Brain Research, 226 (1981) 155–170
Elsevier/North-Holland Biomedical Press

NEUROPHYSIOLOGICAL CONSEQUENCES OF PRESYNAPTIC RECEPTOR ACTIVATION: CHANGES IN NORADRENERGIC TERMINAL EXCITABILITY

SHOJI NAKAMURA, JAMES M. TEPPER, STEPHEN J. YOUNG and PHILIP M. GROVES

Department of Psychiatry (M-003), University of California, San Diego, School of Medicine, La Jolla, CA 92093 (U.S.A.)

(Accepted April 23rd, 1981)

Key words: presynaptic receptors — noradrenergic terminals — neurotransmitter release

SUMMARY

Experiments were carried out to explore the view that activation of presynaptic receptors on the terminals of noradrenergic neurons is accompanied by alterations in their excitability to direct electrical stimulation. Antidromic action potentials evoked from frontal cortex of urethane anesthetized rats were recorded extracellularly from nucleus locus coeruleus. The threshold current necessary to evoke antidromic action potentials varied as a result of infusion of adrenergic agonists and antagonists into frontal cortex within 50 μm of the stimulating electrode. Local infusion of the α-adrenergic agonist clonidine produced a marked decrease in terminal excitability, while the α-antagonist phentolamine produced an increase in terminal excitability and was shown to reverse the effect of the agonist. Infusion of the β-adrenergic agonist isoproterenol was without effect, although the β-antagonist propranolol resulted in a decrease in terminal excitability. Infusions of potassium increased excitability of locus coeruleus terminals. Terminal excitability was seen to vary inversely with the rate of spontaneous or high frequency stimulation-induced firing of locus coeruleus neurons. From these observations, it may be inferred that activation or blockade of α-adrenergic presynaptic receptors results in changes in polarization and/or conductance of the noradrenergic synaptic endings. These results are discussed with respect to phenomena associated with the possible presynaptic regulation of neurotransmitter release.

INTRODUCTION

For some time evidence has been accumulating that the quantity of norepinephrine (NE) released from adrenergic nerve terminals under conditions of electrical

stimulation or potassium-induced depolarization is modulated by presynaptic receptors located on the nerve terminals themselves[16,36]. In addition to α-adrenergic agents, a variety of others including β-adrenergic agents, opiates and prostaglandins of the E series have been proposed to modify NE release by presynaptic receptors in a variety of central nervous system in vitro preparations including frontal and occipital cortex, cerebellar cortex, hypothalamus and other tissues[5–7,9,18–20,28,30–33].

That activation of α-adrenergic receptors on the presynaptic ending results in reduced neurotransmitter release by central noradrenergic neurons has been inferred by numerous investigators[5–7,9,16,20,28,30–33,36]. Further, facilitation of release following presumed presynaptic α-receptor blockade has also been reported[5,7,32,33]. The mechanisms involved in presynaptic receptor mediated regulation of neurotransmitter release have yet to be fully elucidated, although it has been shown that calcium plays an important part in the process[6].

Since the impulse activity of catecholamine containing neurons appears to be inhibited by the cells' own transmitter[1–4,12,13], it is possible that a similar inhibitory influence occurs with activation of receptors on the presynaptic terminals. Thus, if presynaptic receptor activation by transmitter released from the synaptic ending produces alterations in the state of polarization of the terminal, e.g., hyperpolarization, then the terminals of these neurons would be expected to exhibit reduced excitability to direct electrical stimulation. Indeed, preliminary evidence has shown that for dopaminergic neurons of the substantia nigra, amphetamine, which promotes the extracellular accumulation of dopamine, reduces the excitability of the dopaminergic terminals to electrical stimulation while haloperidol, a dopaminergic receptor blocking agent, produces increased excitability to electrical stimulation of the terminal fields[11].

In order to examine this possibility for noradrenergic neurons, antidromic stimulation of the terminal field in frontal cortex of individual noradrenergic neurons of nucleus locus coeruleus was employed to determine if locally administered adrenergic agonists and antagonists would lead to changes in terminal excitability. A preliminary report of some of these results has appeared elsewhere[23].

MATERIALS AND METHODS

Experiments were carried out on 41 male Sprague–Dawley rats weighing between 250 and 450 g. Animals were housed with food and water available ad libitum on a 12 h light/dark cycle.

Subjects were anesthetized with urethane (1.3 g/kg, ip), a tracheal intubation was performed for the purpose of subsequent artificial ventilation, and the rat was placed in a stereotaxic apparatus (oriented according to König and Klippel[15]) using blunt, atraumatic earbars (Kopf Instruments). Body temperature was maintained at 37 $\pm$ 1 °C by a circulating water-filled heating pad electronically coupled to a Yellow Springs telethermometer. The electrocardiogram was monitored continuously on an auxillary oscilloscope during the experiments.

After a local subcutaneous injection of lidocaine HCl, (2% xylocaine), the calvarium was exposed and a small hole drilled for the insertion of a bipolar

stimulating electrode into the dorsal noradrenergic pathway (dorsal bundle). The coordinates for this electrode placement were: 2.0 mm anterior to lambda, 0.8 mm lateral to the midline and 6.0 mm ventral to the cortical surface. The electrode was affixed to the skull with cyanoacrylate glue and dental cement. For stimulation and infusion of the ipsilateral frontal cortex, a hole was drilled at coordinates 3.0 mm anterior to bregma and 2.5 mm lateral to the midline.

In order to facilitate recording from the locus coeruleus, the transverse sinus was ligated. Briefly, a portion of the skull directly over the sinus, approximately 4 mm $\times$ 3 mm, was carefully removed. The dura mater at the rostral and caudal edges of the sinus was carefully reflected and a doubled strand of 6–0 surgical silk suture was passed under the sinus by means of a small wire hook. The caudolateral and rostromedial aspects of the sinus were ligated separately and the sinus was cut in the middle, providing clear access to the locus coeruleus.

For electrical stimulation of both the frontal cortex and the dorsal noradrenergic pathway, bipolar electrodes consisting of insulated stainless steel wires, with exposed tips approximately 0.5 mm apart, were used. Electrical stimuli were generated by a Grass stimulator (Model S88) and passed through a Grass stimulus isolation unit (Model SIU-5) to the stimulating electrodes. Stimulus current was monitored periodically throughout the experiment on one channel of a Tektronix 565 dual-beam oscilloscope. In the frontal cortex, stimulation was applied at the rate of 1/s with a pulse width of between 0.5 and 2.0 ms at a current range of 0.2–2.5 mA, and in the dorsal pathway at the rate of 1/s with a pulse width of 0.5 ms and a current range of 0.10–1.0 mA.

