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COMMENTARY

PRESYNAPTIC REGULATION OF CATECHOLAMINE RELEASE

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ALTHOUGH it is well established that norepinephrine is the neurotransmitter released at adrenergic nerve endings in the peripheral nervous system, the mechanisms involved in the regulation of the amount of transmitter released upon arrival of nerve impulses have been studied only in recent years.

When dealing with experimental evidence concerning norepinephrine release elicited by nerve stimulation, it is important to distinguish *transmitter release* from *transmitter overflow*. As shown in Fig. 1A, release refers to the actual output of transmitter elicited by nerve stimulation. Transmitter overflow, on the other hand, refers

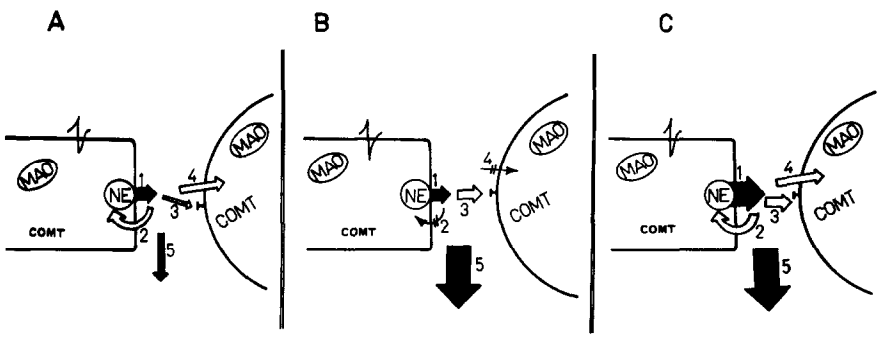

FIG. 1. Schematic representation of transmitter release and transmitter overflow during nerve stimulation. (A) Normal; (B) increase in overflow due to inhibition of sites of loss, no change in transmitter release; (C) increase in overflow due to an actual increase in transmitter release, sites of loss unaffected. 1, Total amount of transmitter released by nerve stimulation; 2, norepinephrine recaptured by neuronal uptake, subsequently deaminated or stored in the vesicles; 3, fraction of the transmitter released available for activation of the receptors of the effector organ; 4, norepinephrine taken up at extraneuronal sites, subsequently metabolized mostly by COMT; 5, overflow: norepinephrine collected during and after the period of nerve stimulation. NE, norepinephrine; MAO, monoamine oxidase; COMT, catechol-*o*-methyltransferase.

to the norepinephrine increase above the resting levels, which is collected in the venous effluent of a perfused organ or in the bathing solution surrounding an isolated organ preparation during and after the period of nerve stimulation. As shown in Fig. 1A, the main sites of loss for norepinephrine released by stimulation are: (a) recapture of the released transmitter through neuronal uptake; (b) extraneuronal uptake of norepinephrine; (c) the metabolizing enzymes, monoamine oxidase and catechol-*o*-methyltransferase; and (d) receptors and other binding sites.

Under normal conditions (i.e. when neither the sites of loss nor the metabolizing enzymes are inhibited), transmitter overflow is an underestimate of the total amount of norepinephrine released by nerve stimulation (Fig. 1A). When one or several of these sites of loss are inhibited, an increase in norepinephrine overflow will be obtained, which is not due to changes in transmitter release (Fig. 1B). Under these conditions, the increase in norepinephrine overflow is only due to the blockade of one or more sites of loss.

An increase in transmitter overflow can also be obtained when the release of norepinephrine is enhanced, even if the sites of loss are not inhibited (Fig. 1C). As will be discussed below, an increase in release can be elicited by drugs interfering with the mechanisms that regulate transmitter output.

Since the first report by Brown and Gillespie,[1] a large number of publications have confirmed that alpha-receptor blocking agents, like phenoxybenzamine or phentolamine, increase the overflow of norepinephrine elicited by nerve stimulation. Initially these findings were interpreted as indicating that the alpha-receptor of the effector organ was an important site of loss for the released transmitter. According to this view, when the alpha-receptors of the effector cell were occupied by the blocking agent, the released transmitter would not be able to combine with the receptors, and thus the overflow would increase without changes in transmitter release. This view was challenged by several authors because phenoxybenzamine, in addition to blocking the alpha-receptors, was a potent inhibitor of neuronal uptake of norepinephrine. Thoenen *et al.*[2] concluded that the increase in transmitter overflow elicited by phenoxybenzamine was unrelated to the ability of this drug to block the alpha-receptors and that it was due to inhibition of neuronal reuptake of the released transmitter. Yet, the puzzling finding was that, when a similar degree or even maximal inhibition of neuronal uptake of norepinephrine was obtained with agents which do not block the alpha-receptors (cocaine or desipramine), little or no increase in transmitter overflow was observed during nerve stimulation.[3–6]

