for such sites is still absent. There is, thus, no evidence that a neurotransmitter can regulate its own release through autoreceptors. Hence, the regulation of transmitter release essentially appears to involve presynaptic heteroreceptors. One cannot exclude the possibility that effector cells also could secrete certain substances, trophic or not, which serve as a signal for receptor sites located on nerve terminals; a good example of this is nerve growth factor (NGF) [44]. Although the present concept is only based on experiments performed in peripheral nerves, one may assume that the same also is true in the brain. Obviously neuronal transmission appears to be more and more a bidirectional process. Furthermore, presynaptic heteroreceptors might convey a drug from nerve terminals to the cell body, a fact which could explain certain long-term effects of drugs. Since the concept of heteroreceptors is a unified model—identity between pre- and postsynaptic sites—the possibility to obtain drugs which will be exclusively selective for presynaptic receptors becomes unlikely; perhaps the use of multiple target drugs would allow transmitter release to be modulated in a more appropriate manner.

REFERENCES

1. S. Z. Langer, *Pharmac. Rev.* **32**, 337 (1981).
2. K. Starke, *Rev. Physiol. Biochem. Pharmac.* **77**, 1 (1977).
3. K. Löffelholz and E. Muscholl, *Naunyn-Schmiedebergs Arch. Pharmak.* **265**, 1 (1969).
4. T. Hökfelt, O. Johansson, A. Ljungdahl, J. M. Lundberg and M. Schultzberg, *Nature, Lond.* **284**, 515 (1980).
5. B. Droz and C. P. Leblond, *J. comp. Neurol.* **121**, 325 (1963).
6. B. Grafstein and D. S. Forman, *Physiol. Rev.* **60**, 1167 (1980).
7. P. Laduron, in *Dynamics of Degeneration and Growth in Neurons* (Eds. K. Fuxe, L. Olson and Y. Zotterman), p. 245. Pergamon Press, Oxford (1974).
8. P. Laduron and F. Belpaire, *Life Sci.* **7**, 1 (1968).
9. A. Dahlström, *Phil. Trans. R. Soc.* (Ser. B), **261**, 325 (1971).
10. J. M. Lundberg, T. Hökfelt, J. Kerventer, G. Pettersson, H. Ahlman, R. Edin, A. Dahlström, S. Nilsson, L. Terenius, K. Uvnäs-Wallenstein and S. Said, *Gastroenterology* **77**, 468 (1979).
11. R. F. T. Gilbert, P. C. Emson, J. Fahrenkrug, C. M. Lee, E. Periman and J. Wass, *J. Neurochem.* **34**, 108 (1980).

12. R. Gamse, F. Lembeck and A. C. Cuello, *Naunyn-Schmiedeberg's Archs Pharmac.* **306**, 37 (1979).
13. P. Laduron, *Archs int. Pharmacodyn. Thér.* **244**, 338 (1980).
14. P. Laduron, *Nature, Lond.* **286**, 287 (1980).
15. P. M. Laduron, *Biochem. Pharmac.*, **33**, 897 (1984).
16. P. M. Laduron, *Fedn Eur. Biochem. Soc. Lett.* **165**, 128 (1984).
17. J. K. Wamsley, M. A. Zarbin and M. J. Kuhar, *Brain Res.* **217**, 155 (1981).
18. K. Gulya and P. Kása, *Neurochem. Int.* **6**, 123 (1984).
19. M. A. Zarbin, J. K. Wamsley and M. J. Kuhar, *J. Neurosci.* **2**, 934 (1982).
20. F. G. Alonso, V. Ceña, A. G. Garcia, S. M. Kirpekar and P. Sánchez-Garcia, *J. Physiol. Lond.* **333**, 595 (1982).
21. W. S. Young, J. K. Wamsley, M. A. Zarbin and M. J. Kuhar, *Science* **210**, 76 (1980).
22. P. M. Laduron and P. F. M. Janssen, *Life Sci.* **31**, 457 (1982).
23. P. M. Laduron, *Brain Res.* **294**, 157 (1984).
24. M. A. Zarbin, J. K. Wamsley, R. B. Innis and M. J. Kuhar, *Life Sci.* **29**, 697 (1981).
25. M. Ninkovic and S. P. Hunt, *Brain Res.* **272**, 57 (1983).
26. M. A. Zarbin, J. M. Palacios, J. K. Wamsley and M. J. Kuhar, *Molec. Pharmac.* **24**, 341 (1983).
27. G. Di Chiara, M. L. Porceddu, L. Vargiu, A. Argiolas and G. L. Gessa, *Nature, Lond.* **254**, 564 (1976).
28. F. Cerrito and M. Raiteri, *Eur. J. Pharmac.* **57**, 427 (1979).
29. S. Hjorth, A. Carlsson, H. Wikström, P. Lindberg, D. Sanchez, U. Hacksell, L.-E. Arvidsson, U. Svensson and J. L. G. Nilsson, *Life Sci.* **28**, 1225 (1981).
30. H. Gozlan, S. El Mestikawy, L. Pichat, J. Glowinski and M. Hamon, *Nature, Lond.* **305**, 140 (1983).
31. S. Yamada, H. I. Yamamura and W. R. Roeske, *Life Sci.* **31**, 1161 (1982).
32. S. M. Kirpekar, J. C. Prat, M. Puig and A. R. Wakade, *J. Physiol., Lond.* **221**, 601 (1972).
33. W. D. Paton, *Br. J. Pharmac.* **12**, 119 (1957).
34. H. W. Koster and D. W. Taylor, *Br. J. Pharmac. Chemothero.* **14**, 209 (1959).
35. S. Z. Langer, *Biochem. Pharmac.* **23**, 1793 (1974).
36. J. C. McGrath, *Biochem. Pharmac.* **31**, 467 (1982).
37. S. Z. Langer and N. B. Schepperson, *Trends pharmac. Sci.* **3**, 440 (1982).
38. T. Tanaka and K. Starke, *Naunyn-Schmiedeberg's Archs Pharmac.* **309**, 207 (1979).
39. C. Pimoule, M. S. Briley and S. Z. Langer, *Eur. J. Pharmac.* **63**, 85 (1980).
40. S. Kalsner, *Can. J. Physiol. Pharmac.* **57**, 717 (1979).
41. J. A. Angus and P. I. Korner, *Nature, Lond.* **286**, 288 (1980).
42. A. G. H. Blakeley, T. C. Cunnane and S. A. Peterson, *J. Physiol., Lond.* **325**, 92 (1982).
43. S. Kalsner, *Br. J. Pharmac.* **78**, 247 (1983).
44. H. Thoenen and M. E. Schwab, *Trends pharmac. Sci.* **1**, 74 (1979).