The animals were immobilized with gallamine triethiodide (Flaxedil, 50 mg/kg, ip) and artificially respired on a Harvard Instruments rodent respirator at 80–90 strokes/min. Responses of individual locus coeruleus neurons were recorded extracellularly with glass micropipettes filled with 2 M KCl, with impedances ranging from 5 to 10 MΩ. Single unit activity was amplified by a Grass preamplifier (Model P-15C), displayed on a Tektronix 565 oscilloscope and simultaneously recorded on magnetic tape for later analysis.

As described previously, electrode placement in the locus coeruleus was verified by the appearance of an antidromically elicited extracellular field response from stimulation of the dorsal pathway[14,21,22]. The field response to dorsal pathway stimulation was elicited in the dorsolateral tegmentum of the pons with a sharp localization in the locus coeruleus. This potential was characterized by a triphasic waveform with an initial positive component followed by a second negative and third positive component, and an onset latency of 5 ms. Responses of individual locus coeruleus neurons exhibited a relatively slow rate of spontaneous firing (0.5–8/s) and an unusually wide waveform (2–5 ms), consistent with previous reports[2,21,22].

Single neuron responses in locus coeruleus elicited by electrical stimulation in the frontal cortex were considered to be antidromic in nature, provided that they exhibited collision with spontaneous discharges[21,22]. In both the frontal cortex and the dorsal pathway, the antidromic threshold was defined as the minimum current sufficient to elicit an antidromic response on 100% of the non-collision trials. In

addition to this value, the proportions of antidromic responses elicited by several lower stimulation currents were determined. These values were obtained by calculating the proportion of antidromic responses in blocks of trials consisting of from 30 to 200 non-collision stimulus presentations at 1/s.

One or more of the following adrenergic agents was dissolved in 0.9 % saline and infused locally into the frontal cortex: phentolamine HCl (Regitine HCl, Ciba–Geigy Corp.), 1 μM, 10 μM; clonidine HCl (Catapres, Boeringer Ingelheim, Ltd., Dist.), 1 μM, 10 μM; isoproterenol HCl (Isuprel HCl, Winthrop Laboratories), 0.1 μM, 1 μM, 10 μM; propranolol HCl (Inderal HCl, Ayerst Laboratories, Inc.), 1 μM, 10 μM. Potassium was infused at concentrations of 50 mM and 100 mM.

In order to observe the local effects of adrenergic agents on the excitability of the terminal fields of locus coeruleus neurons in the frontal cortex, these drugs were infused from the tips of 32-gauge cannulae situated approximately 50 μm from the exposed tips of the bipolar stimulating electrode. The cannulae and stimulating electrode were positioned in the frontal cortex at a depth of approximately 1.5 mm. Drugs were infused by means of an infusion pump (Harvard Apparatus Co., Model no. 975) equipped with a 10 μl Hamilton syringe (Model no. 701) at a rate of 0.06 μl/min for 5 min. Immediately after the completion of each infusion experiment, the infusion cannulae were removed from the frontal cortex and checked for clogging. In some subjects, the terminal fields of more than one cell were infused per animal. In these cases, the stimulating electrode and infusion cannulae were moved to a new site,

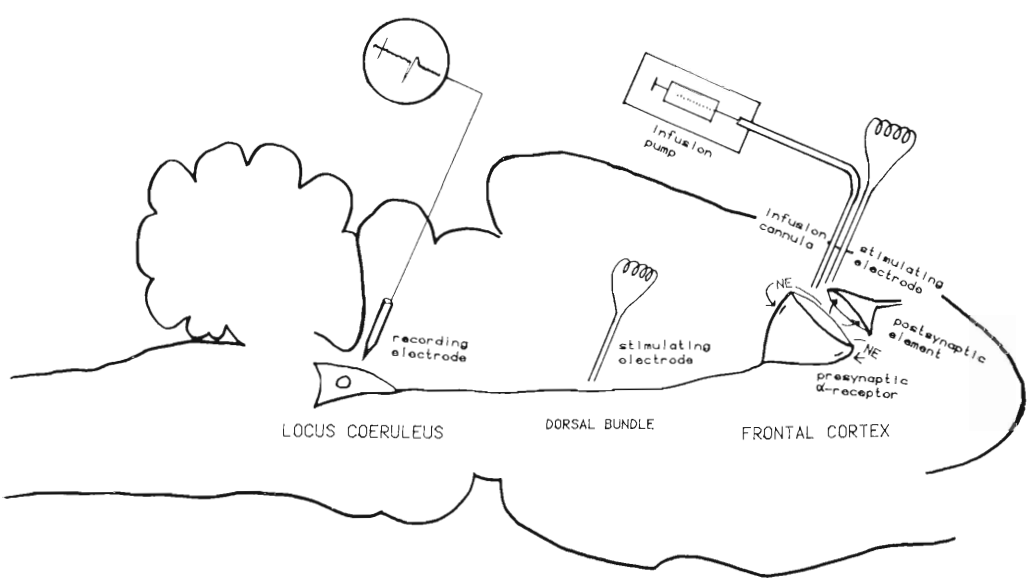

Fig. 1. Schematic diagram of experimental preparation for testing locus coeruleus terminal excitability. Spontaneous and antidromically evoked action potentials were recorded by a micropipette located in n. locus coeruleus. Bipolar stimulating electrodes were implanted into the dorsal noradrenergic pathway (dorsal bundle) and in the terminal field of locus coeruleus neurons in frontal cortex. Drugs were infused into the frontal cortex over a period of 5 min by means of an infusion pump.

at least 2.0 mm distant from the initial site after the completion of the initial infusion and terminal excitability measurements. In control experiments utilizing two stimulating electrodes separated by 2.0 mm, it was found that whereas the thresholds obtained from the infusion site were reliably altered by the local infusion of noradrenergic agents, those obtained from the second electrode, 2.0 mm from the infusion site, remained unchanged in all cases. The experimental paradigm is illustrated schematically in Fig. 1.

RESULTS

A summary of the results of experiments in which various agents were infused directly into frontal cortex is shown in Table I, showing whether the treatment resulted in increased or decreased terminal excitability, or no change. Change in excitability was expressed as per cent of control threshold current. Cases showing changes of less than 10% are shown as no effect in Table I. The latency to the onset of these alterations following local infusion was, on the average, approximately 2 min. None of the infusions exerted any effects on spontaneous firing rate, action potential amplitude or waveform.

