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MINI-REVIEW

Autoreceptors do not regulate routinely neurotransmitter release: focus on adrenergic systems

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Abstract

The theory that neurotransmitter release is regulated locally at the individual terminals of neurons has achieved a rapid and seemingly secure status in our understanding of neuronal function both in the periphery and in the central nervous system. This concept of negative feedback control through the monitoring of the perineuronal concentration of previously released transmitter has been extended to a multiplicity of transmitters and utilized to explain the mechanisms of action of diverse classes of drugs, ranging from antihypertensives to antidepressants. It is my view that negative feedback by terminal and by somadendritic receptors cannot account for the existing body of experimental work. Analyses of the profiles of action of agonists and antagonists, and of the per pulse release of transmitter in the absence of drugs in a variety if peripheral organ systems, as well as in superfused brain slices, demonstrates the need for alternate interpretations of the available data. Evidence is provided that the actions of agonists to inhibit transmitter release and that of antagonists to enhance release occur at different cellular loci and that the purported unitary action of these two classes that is so central to the validity of presynaptic theory is unsupportable.

Keywords: adrenergic mechanisms, autoreceptors, negative feedback, neurotransmitter release.

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This brief article will raise for reconsideration the principal kinds of evidence that currently define the status and validity of presynaptic receptor theory as it pertains to autoregulation of transmitter release through transmitter-mediated negative feedback. To avoid misunderstanding, it should be made clear at the outset that I do not question that terminal neuronal receptors exist; or that neurons often possess populations of receptors responsive to a variety of agonists. Nor do I question that terminal, and even somadendritic, receptors may be highly receptive to a neurons own transmitter: that is when it is provided from an exogenous source. It is even potentially possible that under certain, as yet uncertified conditions, endogenously liberated transmitter may actuate such receptors. Important work has been done by several groups of investigators in this regard. The debate of consequence here involves solely one central question; is there routinely operative in the peripheral and central nervous systems, at the axon terminals and at the soma dendritic regions, the local regulation of neuronal transmitter release by autoreceptors? I do not believe the answer is yes.

It may be difficult for some readers to accept the idea that the reality of autoreceptors sensing and responding to variations in the extraneuronal status of transmitter in the peripheral and central nervous systems can be legitimately questioned, particularly after these many years of intensive research. However, evidence will be presented here to authenticate my assertion that presynaptic receptors are likely to not function ordinarily as autoreceptors in the neuroeffector systems innervated by the autonomic nervous system and that the question of their operation in the central nervous system is far from resolved, regardless of the neurotransmitter system implicated (Starke *et al*. 1989). Although data from my own laboratory will be brought to bear on this problem it is obviously necessary that I deal in detail with pivotal findings of other workers, and that will be done as space allows. My focus will be on adrenergic mechanisms simply because the largest body of available work is with them, but reference will be made to other systems (e.g. dopaminergic, serotonergic) as space permits. It is possible that certain selective neuronal systems may fulfil the needed criteria for feedback but no assurances can be given at present, in this regard.

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Types of neuronal receptors

At the outset of our discussion a distinction needs to be made between receptors that respond to the exogenous administration of a neurons own transmitter and receptors that participate in an endogenous process of autoregulation. I believe that both sides of this debate can agree that the presence of a population of receptors responsive to a neurons own transmitter does not, *ipso facto*, signify the operation of a feedback loop by endogenously released transmitter. This distinction was stressed by me over a decade ago when I put forward the suggestion that not only heteroreceptors, but also homoreceptors, may exist much more frequently then do autoreceptors: the latter term denoting active participation in negative feedback (Kalsner 1990a). A concrete example of this can be given. Alpha$_2$ receptors on sympathetic nerves of vascular tissue may be activated by circulating epinephrine and norepinephrine from the adrenal glands (Abrahamsen and Nedergaard 1989; Kalsner 1990b). But this is not negative feedback via autoreceptors but rather the activation of homoreceptors by blood borne mediators. It can easily be envisioned that such processes exist in the central nervous system with its extraordinary mix of modulators and mediators and the potential for chemical interactions between discrete nerves (Kalsner 2000a; Vizi 2000).

The simple presence of homoreceptors or heteroreceptors on a cell does not even dictate function of any kind. To accept this premise, one has to refer only to the multitudinous presence of muscarinic receptors on the endothelial cells of blood vessels, largely for which no cholinergic innervation, or other evident source of acetylcholine, exists (Kalsner 2000b). Agreement on this issue is integral to the satisfactory resolution of presynaptic receptor theory. At present, a number of laboratories certify the presence of neuronal 'autoreceptors' based on responses of their test preparations to administration of the neurons own transmitter or its analogues, this is clearly inappropriate and highly speculative.

