Role of the α-adrenoceptor in regulating noradrenaline overflow by nerve stimulation

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Commentary by

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It was during the years 1967 to 1969, while working with Marthe Vogt in Babraham, UK, that I studied the metabolism of ^{3}H-noradrenaline released by field stimulation from the isolated nictitating membrane of the cat (Langer, 1970). When I returned to Buenos Aires in 1969, I set up a small unit which was the core of what became the Instituto de Investigaciones Farmacologicas, and I continued my work on the release of NA during postganglionic sympathetic nerve stimulation using several preparations : a) the isolated nictitating membrane of the cat ; b) guinea-pig isolated atria with the accelerans nerves ; c) cat isolated perfused spleen and d) cat perfused hearts with with their accelerans nerves. The aim of the project was carefully to determine full dose-response curves (as I had learned from Ulli Trendelenburg during by postdoctoral years at Harvard) for several key compounds like cocaine, phenoxybenzamine (PBZ) and phentolamine, on NA release elicited by sympathetic nerve stimulation at different frequences of stimulation.

We examined in the isolated nictitating membrane of the cat the concentration effect relationships of PBZ on neuronal and extraneuronal uptake of ^{3}H-NA and on responses and ^{3}H-NA overflow elicited by postganglionic nerve stimulation. To our surprise, we found that PBZ enhanced greatly the overflow of ^{3}H-NA at concentrations which did not inhibit neuronal or extraneuronal uptake of ^{3}H-NA. This unexpected dissociation focussed our attention on the α-adrenoceptor blocking properties of PBZ. Already 15 years earlier, Brown and Gillespie (1957) reported for the cat perfused spleen the marked increase in NA release elicited by nerve stimulation in the presence of PBZ. These authors attributed this effect to the blockade by PBZ of the postsynaptic α-adrenoceptors which mediate the response of the effector organ, suggesting a transsynaptic regulation of NA release. However our results in the cat perfused heart and in guinea-pig atria (where the postsynaptic responses to released NA are mediated by β-adrenoceptors) showed that PBZ and phentolamine still produced a marked increase in NA release during sympathetic nerve stimulation in concentrations which did not inhibit the neuronal or extraneuronal uptake of NA.

In trying to provide a rational interpretation of our puzzling results it became clear to us that we were dealing with α-adrenoceptors modulating the release of NA but located on the membrane of the noradrenergic nerve terminals. These presynaptic inhibitory autoreceptors were acted upon by the released transmitter to modulate its own release. According to the hypothesis proposed in our article, NA released by nerve stimulation, in addition to stimulating postsynaptic receptors mediating the response of the effector organ, activates presynaptic receptors, located on the nerve terminal, which regulate the release of the neurotransmitter through a negative feed-back mechanism. In support of this view we subsequently demonstrated that α-adrenoceptor agonists like clonidine and NA inhibited the release of ^{3}H-NA during nerve stimulation and that this inhibitory effect was blocked by α-adrenoceptor antagonists. In addition, α-adrenoceptor antagonists on their own enhanced the nerve stimulation evoked release of NA, indicating that this negative feed-back regulatory mechanism was operational and physiologically relevant under conditions of transmitter release.

From the scientific isolation of our research lab-

oratories in Buenos Aires in 1969-1970, we feared that an hypothesis that proposed the existence of adrenoceptors on noradrenergic nerve terminals was perhaps too speculative. We hesitated to submit for publication such conclusions which contradicted the well established view that the function of the nerve terminals was restricted to the synthesis, storage, release and inactivation of the neurotransmitter. My peers at the University and my superiors at the National Research Council in Argentina were most disbelieving and my job was put into real jeopardy because I was focussing my resources in working on *pre-synaptic* receptors rather than the well-established *postsynaptic* receptors. There were no faxes then and the telephone was much too expensive from there, but Ulli Trendelenburg encouraged me to build up solid data in his weekly letters wrapped up in oxygen from the upper spheres of European and American science.

With our results using α-adrenoceptor agonists and antagonists on NA release in the guinea-pig atria and in the cat perfused heart (where the postsynaptic receptors are of the β-subtype) we were reassured and summoned up the courage to submit our article to the British Journal of Pharmacology on September 1st, 1971. We mentioned these unpublished data in guinea-pig atria and cat perfused heart in the Discussion of our 1972 paper in Brit. J. Pharmacol. For the anecdote, my continuity on the job in Buenos Aires during 1972 was saved thanks to an international chain of prestigious letters of support that Ulli Trendelenburg engineered just in time (and not by this publication). For all this, and much more, I will always be indebted to him.

Subsequent work with PBZ in the cat perfused spleen demonstrated that this α-adrenoceptor antagonist was 100 times more potent in blocking postsynaptic α-receptors than in blocking the presynaptic receptors that regulate NA release (Dubocovich and Langer, 1974). The proposal to subdivide α-adrenoceptors into α_1- and α_2-subtypes was made (Langer, 1974) and subsequently confirmed by the work from various laboratories.

This article published in 1972 in Brit. J. Pharmacol. was our first full length publication on the existence of presynaptic terminal autoreceptors that regulate the release of NA. These presynaptic inhibitory autoreceptors were shown to be of the α_2-subtype. This negative feed-back loop introduces terminal regulation into the neural secretory event and it is now described for other neurotransmitters like dopamine (D_2/D_3), acetylcholine (M_2), GABA ($GABA_B$), histamine (H_3) and serotonin ($5\text{-}HT_{1D}$ in man and $5\text{-}HT_{1B}$ in rodents).

Presynaptic release-modulating autoreceptors represent suitable targets for pharmacological intervention by exogenous compounds acting as agonists, partial agonists or antagonists. One such example is mirtazepine, an antidepressant which blocks presynaptic α_2-adrenoceptors and enhances the release of NA in the brain (de Boer *et. al.*, 1996).

In summary this article, published in 1972 provided the early experimental evidence for the now well-established concept that neurotransmitters can regulate their own release through a negative feed-back mechanism mediated by terminal presynaptic autoreceptors. According to this concept, in addition to the classical effects of neurotransmitters on postsynaptic receptors, the concentration of the released transmitter in the synaptic cleft can further regulate the release of neurotransmitter and represents a mechanism for the fine tuning of the amplitude of the chemical signal originating from the nerve ending.

As requested by the rules of the Journal at the time, the order of the authors in our article was alphabetical. Dr. Maria A. Enero died a few years ago at a rather young age. Drs R.P. Rothlin and F.J.E. Stefano live in Buenos Aires, where they are professors of pharmacology at the School of Medicine, University of Buenos Aires.

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