Antidromic action potentials evoked by stimulation of the frontal cortical terminal field of a locus coeruleus neuron are depicted in Fig. 2 prior to and following local infusion of the α-adrenergic agonist, clonidine. Illustrated in Fig. 2A are antidromically elicited action potentials prior to drug infusion, in which a minimum current of 0.53 mA was necessary to evoke antidromic potentials on 100% of the non-collision trials. Following local infusion of 10 μM clonidine, the proportion of antidromic invasions drops to approximately 10% (Fig. 2B) and a current of 0.69 mA is now necessary to elicit anti-

TABLE I

The effects of the local infusion of adrenergic and depolarizing agents on the terminal excitability of locus coeruleus neurons

Agent	*N**	*Terminal excitability*		
		Decrease	*Increase*	*No change*
Clonidine	17	14 (37.2% ± 10.2%)**	0	3
Phentolamine	13	0	8 (22.1% ± 8.5%)	5
Isoproterenol	7	0	0	7
Propranolol	10	8 (20.1% ± 3.4%)	0	2
K^+	11	0	7 (17.5% ± 3.6%)	4
Saline	8	0	1 (10.0%)	7

* N indicates the total number of cases tested.

** Numbers in parentheses represent the mean increase/decrease in threshold current as per cent of control values ± S.E.M.

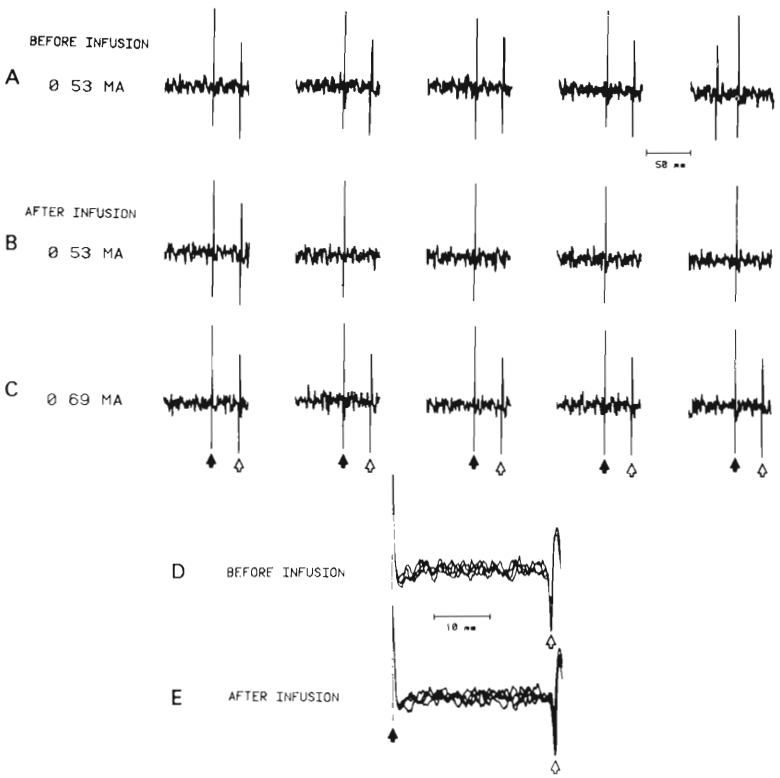

Fig. 2. Action potentials elicited by antidromic stimulation of the terminal field of a locus coeruleus neuron are shown prior to infusion of 0.31 μl of 10 μM clonidine into frontal cortex (A) and following infusion of the drug (B and C). Note the instance of collision in the last trace in A. Before infusion, 0.53 mA stimulus current produced antidromic invasion on 100% of non-collision trials whereas after clonidine infusion this current was nearly ineffective, and an increased stimulus current (0.69 mA) was required to produce 100% invasion. The latency and variability of latency to antidromic invasion are shown prior to (D) and following (E) clonidine infusion. Note the increased latency and variability of latency following drug infusion. Black arrows indicate the stimulus artefact, while white arrows indicate antidromic action potentials.

dromic invasion on 100% of non-collision trials (Fig. 2C). The decrease in terminal excitability produced by the local infusion of clonidine was accompanied by a slight prolongation (0.5–1.0 msec) in the latency to antidromic invasion and an increase in the variability of the antidromic response latency, as shown in Fig. 2D and E.

A typical dose-related decrease in the terminal excitability of an antidromically activated neuron in locus coeruleus following infusions of clonidine into frontal cortex is shown in Fig. 3A. The current necessary to elicit antidromic action potentials is shown on the abscissa, while the proportion of antidromic action potentials evoked on non-collision trials is shown on the ordinate. Note that prior to clonidine infusion, stimulus currents between 0.40 and 0.55 mA were required to evoke antidromic responses on 0–100% of non-collision trials. Following the first drug infusion, the currents necessary to evoke this range of antidromic invasions increased to between 0.80 and 0.92 mA, while a second infusion resulted in a further decline in terminal