Key evidence

The minimal evidence needed to meaningfully propose ongoing autoinhibition in a neuronal system under study is a finding that antagonists of the relevant presynaptic receptors increase stimulation-induced transmitter release, and do so in direct proportion to the intensity of the stimulation parameters, reflecting the graduating consequences of receptor blockade. Additionally, of course, all conceivable alternate explanations for the potentiation must be carefully ruled out. Auxiliary evidence would be a pattern of declining per pulse release of transmitter with increased stimulation intensity (frequency or pulse number) in the absence of antagonist. Further, inhibition of stimulation-induced release by a fixed concentration of exogenously administered agonist is expected to show declining efficacy as the frequency or pulse train number increases, reflecting competition for receptor sites from increasing amounts of endogenously released transmitter.

Of unique weight in the validation of presynaptic receptor theory is the requirement that transmitter released by a single stimulation pulse not be potentiated by presynaptic antagonists because such conditions do not allow for a contribution by previously released transmitter. It is well established that transmitter release does not even occur until late in the depolarization/repolarization cycle (Kandel and Siegelbaum 2000). Each of these points will be covered briefly below.

Single pulse data

Some years ago, my laboratory reported that the irreversibly acting alpha adrenergic antagonist phenoxybenzamine tripled both the contractile response and the stimulation-induced efflux of norepinephrine in the guinea pig vas deferens, when only a single 1-ms stimulation pulse was delivered, and that potentiation was apparent both in the presence and absence of a functional uptake system (Kalsner 1979). The uptake blocker, cocaine, when used, was at a concentration that inhibits over 90% of neuronal uptake (8.8×10^{-6} M; Iversen 1965). In the intervening years, several other investigators have reported antagonist-induced increases in norepinephrine or dopamine transmitter release following a single stimulation pulse or with pseudo one-pulse stimulation (Blakeley *et al*. 1984; Mayer *et al*. 1988). My laboratory found significant potentiation of the release of norepinephrine and of 5-hydroxytryptamine by the antagonists yohimbine and methiothepin, respectively, following the delivery of a single pulse or pseudo-one pulse in slices of rabbit hippocampus (Fig. 1a). Such studies have been quite limited, however, because transmitter release with a single pulse is at or below detection limits in most test preparations (see Fig. 1a, 5-HT).

Advocates of feedback theory maintain that 'a small degree of tonic inhibition', attributable to stray transmitter, is sufficient to explain enhancement of release by receptor antagonists with a single pulse (Mayer *et al*. 1988). This interpretation requires more demonstrative evidence, and also raises an obvious problem. If, for example, a tripling of transmitter release and response size in the vas deferens by an antagonist following one pulse (Kalsner 1979) results from blockade of the effects of transmitter leaking from neurons, then the feedback system operates near maximally without neuronal depolarization and with a synaptic concentration insufficient to elicit even minimal postsynaptic responses. Further insight into this dilemma for presynaptic theory is obtained by examining release with the delivery of a few pulses and with pseudo one-pulse stimulation, and this is done below.

Pseudo one-pulse release

In a seminal study, stimulation with one pulse and with four pulses at 1 Hz was used along with pseudo one-pulse