In addition to the neuronal uptake process for norepinephrine ($uptake_1$), a second uptake mechanism for the neurotransmitter was described by Iversen.[7] This extraneuronal uptake mechanism ($uptake_2$) represents the access of the transmitter to the postsynaptic metabolizing enzymes and it is inhibited by phenoxybenzamine.[8–10]

In studies on transmitter overflow carried out with ^{3}H-norepinephrine, it was discovered that under control conditions a significant fraction of the transmitter released by nerve stimulation is collected as metabolites.[11–14] Since phenoxybenzamine prevented the metabolism of ^{3}H-norepinephrine released during nerve stimulation, inhibition of extraneuronal uptake by this drug was postulated as another factor contributing to the increase in transmitter overflow obtained in the presence of phenoxybenzamine.[11,15] Yet, when a quantitative analysis was carried out on the overflow of total radioactivity obtained in the presence of phenoxybenzamine it was concluded that, in addition to inhibition of neuronal and extraneuronal uptake of norepinephrine, phenoxybenzamine increases the output of norepinephrine elicited by nerve stimulation.[11] A similar conclusion was reached by Starke *et al.*[16] for phenoxybenzamine and for phentolamine, because these drugs increased transmitter overflow elicited by nerve stimulation in concentrations which did not inhibit either neuronal or extraneuronal uptake. Moreover, De Potter *et al.*[17] reported an increase

in dopamine beta-hydroxylase during release elicited by nerve stimulation in the presence of phenoxybenzamine, thus confirming that this drug enhances transmitter release during nerve stimulation.

The increase in transmitter release obtained by exposure to phenoxybenzamine was observed in the range of concentrations of the drug eliciting blockade of the alpha-receptors.[18] However, a causal relationship between the block of the responses of the effector organ by phenoxybenzamine and the increase in transmitter release

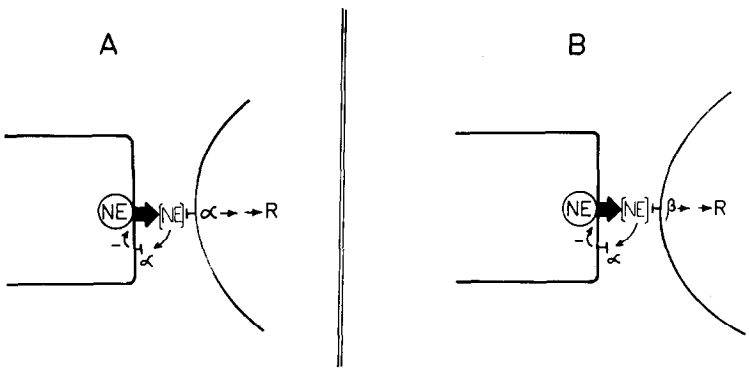

FIG. 2. Negative feedback mechanism for norepinephrine released by nerve stimulation. Response (R) of the effector cell mediated through alpha-receptors (A), and through beta-receptors (B). Note that, irrespective of the nature of the postsynaptic adrenergic receptor, the presynaptic receptor involved in the regulation of transmitter release is an alpha-receptor.

was excluded because similar results were obtained in isolated atria[19] and in the perfused rabbit heart,[16] where the adrenergic receptors that mediate the response of the effector organ are of the beta type. These results led to the hypothesis of a presynaptic regulation of norepinephrine release through a negative feedback mechanism mediated by adrenergic alpha-receptors. According to this hypothesis, the norepinephrine released by stimulation, once it reaches a threshold concentration in the synaptic gap, would activate presynaptic alpha-receptors, triggering a negative feedback mechanism that inhibits further release of the transmitter[18–22] (Fig. 2). Compatible with this hypothesis is the fact that alpha-receptor agonists inhibit transmitter release by stimulation,[22–24] while alpha-receptor antagonists enhance norepinephrine release[11,17,18,20,22,24,25] regardless of the alpha or beta type of the adrenergic receptor that mediates the response of the effector organ (Fig. 2).