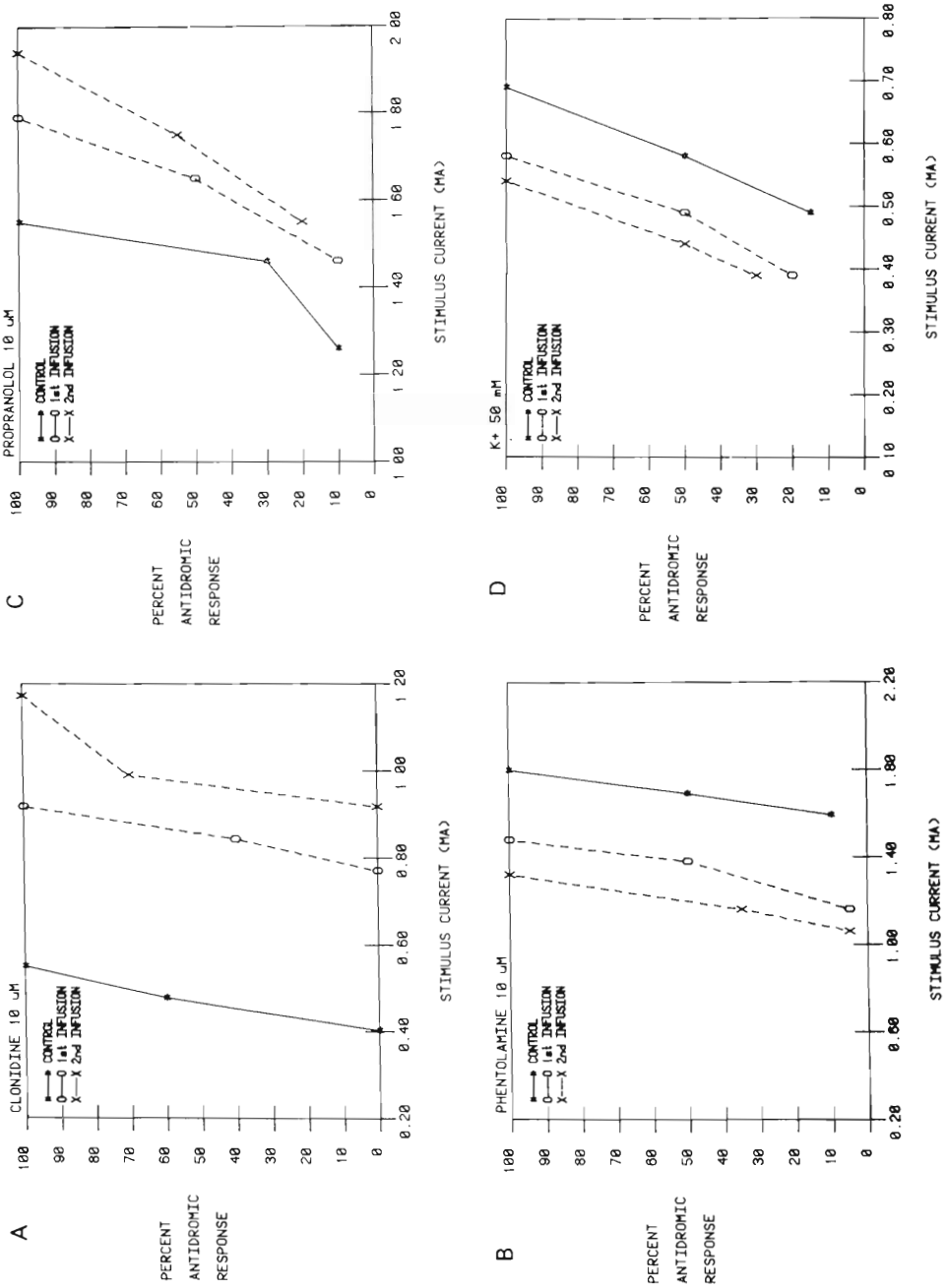

Fig. 3. Dose-related effects of local infusions of various agents into frontal cortex on the excitability of locus coeruleus terminals. In A, infusion of 0.31 μl of 10 μM clonidine produces a marked decrease in the excitability of the terminal field of a locus coeruleus neuron, as revealed by the increase in stimulus currents necessary to produce antidromic invasion. A second infusion produces a further reduction in terminal excitability. In B, local infusion of 0.31 μl of 10 μM phentolamine produces increased terminal excitability of a locus coeruleus neuron as shown by the uniform reduction in stimulus currents needed to produce antidromic activation. A second infusion produces a further increase in terminal excitability. In C, 0.31 μl of 10 μM propranolol infused into frontal cortex produces decreased terminal excitability of a locus coeruleus neuron and a second infusion produces a further decrease in terminal excitability. In D, infusion of 50 mM potassium leads to increased terminal

excitability as shown. Clonidine was infused into the terminal fields of 17 locus coeruleus neurons at a concentration of 1 μM or 10 μM. As depicted in Table I, clonidine infusion resulted in a decrease in terminal excitability of 14 neurons ($\bar{x}$ = 37.2% $\pm$ 10.2%) and had no effect on the remaining 3 cells.

Local infusion of the α-adrenergic antagonist, phentolamine, produced an effect opposite to that of the agonist, as illustrated for one locus coeruleus neuron in Fig. 3B. Prior to phentolamine infusion, this neuron could be antidromically activated on between 0 and 100% of non-collision trials by stimulus currents ranging from 1.50 to 1.80 mA. Following infusion of 10 μM phentolamine, currents effective in eliciting 0–100% antidromic invasion decreased uniformly to the left and ranged between 1.20 and 1.48 mA, an increase in terminal excitability of approximately 22%. A second infusion resulted in a further increase in terminal excitability of about 12%, illustrating that the effect of phentolamine is dose-related. Phentolamine was infused into frontal cortex at concentrations of 1 μM or 10 μM in 13 experiments. In 8 of these cases, phentolamine produced an increase in terminal excitability ($\bar{x}$ = 22.1% $\pm$ 8.5%), while in the remaining 5 cases no change in terminal excitability was observed.

In an effort to determine if phentolamine could reverse the effect of prior clonidine infusion, experiments were carried out on 8 neurons in which prior clonidine infusion had resulted in a marked decrease in terminal excitability. In 6 cases, phentolamine partially or totally reversed the decreased terminal excitability produced by prior infusion of clonidine. In two of these cases, phentolamine not only reversed the effect of clonidine but also increased terminal excitability beyond control levels. In

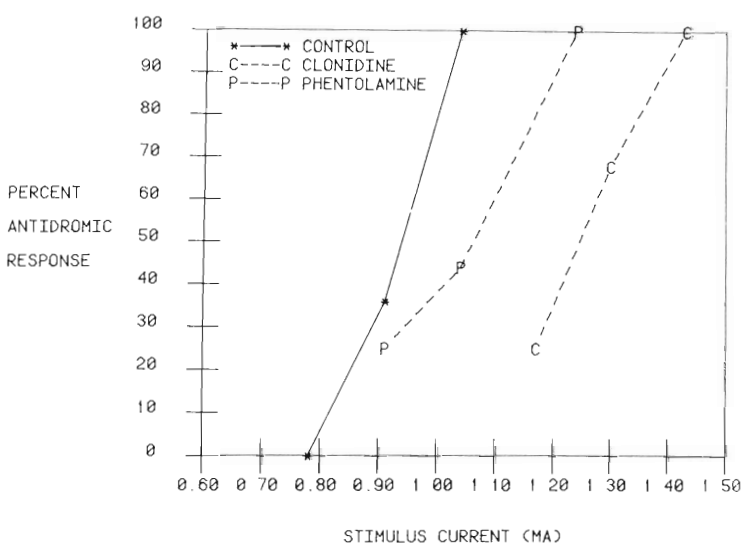

Fig. 4. The reversal by 10 μM phentolamine of decreased terminal excitability produced in a locus coeruleus neuron by prior infusion of 10 μM clonidine into frontal cortex. Stimulus currents necessary to produce antidromic invasion ranged between 0.90 and 1.0 mA prior to drug infusion. Following clonidine infusion, stimulus currents between 1.1 and 1.4 mA were required to produce comparable proportions of antidromic response. A subsequent infusion of phentolamine reversed the change in excitability produced by clonidine.

the remaining 2 cases, phentolamine did not affect the decreased terminal excitability produced by prior clonidine infusion. A typical decrease in terminal excitability produced by clonidine infusion and its partial reversal by subsequent phentolamine infusion are shown in Fig. 4.