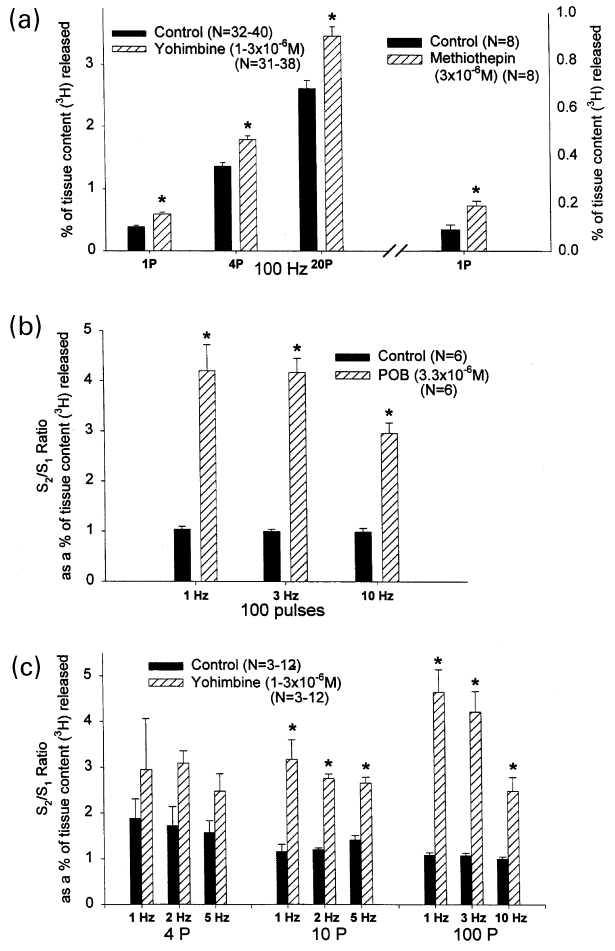

Fig. 1 Effects of frequency and pulse number on the release of [^{3}H]norepinephrine and [^{3}H]5-HT in rabbit hippocampal slices. (a, left) Release of norepinephrine from hippocampal slices stimulated with one pulse and with four and 20 pulses at 100 Hz, in the presence or absence of yohimbine; (a, right), release of 5-HT from ,hippopcampal slices stimulated with one pulse in the presence or absence of methiothepin. (b) Effects of phenoxybenzamine on the stimulation-induced release of norepinephrine. (c) Effects of yohimbine under multiple test conditions on the stimulation-induced release of norepinephrine. Data are shown either as percent of ,tissue content released with stimulation or as S_2/S_1 ratios with S_1 representing the initial values obtained in the relevant preparations before the introduction of antagonists. Control tissues were untreated during S_2. Asterisks indicate treated values significantly different from corresponding controls.

stimulation (four pulses at a frequency too rapid to engage autoinhibition, namely 100 Hz, Singer 1988) to examine feedback in noradrenergic and dopaminergic systems in rabbit brain cortex and caudate nucleus (Mayer *et al*. 1988). The release of dopamine and norepinephrine with four pulses at 1 Hz 'did not exceed that elicited by a single pulse' whereas with four pulses at 100 Hz norepinephrine output was 5.1-fold higher than with a single pulse, seeming to provide striking support for feedback theory. These results were recently confirmed in mouse hippocampus (Trendelenburg *et al*. 1999) leading the authors to assert that 'when stimulated by two or more pulses autoinhibition develops and depresses transmitter release by the second and later pulses.' There are significant interpretational concerns that should be raised.

If pseudo one-pulse release is insufficient to engage autoinhibition (Mayer *et al*. 1988; Singer 1988; Trendelenburg *et al*. 1999), and autoinhibition is presumed to account substantially for the observed decrements in release with multiple pulses, why was release of dopamine with four pulses at 100 Hz only 1.4 times that of one pulse rather than four times one pulse (Mayer *et al*. 1988)? Further, if total stimulation-induced release of dopamine or norepinephrine with two and four pulses at 1 Hz is only marginally greater than the release achieved with a single pulse must we not conclude that during routine stimulation, release by the first pulse in a train activates the presynaptic receptor population in such numbers as to not only reduce, but virtually shut down release by the second and subsequent pulses, and that this occurs in the face of continued neuronal depolarizations. This important issue is dealt with further below, first in a consideration of peripheral neurotransmission and then central transmission.

Train length, frequency and transmitter release in the periphery

Irrefutable evidence demonstrates that peripheral end organ responses increase reliably with increasing nerve frequency and/or pulse train number (see Farnebo and Malmfors 1971; Hughes 1972; Henderson *et al*. 1975; Chan and Kalsner 1980; Kalsner 1985a, 1990c; Yamamoto *et al*. 1990). This is obvious in the graduated responses of diverse organ systems to autonomic and motor nerve stimulation, and has been observed by many investigators over many years. Increases in frequency yield increased response magnitudes through compression of the release time for a given pulse number, e.g. from 300 s to 20 s, and increased pulse number increases the total quantity of transmitter released. Both procedures ensure higher peak synaptic transmitter densities, even if some tangible decline occurs in per pulse transmitter release.