The negative feedback mechanism for norepinephrine release should be expected to operate most effectively when the quantity of transmitter released by each nerve impulse is high. In agreement with this view, it has been recently shown that when the endogenous norepinephrine stores are depleted, the effectiveness of phenoxybenzamine in increasing the overflow of ^{3}H-norepinephrine during nerve stimulation is almost completely lost.[26] Therefore it appears that, when the concentration of released norepinephrine in the synaptic gap falls below a certain threshold, it fails to trigger the negative feedback mechanism that regulates transmitter release.

Tyramine elicits norepinephrine release by displacing the transmitter from vesicular binding sites. In contrast to release elicited by potassium or by nerve stimulation,

the norepinephrine release obtained by exposure to tyramine is not calcium dependent. It is of interest that the presynaptic negative feedback mechanism that regulates norepinephrine release is operative for release elicited by nerve stimulation or by potassium, while this mechanism is not involved in the regulation of transmitter release elicited by tyramine.[27] It is possible that the presynaptic feedback mechanism modifies the availability of calcium ions for the release process and that this is the reason why release elicited by tyramine is not influenced by this regulatory mechanism.

When the potency of phenoxybenzamine in blocking the pre- and postsynaptic alpha-receptors was tested in the perfused cat spleen, it was found that a significant reduction in responses to nerve stimulation was obtained in the presence of low concentrations of phenoxybenzamine, although transmitter release was not increased under these experimental conditions.[28] The differences between the concentration of phenoxybenzamine required for blockade of the postsynaptic alpha-receptors and that necessary to enhance transmitter release during nerve stimulation was 30-fold.[28] Similar results were obtained in experiments in which the overflow of dopamine beta-hydroxylase was determined (L. X. Cubeddu, E. M. Barnes, S. Z. Langer and N. Weiner, unpublished observations). These results are compatible with the view that the pre- and the postsynaptic alpha-receptors are not identical. Perhaps the postsynaptic alpha-receptor that mediates the response of the effector organ should be referred to as α_1, while the presynaptic alpha-receptor that regulates transmitter release should be called α_2.

Recently it was found that, in addition to norepinephrine and several alpha-receptor agonists, dopamine also inhibits transmitter release during nerve stimulation.[29] While norepinephrine and dopamine were nearly equipotent in inhibiting ^{3}H-norepinephrine release during nerve stimulation, norepinephrine was 50 times more potent than dopamine on the postsynaptic alpha-receptor.[29] These differences in affinity for agonists between the pre- and the postsynaptic alpha-receptors appear to be due to the fact that norepinephrine and dopamine activate different presynaptic inhibitory receptors. While dopamine and norepinephrine act on the same postsynaptic alpha-receptor in the cat's nictitating membrane,[30] presynaptic inhibition of transmitter release in the presence of exogenous norepinephrine is blocked by phentolamine. On the other hand, inhibition by dopamine is blocked by chlorpromazine or pimozide, but not phentolamine.[31] Since neither chlorpromazine nor pimozide increase transmitter release elicited by nerve stimulation, it can be concluded that in this tissue the dopaminergic presynaptic receptors do not play a physiological role in the regulation of transmitter release by nerve impulses.

Although physiologists and pharmacologists generally think of receptors only on the membrane of the effector cell, it appears that we should accept the idea of multiple receptor sites in the membrane of the nerve ending as well. In adrenergic nerve endings, in addition to the alpha and dopaminergic receptors, there are muscarinic receptors which also are inhibitory on transmitter release[32] and nicotinic receptors which elicit norepinephrine release. The muscarinic inhibitory presynaptic receptors have been described earlier by Löffelholz and Muscholl[33] in the rabbit heart. Figure 3 shows schematically the pre- and postsynaptic receptor sites in the neuroeffector junction of the cat's nictitating membrane.