In those cases where phentolamine increased terminal excitability, the latency of the antidromic response was typically reduced by approximately 0.5–1.0 ms, and the variability of antidromic response latency also decreased. In general, those treatments which increased terminal excitability led to a decrease in the latency of the antidromic response and in the variability of antidromic response latency. Conversely, those treatments which resulted in a decrease in terminal excitability typically produced an increase in the antidromic response latency and variability.

In order to determine if β-adrenergic receptor activation and blockade could induce changes in noradrenergic terminal excitability, the β-receptor agonist isoproterenol and the β-receptor antagonist, propranolol, were infused into frontal cortex while testing antidromic excitability of locus coeruleus neurons. Isoproterenol was infused into the terminal field of 7 locus coeruleus neurons at concentrations of 0.1 μM, 1 μM and 10 μM and produced no change in terminal excitability. The antagonist, propranolol, however, led to a decrease in terminal excitability in 8 cases ($\bar{x} = 20.1\% \pm 3.4\%$) and had no effect in 2 additional experiments. A typical example of propranolol-induced decrease in terminal excitability is depicted in Fig. 3C.

If increased terminal excitability were resulting from depolarization of noradrenergic terminals, then local infusion of a depolarizing agent such as potassium should produce increased terminal excitability. In 11 experiments, potassium was infused into frontal cortex at concentrations of 50 or 100 mM. A typical example of the increased terminal excitability following 50 mM potassium infusion into frontal cortex is shown

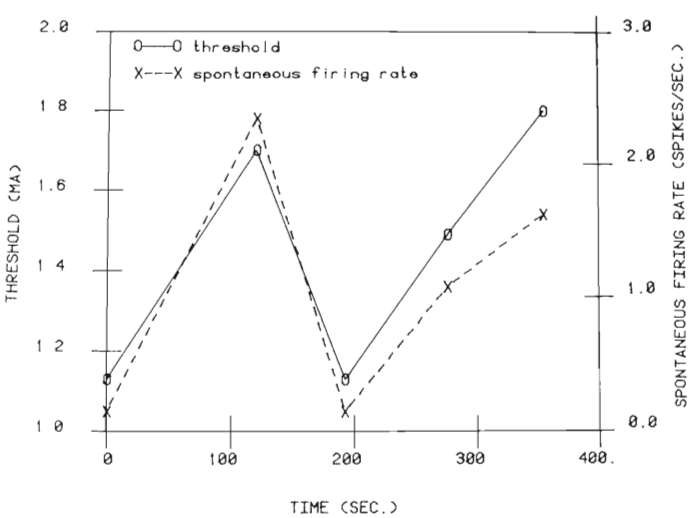

Fig. 5. Relation between spontaneous firing rate and terminal excitability for one locus coeruleus neuron. Note that the threshold for invasion of the neuron to antidromic stimulation (left ordinate) varies directly with changes in spontaneous firing rate (right ordinate).

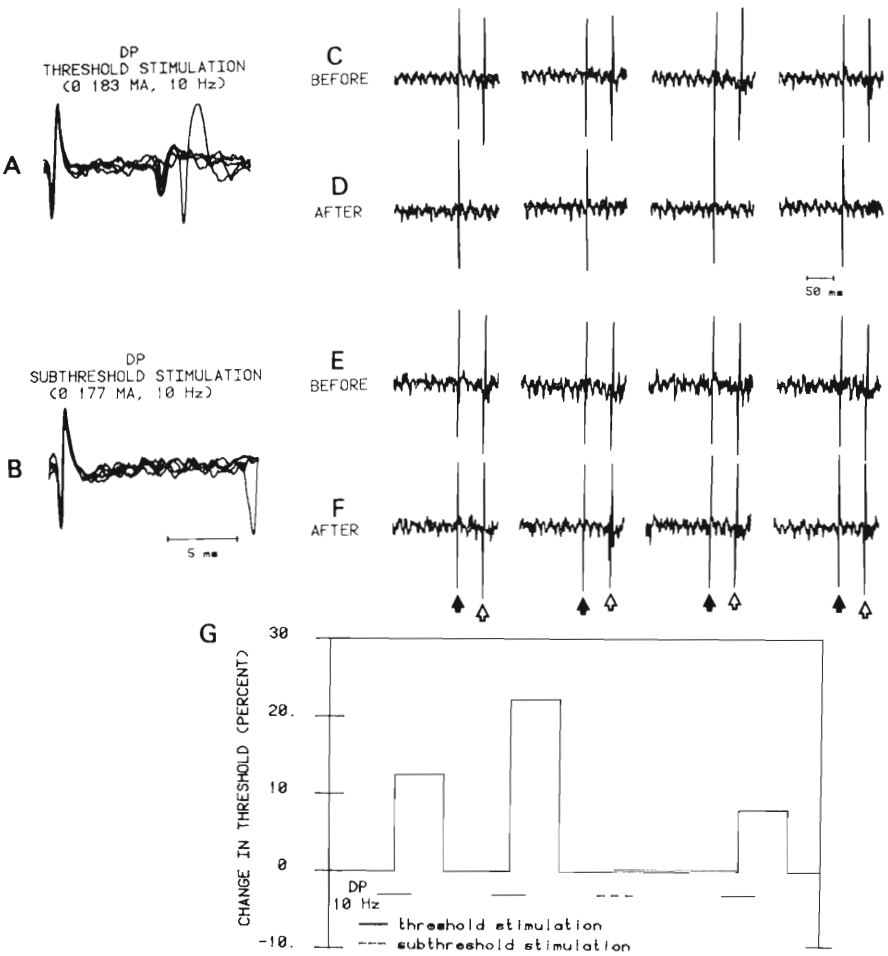

Fig. 6. High frequency stimulation (10 Hz for 10 s) of the dorsal noradrenergic pathway (DP) is followed by a decrease in the excitability of the terminal field of a locus coeruleus neuron. In A, high frequency stimulation at 0.183 mA is sufficient to produce 100% antidromic invasion. Prior to high frequency stimulation, antidromic action potentials are evoked to single test stimuli on 100% of non-collision trials (C) whereas following high frequency stimulation, this stimulus current is not sufficient to produce antidromic invasion (D). Stimulation of the dorsal pathway at a current just below the threshold necessary to evoke antidromic invasion (B) produces no change in terminal excitability (compare traces in E to those in F). Solid arrows indicate stimulus artefact, while white arrows indicate antidromic action potentials. The graph in G summarizes the results of several series of experiments in which high frequency stimulation of the dorsal pathway at a current sufficient to produce antidromic responding (solid line) produces decreased terminal excitability, whereas sub-threshold stimulation, which does not produce antidromic responding (dashed line), has no effect on terminal excitability as determined by subsequent antidromic test stimuli.

in Fig. 3D. Prior to infusion, currents ranging from 0.48 to 0.68 mA were required to evoke between approximately 10 and 100% antidromic invasion. Following the first infusion of potassium, excitability was markedly increased such that similar proportions of antidromic responses could now be evoked by currents ranging from 0.38 to

0.52 mA. A second infusion resulted in a further increase in terminal excitability, as shown.