It is clear that in the periphery release with an initial pulse, or with a small number of pulses, or for that matter with even a moderate to large number, does not cancel out release by subsequent pulses. My work has shown that the per pulse release of norepinephrine is generally well maintained and does not precipitously decrease with increasing stimulation parameters (for a review see Kalsner 1985a, 1990c, 2000c). For example, cattle iris preparations stimulated with 10, 20, 50 and 100 pulses at 2 Hz released transmitter in direct proportion to pulse number and this was also reflected in the increasing magnitude of the adrenergic beta receptor-mediated smooth muscle response (Kalsner

1983). Per pulse transmitter release does not decline materially in arterial tissue with increasing contractions, even when stimulated with 300 pulses over the broad physiological range of frequencies (1, 2, 5, 10 and 15 Hz; Chan and Kalsner 1980). Progressively increasing contractions were recorded in perfused rabbit carotid arteries with increasing frequency (1.5–24 Hz) and this was accompanied by a constant per pulse release of norepinephrine (Yamamoto *et al*. 1990). Again, in guinea pig vas deferens, and with a small pulse number, release of norepinephrine with four pulses at 5 Hz is four times that of one pulse (Kalsner 1979), not at all like the values reported for hippocampus. Per pulse norepinephrine output from the rabbit vas deferens and portal vein increases with increasing frequency of stimulation (Hughes 1972), as does that in the mouse vas deferens with increasing pulse number (Farnebo and Malmfors 1971). With few exceptions, transmitter release characteristics and effector responses outside the CNS do not reveal the potent operation of presynaptic receptor mediated terminal inhibition.

Agonists and antagonists in the periphery

A central tenet of presynaptic receptor theory requires that agonists become less effective in inhibiting stimulation-induced transmitter release as stimulation frequency increases. It is an obligatory consequence of competition between an increasing biophase level of endogenously liberated transmitter and a fixed amount of exogenous agonist for a common population of receptor sites. However, this pattern is not predictably seen in peripheral neuroeffector systems (Kalsner 1990b), and when it is seen it also appears to be common to agonists acting on unrelated heteroreceptor systems (e.g. Chan and Kalsner 1980).

Further, the extent of potentiation of transmitter release by receptor antagonists (e.g. yohimbine) in the periphery seems to be indifferent to, rather than dependent on, the varied dimensions of the synaptic space in diverse organ systems, and consequently with few exceptions, to the synaptic transmitter density (Kalsner 1990b,c; Yamamoto *et al*. 1990). Some of the experimental designs utilized by my laboratory to establish this view have included blockade of neuronal and extraneuronal uptake and modifications in applied voltage, in stimulation pulse duration, in extracellular calcium levels, as well as in frequency and pulse train lengths (e.g. various blood vessels, ureter, heart, vas deferens, uterus, iris, spleen; Kalsner 1984, 1985a).

Another shibboleth of presynaptic theory is that antagonists become more effective potentiators of transmitter release as the agonists become less effective inhibitors of stimulation-induced release. This is because the two effects are presumed to represent two sides of the same coin, namely reciprocally linked interactions with a common set of receptors. This does not hold up to review. A number of reports prominently dissociate the profile of effects of agonist and antagonist on transmitter efflux raising additional interpretive uncertainties for presynaptic theory (Kalsner 1980, 1982; Kalsner *et al*. 1980; Yamamoto *et al*. 1990).

This lack of concordance between the effects of agonist and antagonist can not be attributed, with confidence, to increased competition from released transmitter at higher stimulation intensities breaking through adrenergic receptor blockade and distorting the relationship. This is because even the covalently bound non-competitive adrenergic antagonist, phenoxybenzamine, increased release of norepinephrine most at the lowest frequency of 1 Hz, not the highest, whereas the increases in release at 2, 5, 10 and 15 Hz did not differ from each other in vascular tissue (Chan and Kalsner 1979), nor in slices of rabbit hippocampus (Fig. 1b). Again, phenoxybenzamine potentiated norepinephrine release to a diminishing, rather than to an increasing, extent in guinea pig atria when 100 pulses were given over the physiological range of frequencies (0.5, 1, 2, 5 and 10 Hz; Kalsner *et al*. 1980).

Central versus peripheral nervous systems

Advocates assert that ‘presynaptic receptors play a cardinal role in the regulation of noradrenergic transmission in the CNS’ (Dennis *et al*. 1987; Starke *et al*. 1989). Findings with central neurons do seem to provide more convincing evidence for the operation of feedback than do data with peripheral systems. Much of the enthusiasm stems from the types of observations, alluded to above: notably that in some brain regions transmitter release decreases profoundly with only the most modest increases in pulse train length, implicating autoinhibition, and further, that in certain brain regions transmitter efflux is potentiated distinctly by receptor antagonists (Cubeddu and Hoffman 1982; Mayer *et al*. 1988; Singer 1988; Valenta *et al*. 1988; Trendelenberg *et al*. 1999).