Another important factor in this presynaptic regulatory mechanism is the effective-

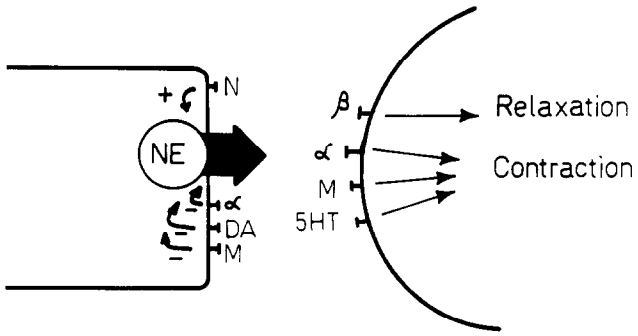

FIG. 3. Schematic representation of the pre- and postsynaptic receptor sites in the neuroeffector junction of the cat's nictitating membrane. α, Alpha-adrenergic receptor; β, beta-adrenergic receptor; M, muscarinic cholinergic receptor; N, nicotinic receptor; DA, dopaminergic receptor; 5-HT, serotonin receptor.

ness of the neuronal uptake of norepinephrine in reducing the concentration of the transmitter in the vicinity of the outer surface of the nerve ending. As shown diagramatically in Fig. 4, it is likely that a concentration gradient develops for released norepinephrine within the synaptic gap. Due to the high effectiveness of neuronal uptake, the reduction in the concentration of norepinephrine should be more pronounced, the closer the transmitter is to the nerve ending, resulting in a concentration gradient within the synaptic gap whereby the lowest concentration is achieved near the outer surface of the nerve ending (Fig. 4A). Under these conditions, when neuronal uptake of norepinephrine is inhibited by cocaine or desipramine, the above mentioned concentration gradient is abolished (Fig. 4B). Therefore, when neuronal uptake is impaired, presynaptic inhibition of transmitter release would be enhanced because a higher concentration of norepinephrine is now available for the activation of the presynaptic alpha-receptors (Fig. 4B). It is likely that this is the reason why cocaine or desipramine is not very effective in increasing transmitter overflow, in spite of being very potent inhibitors of neuronal uptake.[18]

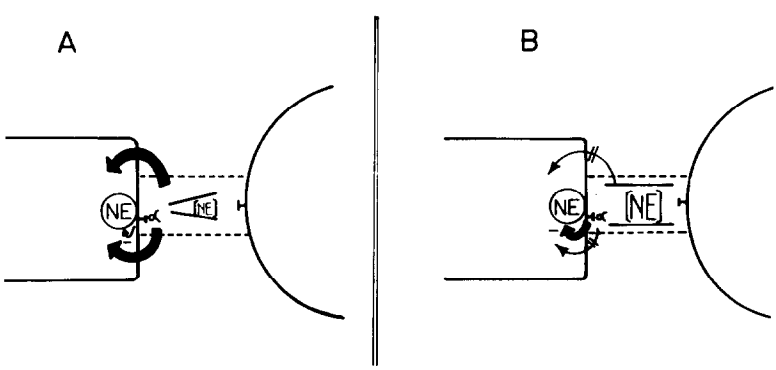

FIG. 4. Diagram of the concentration gradient for norepinephrine released by nerve stimulation in the synaptic gap. (A) Normal; (B) inhibition of neuronal uptake. Note that in the normal tissue neuronal uptake selectively reduces the concentration of released norepinephrine in the vicinity of the nerve ending. When uptake is inhibited, the concentration gradient is abolished, enhancing the presynaptic inhibition of transmitter release.

Compatible with the hypothesis of a concentration gradient of norepinephrine in the synaptic gap is the fact that exogenous norepinephrine does not inhibit transmitter release during nerve stimulation unless neuronal uptake is either saturated[28] or inhibited by cocaine.[23] However, if the alpha-receptor agonist employed is not a substrate for neuronal uptake, as is the case with clonidine, norepinephrine release by nerve stimulation can be inhibited by this drug even in the absence of cocaine.[20,24] It is likely that when neuronal uptake is not inhibited, the transmitter released by nerve stimulation has a higher chance than exogenous norepinephrine of reaching the threshold concentration required to activate the presynaptic inhibitory alpha-receptors.

It is possible that cyclic AMP might be involved in the mechanism of norepinephrine release by nerve stimulation. Recently, it has been reported that agents which inhibit phosphodiesterase, like papaverine or dipyridamole, increase norepinephrine release during nerve stimulation in the cat's nictitating membrane and in isolated guinea pig atria.[29,34] Similar findings have been reported for theophylline and for dibutryl cyclic AMP in the guinea pig vas deferens[35] and for monobutyryl cyclic AMP and several phosphodiesterase inhibitors in the perfused cat spleen.* In the bovine adrenal medulla, release of catecholamines is elicited by inhibition of phosphodiesterase.[36] Yet, it appears that cyclic AMP is not involved in the regulatory mechanism which is mediated through presynaptic alpha-receptors, because the effects of phentolamine or phenoxybenzamine on transmitter release are not affected in the presence of cyclic AMP analogs or when the phosphodiesterases are inhibited.[34]*