Infusion of 50 mM potassium resulted in increased terminal excitability in 5 cases ($\bar{x} = 15.8\% \pm 2.3\%$) while having no effect in 4 others. Infusion of 100 mM potassium resulted in increased terminal excitability in both cases in which it was attempted ($\bar{x} = 35.4\% \pm 2.1\%$).

In order to control for various non-pharmacological effects of local infusion such as volume or pressure changes at the infusion site, local infusion of 0.9% saline was performed in 8 experiments. In 7 of these cases, saline infusions had no effect on terminal excitability; however, in 1 case there was a 10% increase in terminal excitability. It is worth noting that the latency to this effect was approximately 4 min following infusion, which is nearly twice as long as the latencies to excitability changes induced by other infusion treatments.

In the course of the experiments, the terminal excitability of locus coeruleus neurons was frequently found to vary with changes in the spontaneous firing rate. Invariably, decreased terminal excitability was associated with increased spontaneous firing rate, and conversely, increased terminal excitability with decreased spontaneous firing rate. A typical example of impulse related changes in the terminal excitability is shown in Fig. 5. The initial threshold for antidromic invasion in this neuron was 0.113 mA at a spontaneous firing rate of 0.1/s. When the firing rate spontaneously increased to 2.6/s, the threshold increased to 0.17 mA. Subsequent decreases and increases in the spontaneous firing rate led to similar changes in the threshold.

Based on this observation, it seemed possible that terminal excitability could be reduced by increasing the frequency of impulses reaching the terminals. In order to test this possibility, the threshold for the antidromic response from frontal cortex was compared before and after 10 Hz stimulation of the dorsal pathway. This result is shown in Fig. 6. After 10 Hz stimulation of the dorsal pathway by a threshold current of 0.183 mA (traces shown in Fig. 6A), stimulation of the frontal cortex at a current which was effective before 10 Hz stimulation (traces in Fig. 6C) no longer activated the antidromic response (Fig. 6D). In contrast, 10 Hz stimulation of the dorsal pathway by a subthreshold current (0.177 mA) (traces in Fig. 6B) only slightly lower than the threshold current did not cause any change in the firing probability of the frontal cortex-induced antidromic response (see traces in Fig. 6E and F). This result is summarized in Fig. 6G, in which the threshold for the antidromic response from frontal cortex can be seen to be increased repeatedly after 10 Hz threshold stimulation of the dorsal pathway, but not after subthreshold stimulation.

High frequency stimulation of the terminal field also produced changes in terminal excitability, as illustrated for one locus coeruleus neuron in Fig. 7. High frequency stimulation (10 Hz) was delivered to the frontal cortical terminal field of a locus coeruleus neuron for 10 s. There was an initial increase in the antidromic excitability during stimulation, followed by a decrease which lasted for periods ranging from 5 to 20 s following termination of stimulation (Fig. 7A, B, C). The increase in terminal excitability during high frequency stimulation is clearly illustrated in Fig. 7D and E. Fig. 7D depicts the absence of any antidromic responses to a

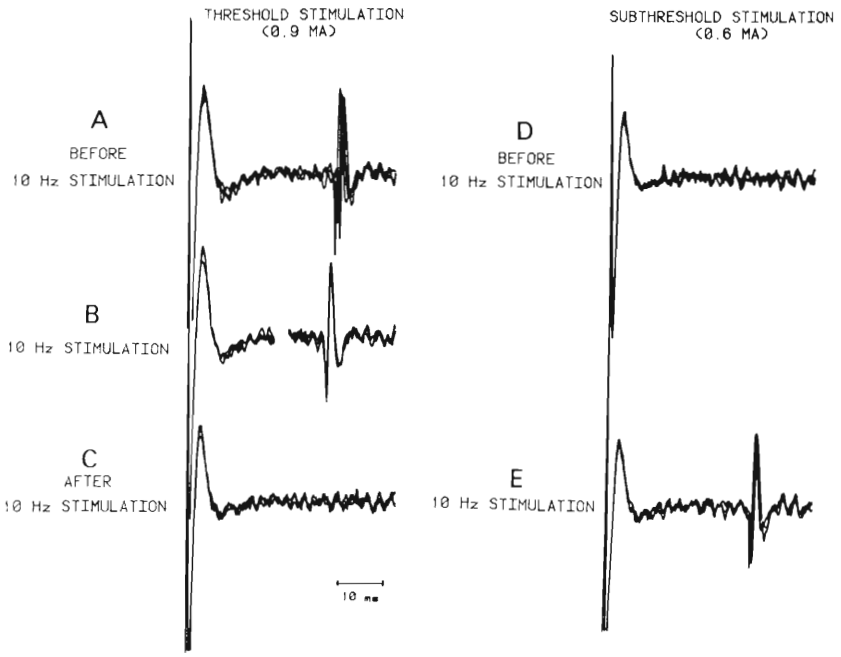

Fig. 7. High frequency stimulation of frontal cortex (10 Hz for 10 s) alters locus coeruleus terminal excitability. In A, antidromic action potentials are shown prior to high frequency stimulation. During stimulation, excitability increases with a concomitant decrease in antidromic response latency and variability (B) and, following termination of stimulation, the terminal excitability decreases markedly for 5–20 s (C). In D, a stimulus current of 0.60 mA is insufficient to elicit any antidromic responses when delivered at 1 Hz, but is at threshold when delivered at 10 Hz (E).

stimulus current of 0.60 mA delivered at 1/s. However, when this same current is delivered at 10/s there is 100% antidromic response (Fig. 7E).