In a key study, Valenta *et al*. (1988) noted little difference in stimulation-induced overflow of norepinephrine in rat cerebral cortex with 1 Hz stimulation regardless of whether 1, 2, 4 or 16 pulses was administered. In their study enhancement of stimulation-induced transmitter efflux by the antagonists idazoxan and rauwolscine reached maximal values with just two pulses and remained constant up to 10 Hz. At higher frequencies, however, the magnitude of potentiation decreased, even when the antagonist concentration was increased 10-fold. These workers reasoned that there was ‘near-maximal activation of autoinhibition after a single stimulus, and a loss of autoinhibition above 10 Hz.’ I regard this as a difficult and far reaching scenario to envision in terms of central nervous system neurotransmission.

In seeming conflict with their own interpretations, Valenta *et al*. (1988) also found that the agonist clonidine inhibited transmitter efflux to a similar extent regardless of whether one pulse or 10 pulses was given. Not at all in

keeping with a scenario of minimal competition from endogenous amine for receptor sites with one pulse and intense competition with two or more pulses. Particularly, as two or four pulses was deemed sufficient to close down release. Of particular relevance here is my finding with rabbit hippocampal slices that norepinephrine-induced inhibition of [^{3}H]norepinephrine release showed no substantial decrement as pulse number increased from 1 to 2 to 4, and to 16. Release was inhibited by 99.5 ± 4.1%, 91.8 ± 1.5%, 94.1 ± 1.5% and 88.0 ± 2.0% ($n = 8$), at each of the pulse numbers, respectively. This finding is in total opposition to the expressed tenets of presynaptic theory.

Another non-conforming observation of the Valenta group was that the antagonist idazoxan increased transmitter release by a second pulse even when an extended interval of 50 s was allowed to elapse before delivery of the second of two pulses. The authors attribute this to prolonged feedback inhibition induced by the initial pulse, but provide no substantiation for such a provocative conclusion. Such an interpretation would require that presynaptic receptors have activation characteristics that are disruptive rather than regulatory in the neurotransmission process. Further, such an extended activation process would render these sites unique and not comparable to central postsynaptic alpha receptors or to peripheral adrenergic alpha$_2$ presynaptic receptors. Alternative interpretations should be encouraged.

Still another dilemma for presynaptic theorists stems from the work of Curet and Montigny (1989). They found that the postsynaptic neuronal response to loceus coerulus stimulation at 1 Hz was five times greater than that seen at a much higher frequency (5 Hz). Further, stimulation at 0.5 Hz yielded a larger postsynaptic response than did 1 Hz. In line with the demands of feedback theory they were led to propose that the firing rate of noradrenergic neurons is not a significant factor in regulating neurotransmission, but instead that 'its regulation might be mainly achieved locally by factors such as the degree of activation of autoreceptors located on norepinephrine nerves'. But such a system is not autoregulation but, again, a seemingly counterproductive cancellation of transmission. Central noradrenergic neurons as well as those of the serotonergic and other modulating systems discharge spontaneously at rates of about 1–10 Hz or show rhythmic burst firing (Curet and de Montigny 1989; McCormick 1999), and the likelihood of an acute short circuiting of terminal release and the postsynaptic response from a lone depolarization strains credibility.

To buttress the rationality of feedback theory, and the concept of autoinhibition as a meaningful regulatory system, operating over a substantial pulse range, it has been asserted that in mouse hippocampus 'at high pulse numbers large release-enhancing effects of rauwolsine', are seen (Trendelenburg *et al*. 1999). But this does not appear to be sustained by their data which instead points to a uniform degree of potentiation, regardless of pulse number. The release by one pulse (0.4222% of tissue content) was not potentiated by rauwolscine in their study, but release by two pulses, which was 1.2 times that of one pulse (0.50664%), was increased by the antagonist by 85%. This increase must then be totally attributable to the second pulse alone (from 0.08444% of tissue content to 0.50664% × 1.85–0.4222% or 0.4951% of tissue content. This yields a potentiation of the second of two pulses to 5.9 times its non-rauwolscine value. (Other methods of calculation may yield an even greater magnification.) Similarly as release with four pulses is again only 1.2 times the single pulse, the release for each of the second, third and fourth pulses is 0.08444% ÷ 3 = 0.02815% of tissue content each. Rauwolscine increased by 95% the four pulse output to 1.95 × 0.50664% = 0.9879% of tissue content. Thus, the second, third and fourth pulses were increased to 0.9879% − 0.4222% ÷ 3 = 0.1886 or 6.7 times untreated controls. Similar calculations done with the 16 and 64 pulse data yield potentiations of about 6.1 and 4.4 times the untreated values. These data reveal a potentiation by rauwolscine that is indifferent to the number of pulses delivered, whether it be two or 64 pulses rather than an index of synaptic transmitter density or pulse number.