It appears that a presynaptic regulatory mechanism similar to that described in the periphery may operate in the central nervous system as well. In the rat cerebral cortex, the regulatory mechanism for norepinephrine release is mediated via alpha-receptors.[37] On the other hand, in neostriatal slices, the regulatory mechanism for dopamine release appears to be mediated through dopaminergic receptors.[37]

The presynaptic regulatory mechanism for norepinephrine release during nerve stimulation might be of clinical interest in connection with the treatment of hypertension. Clonidine, an alpha-receptor agonist which is very effective in reducing norepinephrine release by nerve stimulation in the periphery, is employed clinically to reduce high blood pressure. This hypotensive effect of clonidine appears to be mediated through inhibitory alpha-receptors in the central nervous system[38] and in the periphery.[39] It might be of interest to study whether the alpha-receptor-mediated presynaptic inhibition of norepinephrine release in the blood vessels is affected in the different models of experimental hypertension.

As pointed out earlier, drugs which inhibit neuronal uptake of norepinephrine should be expected to reduce transmitter output because they facilitate presynaptic inhibition. On the other hand, agents that block the receptors involved in the regulatory mechanism may enhance the release of the transmitter. These interactions are helpful in explaining some of the effects of the central dopaminergic agonists and of the antipsychotic dopaminergic blocking agents. The increase in dopaminergic metabolite concentration and dopamine turnover in the corpus striatum caused by several phenothiazines and by haloperidol[40–42] may be related to the blockade of central presynaptic dopaminergic receptors induced by these drugs. In addition, a similar

* L. X. Cubeddu, N. Weiner and S. Z. Langer, unpublished observations.

explanation can be put forward to account for the increase in the firing rate observed in central dopaminergic neurons exposed to antipsychotic phenothiazines or to haloperidol.[43]

It is possible that presynaptic effects on the release of neurotransmitters may be involved in the mechanism of action of hallucinogenic drugs like D-lysergic acid diethylamide (LSD). This drug inhibits postganglionic motor transmission in the guinea pig vas deferens and this effect appears to involve the activation of presynaptic alpha-receptors.[44] In addition to these effects in the periphery, LSD inhibits ^{3}H-5-HT release from cerebral cortex slices.[37]

A second mechanism has been proposed for the regulation of norepinephrine release by nerve stimulation. This hypothesis is based on the inhibitory effects of prostaglandins of the E series on transmitter release elicited by nerve stimulation[45–47] and on the release of prostaglandins coinciding with the periods of adrenergic nerve stimulation.[48,49] Several authors reported that, when the adrenergic nerves are stimulated, prostaglandins are released either from the effector cells or from the same nerve endings, thus leading to inhibition of norepinephrine release.[50,51] According to this hypothesis, the prostaglandins would mediate an endogenous feedback inhibition of norepinephrine release by nerve stimulation. Recent evidence appears to indicate that the feedback inhibition mediated through the prostaglandins would be independent from the regulatory mechanism which is mediated through presynaptic alpha-receptors.[52,53]

The origin of the prostaglandins released during adrenergic nerve stimulation is not clear. While Stjärne[54] postulated that the prostaglandins that regulate norepinephrine release in the guinea pig vas deferens are of neural origin, Junstad and Wennmalm[55] concluded that prostaglandin release from the isolated rabbit heart does not derive from the adrenergic nerves.

As discussed above, the increase in transmitter release obtained in the presence of alpha-receptor blocking agents is very large, 10- to 20-fold. In contrast to these results inhibition of the synthesis of prostaglandins produces only a very small or no increase in transmitter release during nerve stimulation.[51,56,57]* Consequently, it appears that the proposed feedback mechanism mediated through prostaglandins does not play a major physiological role in the regulation of adrenergic neurotransmission.

Recently, Szerb and Somogyi[58] reported a negative feedback mechanism for acetylcholine released by nerve stimulation from cerebral cortical slices, which appears to be mediated through muscarinic receptors. It is possible that presynaptic inhibition of transmitter release through a negative feedback mechanism which is mediated through the neurotransmitter itself represents a more general type of phenomenon and might occur not only for norepinephrine, dopamine and acetylcholine but for other neurotransmitters as well. This is an intriguing possibility which is worth exploring experimentally.

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