While stimulation of the terminal field or dorsal pathway induced changes in terminal excitability of locus coeruleus neurons, no changes in the excitability of noradrenergic axons to stimulation of the dorsal pathway resulted from these manipulations, showing that these changes are specific for locus coeruleus terminal fields.

DISCUSSION

The results of our experiments suggest that stimulation of α-adrenergic presynaptic receptors by the α-agonist, clonidine, infused directly into the terminal region of locus coeruleus neurons, leads to a marked dose-dependent decrease in terminal excitability to direct electrical stimulation. Further, blockade of these receptors by direct local application of the α-antagonist, phentolamine, leads to an increase in terminal excitability. These results are consistent with those from biochemical studies showing that activation of α-receptors on noradrenergic terminals inhibits electrical stimulation or potassium-induced release of norepinephrine, while blockade of these

presumed presynaptic receptors increases the release of noradrenaline[5–7,16,20,31–33]. It is therefore possible that changes in terminal excitability reveal underlying processes that may play a part in the presynaptic regulation of neurotransmitter release shown in biochemical experiments. It is also conceivable that processes revealed by this paradigm are involved in the regulation of neurotransmitter biosynthesis, since both of these presynaptic phenomena have been suggested to occur in another catecholaminergic system, the nigro-neostriatal dopaminergic projection originating in the pars compacta of the substantia nigra[11,35].

While it has been proposed that β-adrenergic presynaptic receptors may regulate neurotransmitter release at noradrenergic terminals, such regulation has not been consistently reported[16,28,32,33]. Infusion of the β-antagonist, propranolol, led to a significant decrease in terminal excitability, while infusion of the β-agonist, isoproterenol, had no effect on terminal excitability. It is conceivable that β-adrenergic presynaptic receptors do exist on the noradrenergic terminals but are fully occupied by the endogenous ligand under the present experimental condition, since the antagonist, but not the exogenously applied agonist, was effective in altering terminal excitability. If β-adrenergic presynaptic receptors do exist on the terminals of noradrenergic neurons, the biochemical and neurophysiological results suggest that their regulatory influence opposes that of the α-adrenergic presynaptic receptors, although the evidence for this is inconclusive at present.

Further evidence for presynaptic feedback by norepinephrine released from the synaptic ending was obtained in the present experiments. Terminal excitability was found to covary with impulse traffic in noradrenergic neurons, increased impulse activity producing decreased terminal excitability. Such alterations would certainly be expected since the amount of norepinephrine released from the synaptic terminals, and therefore the amount of ligand available for presynaptic receptor activation, should vary directly with impulse traffic. Further evidence in favor of this view was derived from experiments in which similar changes in terminal excitability occurred when impulse traffic was manipulated directly by repetitive stimulation of the noradrenergic axons in the dorsal noradrenergic bundle or high frequency stimulation of the terminal field itself. In both cases, terminal excitability was decreased following increased impulse traffic, a result similar to pharmacological activation of the receptor by the local application of clonidine. In contrast, increased impulse traffic produced by high frequency stimulation had no effect on the excitability of noradrenergic axons in the dorsal pathway. Thus, we conclude that the changes in terminal excitability observed here are specific to the terminal field of locus coeruleus neurons and do not represent alterations in the excitability of noradrenergic axons.

Changes in the excitability of the terminal field are often attributed to alterations in the state of polarization of the terminal, increased excitability being attributed to depolarization of the synaptic ending and decreased antidromic excitability being attributed to hyperpolarization of the terminal field[8,34]. This interpretation is consistent with our observations that local infusion of potassium led to increased terminal excitability, a consequence attributable to this agent's depolarizing effects, although it is also possible that the excitability changes observed from administration of

adrenergic agents may depend more critically on other membrane properties such as conductance.

At least in the case of primary afferent depolarization, which is believed to underlie presynaptic inhibition in mammalian spinal cord as well as several invertebrate systems in which this phenomenon has been characterized[8,17,24,34], decreased transmitter release is believed to be accompanied by depolarization of the terminal field. On the other hand, the results of the present experiments would seem to indicate that decreased transmitter release may be accompanied by a hyperpolarization of the synaptic ending. Indeed, there are now several chemical synapses in which hyperpolarization of the presynaptic element seems to accompany presynaptic inhibition[24,25,27,29].

In at least one system where presynaptic inhibition has been demonstrated, the decreased transmitter release has been attributed to decreased calcium influx[6,25]. Although presynaptic inhibition is currently believed to occur heterosynaptically by means of axo-axonic synapses, activation of presynaptic receptors on the terminals of catecholaminergic neurons seems to occur without the intervention of interneurons or axo-axonic synapses, since examination of catecholaminergic synaptic endings has never produced morphological evidence for such a mechanism[10,26].

Several possible mechanisms exist which could regulate calcium entry into the synaptic ending, including a direct neurotransmitter effect on the calcium ionophore, an alteration consistent with a reduced voltage-dependent calcium current and others. Decreased neurotransmitter release during presynaptic inhibition in the spinal cord has been attributed to depolarization of the synaptic ending and an associated reduction in action potential amplitude invading the terminal[8,24,34]. On the other hand, if the action potential were to spread passively across the synaptic terminal, then depolarization would favor increased voltage-dependent calcium influx and increased neurotransmitter release, while hyperpolarization of the synaptic terminal would favor decreased transmitter release. Further experiments on the relation of changes in terminal excitability and presynaptic regulation of transmitter release should continue to clarify the mechanisms of presynaptic inhibition, including alterations in membrane polarization, conductance and other biophysical parameters relevant to transmitter release and its neuropharmacological basis.

ACKNOWLEDGEMENT

This work was supported in part by Grant DA 02854-01 and Research Scientist Development Award DA 00079-08 from the National Institute on Drug Abuse to Philip M. Groves.

REFERENCES

1 Aghajanian, E. K. and Bunney, B. S., Dopamine 'autoreceptors': Pharmacological characterization by microiontophoretic single cell recording studies, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 297 (1977) 1–7.