In this context, it is important to convey here that a number of workers do describe a pattern of per pulse release in brain preparations that is not consonant with an abrupt shutdown of central neurotransmission with two pulses, or even with the basic tenets of feedback regulation. For example, the stimulation-induced release of [^{3}H]dopamine does not decline in rabbit striatal slices with increasing pulse train length (Cubeddu and Hoffman 1982). Instead, a 'highly significant positive correlation between the total number of pulses applied (30, 60, 90 and 360) and the percentage of tissue radioactivity released by stimulation', was described by the authors over frequencies of 0.3 Hz to 10 Hz.

On the other hand, although a sharp decline in per pulse release of acetylcholine was noted when 120 pulses was given at increasing frequencies between 0.3 and 3.0 Hz (James and Cubeddu 1984), the presynaptic muscarinic antagonist atropine increased only slightly the efflux of acetylcholine at both the lower and the higher test frequencies, contrary to feedback theory. These authors rather than constraining their interpretation to fit feedback theory concluded that 'negative frequency-release relationships for striatal cholinergic neurons may reflect an intrinsic characteristic of these neurons'.

Antagonist interactions with dopaminergic transmission provide a convincing case for operational feedback in the central nervous system according to a major review (Starke *et al*. 1989). But an examination of the purportedly key literature quoted by them does not unilaterally sustain their views. Dwoskin and Zahniser (1986) reported that the evoked release of dopamine in rat striatal slices is augmented by the dopamine presynaptic receptor antagonist sulpiride but that 'augmentation of evoked ^{3}H-release by

sulpiride is related inversely and not directly to the number of depolarizing pulses delivered'. This is, of course, contrary to the stipulations of feedback theory. Another report described by Starke *et al.* (1989) as supportive of feedback concluded, however, that presynaptic receptors in prefrontal cortex of rats do not show modulation at their 'normal firing rates' and that these receptors are 'functionally inactive' (Hoffman *et al*. 1988).

We investigated the effects of frequency on per pulse transmitter release in rabbit hippocampal slices. We found no predictable decrement in per pulse release of norepinephrine or of 5-HT with the delivery of 10 or 30 or even 100 pulses, and with frequencies ranging from 0.2 Hz to 10 Hz. We also found that the non-equilibrium covalently bound phenoxybenzamine potentiates to a similar extent norepinephrine release when 100 pulses are given at 1, 3 or 10 Hz (Fig. 1b). Also, the pattern of potentiation of release by yohimbine ($1–3 \times 10^{-6}$ M) was not predictably dependent on frequency when examined with four, 10 or 100 pulses (Fig. 1c and Kalsner and Abdali, to be published).

Limitations of knockout experiments

Experiments with mice in which the adrenergic $alpha_2$ A/D gene has been disrupted has been interpreted as supportive of the principal role of feedback operation by autoreceptors (Trendelenburg *et al*. 1999). The marked suppression of norepinephrine release with increasing pulse train length seen in brain and heart preparations of control (wild-type) mice is muted at the longest pulse train numbers in KO mice. Additionally, the adrenergic antagonist rauwolscine, employed for this study, did not potentiate transmitter efflux meaningfully in brain and heart preparations of KO mice. These results deserve discussion.

Stimulation of a slice of brain tissue releases a number of neuroactive 'entities' that are potentially available to interact with the transmitter system under study. Their impact, if any, on the test system, and its pattern of per pulse release, could only occur with multiple pulses, not during the delivery of the initial pulse. The first pulse in a train is in a real sense immune from interactions with other neurons, or the consequences of local circuits, which are activated during wholesale stimulation of the slice. Consequently, transmitter release with even a short train of pulses cannot be analyzed solely in the context of a solitary transmitter system governed by local feedback. Further, $alpha_2$ receptors functioning presynaptically or postsynaptically, on neuronal systems within the preparation, and themselves targets of adrenergic antagonist drugs, may well impact the studied system. My own work shows that hippocampal 5-HT neurons contain $alpha_2$ adrenergic receptors that significantly modify 5-HT release, and the impact of this activity on neighboring systems is unresolved. KO mice obviously are functionally disrupted not only at noradrenergic terminals, but at all sites that possess the relevant pre- and postsynaptic receptors.

An additional difficulty with the study in KO mice is that the agonist used to assess the presence of $alpha_{2a}$ type receptors, medetomidine, retained approximately 40% of its capacity to inhibit transmitter release in the hippocampal slices of KO mice. This occurred although the antagonist rauwolscine retained only minor capacity to potentiate transmitter release in these particular tissues. Presynaptic theory would anticipate concordance between the loss of agonist and the loss of antagonist efficacy, and the differential is disconcerting.

In the peripheral nervous systems, the complications are obviously less pervasive and deserve attention. Trendelenburg *et al.* (1999) reported the loss of autoreceptor function in the atria of KO mice based on what they call the 'strict proportionality' in norepinephrine release demonstrated in these atria; release by two and four sets of POPS (20 pulses/50 Hz) was 2.0 and 3.8 times, respectively, that of 1-POP. However, in wild-type atria with fully functional alpha2a inhibitory receptors the corresponding values were not clearly indicative of release suppression, but also roughly proportional to POP number. The ratios for two and four sets were 1.7 and 2.7 times the 1 -POP value, not substantially different from those seen in KO mice. The authors note that rauwolscine was no longer effective in atria of KO mice but its pattern of potentiation in the atria of control wild-type mice is not reassuring. The pseudo one-pulse stimulation and the trains of two or four POPs were all increased to a similar extent by rauwolscine in these atria. Intriguing questions are: As relative proportionality in release was evident in the atria of wild-type mice, why does rauwolscine work prominently in control mice? Why does rauwolscine potentiate efflux with one POP, which should be exempt from autoinhibition? Why does rauwolscine potentiate efflux with multiple POPs to a common extent, rather than in proportion to the density of extraneuronal transmitter?

Reconsiderations

It is clearly critical to the viability of negative feedback theory that the linkage be inseparable between potentiation of stimulation-induced transmitter release by antagonists and their blockade of agonist-induced inhibition of release, certifying that they are expressions of the same unitary event. My laboratory has in the past provided evidence that these two effects represent largely discrete actions in noradrenergic systems. Such studies have included the contrasting expressions of agonist and antagonist behavior under conditions of altered temperature (Kalsner 1990c), and the differential effects of heteroreceptor agonists (i.e. acetylcholine) on adrenergic agonist and antagonist performance (Kalsner and Quillan 1988). Further, a concentration of yohimbine (3×10^{-7} M) that enhanced transmitter release in guinea pig ureter did not substantially reduce

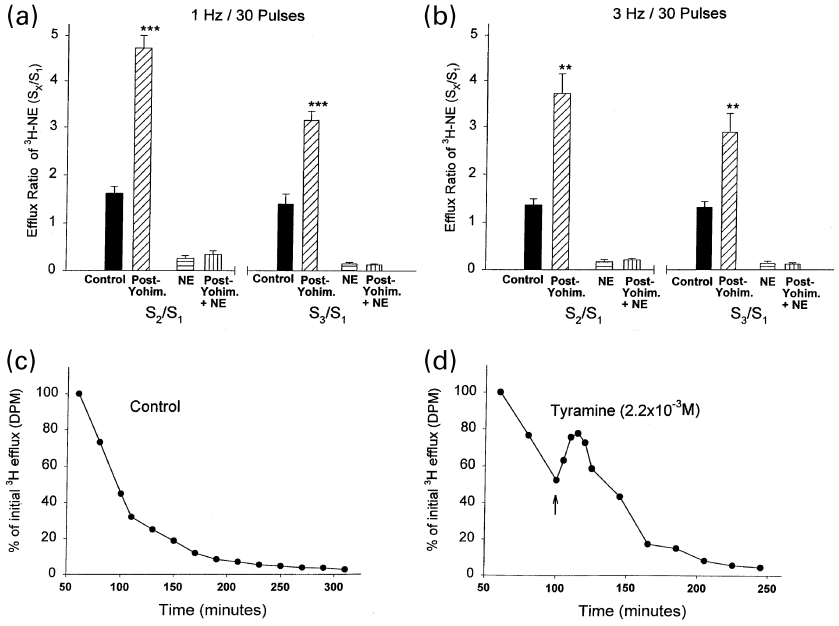

Fig. 2 Yohimbine and norepinephrine interactions (a and b). Yohimbine effects on stimulation-induced [^{3}H]norepinephrine efflux and on norepinephrine-induced inhibition of efflux. All rabbit hippocampal slices were stimulated initially (S_1) at 1 Hz and 3 Hz with 30 pulses and then divided into four treatment groups, as shown, and re-stimulated at 1 Hz and 3 Hz (S_2) and again a third time (S_3). Exposure to yohimbine (3×10^{-6} M) was for 35 min after S_1 in the indicated tissues (post yohimb group) followed by its removal from the superfusate 75 min prior to S_2 and 140 min prior to S_3. Norepinephrine, where indicated, was added to the superfusate 15 min prior to S_2 and S_3. Mean values are expressed as the ratio of transmitter efflux in S_2 or S_3 compared with initial control values (S_1). The control group received neither yohimbine nor norepinephrine at any time. Asterisks indicate mean values significantly different from corresponding controls. The norepinephrine-treated groups did not differ significantly from each other. (c and d) Efflux of [^{3}H]yohimbine from superfused hippopcampal slices in the absence (c) and presence of tyramine (d) and amphetamine. Typical responses are shown. Arrows indicate onset of superfusion with indicated amines.