2 Aghajanian, G. K., Cedarbaum, J. M. and Wang, R. Y., Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons, *Brain Research*, 136 (1977) 570–577.
3 Bunney, B. S., Aghajanian, G. K. and Roth, R. H., Comparison of the effects of L-Dopa. amphetamine and apomorphine on firing rate of rat dopaminergic neurons, *Nature New Biol.*, 245 (1973) 123–125.
4 Cedarbaum, J. M. and Aghajanian, G. K., Catecholamine receptors on locus coeruleus neurons: pharmacological characterization, *Europ. J. Pharmacol.*, 44 (1977) 375–385.
5 De Langen, C. D., Hogenboom, F. and Mulder, A. H., Presynaptic noradrenergic alpha-receptors and modulation of ^{3}H-noradrenaline release from rat brain synaptosomes, *Europ. J. Pharmacol.*, 60 (1979) 79–89.
6 De Langen, C. D. and Mulder, A. H., On the role of calcium ions in the presynaptic alpha-receptor mediated inhibition of ^{3}H-noradrenaline release from rat brain cortex synaptosomes, *Brain Research*, 185 (1980) 399–408.
7 Dismukes, R. K. and Mulder, A. H., Cyclic AMP and alpha-receptor mediated modulation of noradrenaline release from rat brain slices, *Europ. J. Pharmacol.*, 39 (1976) 383–388.
8 Eccles, J. C. and Krnjevic, K., Potential changes recorded inside primary afferent fibers within the spinal cord, *J. Physiol. (Lond.)*, 149 (1959) 250–273.
9 Farnebo, L. O. and Hamberger, B., Drug induced changes in the release of ^{3}H-monoamines from field stimulated rat brain slices, *Acta physiol. scand.* Suppl. 371 (1971) 35–41.
10 Groves, P. M., Synaptic endings and their postsynaptic targets in neostriatum: synaptic specializations revealed from analysis of serial sections, *Proc. nat. Acad. Sci. (U.S.A.)*, 77 (1980) 6926–6929.
11 Groves, P. M., Fenster, G. A., Tepper, J. M., Nakamura, S. and Young, S. J., Changes in dopaminergic terminal excitability induced by amphetamine and haloperidol, *Brain Research*, (1981) submitted.
12 Groves, P. M., Staunton, D. A., Wilson, C. J. and Young, S. J., Sites of action of amphetamine intrinsic to catecholaminergic nuclei: catecholaminergic presynaptic dendrites and axon terminals, *Progr. Neuropsychopharmacol.*, 3 (1979) 315–335.
13 Groves, P. M., Wilson, C. J., Young, S. J. and Rebec, G. V., Self-inhibition by dopaminergic neurons, *Science*, 190 (1975) 522–529.
14 Huang, Y. H. and Maas, J. W., Stimulation of the dorsal noradrenergic bundle and field potentials in the locus coeruleus, *Brain Research*, 115 (1976) 91–94.
15 König, J. F. R. and Klippel, R. A., *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Part of the Brain Stem*, Williams and Wilkins, Baltimore, MD, 1963.
16 Langer, S. Z., Presynaptic receptors and their role in the regulation of transmitter release, *Brit. J. Pharmacol.*, 60 (1977) 481–497.
17 Levy, R. A., Presynaptic control of input to the central nervous system, *Canad. J. Physiol. Pharmacol.*, 58 (1980) 751–766.
18 Montel, H., Starke, K. and Taube, H., Influence of morphine and naloxone on the release of noradrenaline from cerebellar cortex slices, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 288 (1975) 427–433.
19 Montel, H., Starke, K. and Weber, F., Influence of morphine and naloxone on the release of noradrenaline from rat brain cortex slices, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 283 (1974) 357–369.
20 Mulder, A. H., Wemer, J. and de Langen, C. D. J., Presynaptic receptor-mediated inhibition of noradrenaline release from brain slices and synaptosomes by noradrenaline and adrenaline. In S. Z. Langer, K. Starke and M. L. Dubocovich (Eds.), *Presynaptic Receptors, Advances in the Biosciences, Vol. 18*, Pergamon Press, Oxford, 1979, pp. 219–224.
21 Nakamura, S., Some electrophysiological properties of neurons of rat locus coeruleus, *J. Physiol. (Lond)*, 267 (1977) 641–658.
22 Nakamura, S. and Iwama, K., Antidromic activation of the rat locus coeruleus neurons from hippocampus, cerebral and cerebellar cortices, *Brain Research*, 99 (1975) 373–376.
23 Nakamura, S., Tepper, J. M., Young, S. J. and Groves, P. M., Modifications in the excitability of locus coeruleus synaptic terminals by adrenergic agents, *Neurosci. Abstr.*, 6 (1980) 449.
24 Nicoll, R. A. and Alger, B. E., Presynaptic inhibition: transmitter and ionic mechanisms, *Int. Rev. Neurobiol.*, 21 (1980) 217–258.
25 Nicholls, J. and Wallace, B. G., Modulation of transmission at an inhibitory synapse in the central nervous system of the leech, *J. Physiol. (Lond.)*, 281 (1978) 157–170.
26 Olschowka, J. A., Grzanna, R. and Molliver, M. E., The distribution and incidence of synaptic contacts of noradrenergic varicosities in the rat neocortex: an immunocytochemical study, *Neurosci. Abstr.*, 6 (1980) 352.

27 Shapiro, E., Castellucci, V. F. and Kandel, E. R., Presynaptic inhibition in *Aplysia* involves a decrease in the Ca^{2+} current of the presynaptic neuron, *Proc. nat. Acad. Sci. (U.S.A.)*, 77 (1980) 629–633.
28 Shenoy, A. K. and Ziance, R. J., Comparative regulation of potassium and amphetamine induced release of ^{3}H-norepinephrine from rat brain via presynaptic mechanism, *Life Sci.*, 24 (1979) 255–264.
29 Shimahara, T. and Tauc, L., Multiple interneuronal afferents to the giant cells in *Aplysia*, *J. Physiol. (Lond.)* 247 (1975) 299–319.
30 Starke, K., Presynaptic receptors and the control of noradrenaline release, *TIPS*, 6 (1980) 268–271.
31 Starke, K. and Montel, H., Alpha-receptor mediated modulation of transmitter release from central noradrenergic neurons, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 279 (1973) 53–60.
32 Starke, K., Taube, H., D. and Borowski, E., Pre- and post-synaptic receptors in catecholaminergic transmission, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 297 (1977) 43–44.
33 Taube, H. D., Starke, K. and Borowski, E., Presynaptic receptor systems on the noradrenergic neurons of rat brain, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 299 (1977) 123–141.
34 Wall, P. D., Excitability changes in afferent fiber terminations and their relation to slow potentials, *J. Physiol. (Lond.)*, 142 (1958) 1–21.
35 Walters, J. R. and Roth, R. H., Dopaminergic neurons: drug-induced antagonism of the increase in tyrosine hydroxylase activity produced by cessation of impulse flow, *J. Pharmacol. exp. Ther.*, 191 (1974) 82–91.
36 Westfall, T. C., Local regulation of adrenergic neurotransmission, *Physiol. Rev.*, 57 (1977) 659–729.