the inhibitory effect of norepinephrine or that of the imidazoline deritivative oxymetazoline (Kalsner 1982). Similarly, yohimbine did not effectively antagonize the inhibitory effect of clonidine or norepinephrine in dog saphenous vein in concentrations which enhanced transmitter release (Baker *et al*. 1984). My own work determined that yohimbine is an unpredictable antagonist of clonidine in guinea pig atria although yohimbine potentiated transmitter release (Kalsner 1985b).

New work from my laboratory has now separated unambiguously the blocking and the potentiating effects of yohimbine (Fig. 2). Potentiation of norepinephrine release in field stimulated slices of rabbit hippocampus by yohimbine persisted following perfusion with yohimbine-free Krebs, and declined only slowly over a period of hours. However, the capacity of norepinephrine to inhibit transmitter release, initially blocked by yohimbine, was largely, and even totally, restored soon after elimination of the blocker from the perfusate (Fig. 2). In fact, taking into account the magnification of transmitter release by yohimbine the degree of agonist-induced inhibition of release pointed to a sensitization of the inhibitory mechanism rather than a blockade of it. Similar results were obtained in peripheral vascular tissue (Kalsner and Abdali, to be published).

To assess mechanisms, hippocampal slices were incubated for 60 min with [^{3}H]yohimbine ($2–3 \times 10^{-8}$ M) instead of [^{3}H]norepinephrine and the efflux of yohimbine in superfused preparations recorded over time. As shown in Fig. 2(c), yohimbine was released progressively over several hours. Field stimulation of slices did not increase the efflux rate of yohimbine, but superfusion with the indirectly acting amines (Furchgott *et al*. 1963; Muscholl 1972), tyramine (Fig. 2d) and also methamphetamine, led to increased release of [^{3}H]yohimbine. Tyramine and methamphetamine infusions over 15 min increased the efflux of tritium to peaks of $126.6 \pm 10.3\%$ and $120.5 \pm 3.6\%$, respectively, of predrug values, compared with a fall in ^{3}H-efflux values of $64.1 \pm 7.1\%$ in control preparations, over the corresponding time period ($n = 4$ in each group).

The location of the site through which yohimbine potentiates transmitter release is unknown. But it should be noted that yohimbine (and rauwolscine) is an alkaloid related to reserpine, a compound that is known to act intracellularly at adrenergic nerve terminals to alter transmitter release (Lefkowitz *et al*. 1996). Experiments with a very low concentration of yohimbine (3×10^{-8} M) and a prolonged incubation time of 90 min permitted a clear separation of the enhancing and inhibiting effects of

yohimbine, pointing to a time-dependent accumulation at an intracellular locus.

A variety of evidence has been presented elsewhere that reinforces the inadequacies of presynaptic receptor theory and its inability to account realistically for the interactions of agonists and antagonists with terminal neuronal receptors (see Kalsner 1990a, b, c, 2001). In this context, it should be emphasized that the presynaptic receptor populations undoubtedly operate through second messenger systems, which are worthwhile objects of research, but no physiological relevance can be presumed from such mechanistic considerations.

Feedback regulation has been extended in recent years to account for the somatodendritic liberation of transmitter in the central nervous system. Evidence was provided by me in a recent article (Kalsner 2000a) that negative feedback does not function at somatodendritic sites either to set firing rate or to regulate transmitter density, and the conclusion was reached that the attribution of the effectiveness of neuroactive drugs such as antidepressants, and certain anti-anxiety drugs to desensitization of raphe 5-HT inhibitory receptors is unlikely.

It is becoming increasingly obvious to this writer that the admittedly compelling theory of local feedback regulation at somadendritic and terminal neuronal sites is unable to account satisfactorily for a substantial body of theoretical and experimental nonconforming or discrepant evidence. Heepe and Starke (1985) appear to appreciate the difficulties in accommodating the theory to function in the central nervous system and periphery and have referred to autoregulation as 'a mechanism in search of a purpose.' This author wholeheartedly agrees with that cogent observation.

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