Noradrenergic Modulation of Sensorimotor Processes in Intact Rats: The Masseteric Reflex as a Model System¹

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Abstract

The masseteric jaw closure reflex was utilized as a model system with which to gauge the functional activity of central noradrenergic neurons. This system was chosen because it is a simple monosynaptic reflex the neuronal substrate of which receives a dense noradrenergic input. The modulatory effects of norepinephrine (NE) on this response in the intact, chloral hydrate-anesthetized rat were studied with a variety of pharmacological strategies.

Initially, a reflex facilitation was obtained with the catecholamine precursor L-DOPA. Manipulations with greater specificity of action on the noradrenergic system were then employed. First, we used the presynaptic α -2 noradrenergic agonist clonidine, which acts to decrease noradrenergic transmission. Clonidine attenuated the amplitude of the reflex, and this suppression was blocked by pretreatment with the α -2 antagonist yohimbine. The effects of yohimbine itself were then examined, and a biphasic effect was obtained. At low doses, at which it preferentially acts as an antagonist at presynaptic α -2 receptors and increases noradrenergic transmission, yohimbine enhanced the reflex. At higher doses, at which it also displays postsynaptic α -1 antagonist activity, yohimbine depressed the reflex. This reflex modulation by yohimbine was blocked by pretreatment with the α -1 antagonist prazosin.

The anatomical site of the observed effect was then localized to the direct noradrenergic innervation of the reflex circuitry by locally destroying, with 6-hydroxydopamine, the noradrenergic terminals in the trigeminal motor nucleus mediating the response. This significantly attenuated the reflex modulation by yohimbine, without affecting elicitation of the reflex itself. Thus, it was concluded that NE facilitates the masseteric reflex, and this facilitation is mediated directly by the noradrenergic input into the motor nucleus. To our knowledge, these represent the first studies to demonstrate modulation of a simple behavioral response by NE, at a specified site mediating that response, in intact animals. The relation of these studies to demonstrations of cellular modulation by NE is discussed.

Recently, much progress has been made in understanding various aspects of the brain noradrenergic neuronal system. Knowledge of the anatomy, pharmacology, and physiology of CNS norepinephrine (NE) has been greatly expanded, as has a more recent understanding of the effects of NE on its target neurons. Nevertheless, despite many investigations examining the role of NE in physiology and behavior, no clear and consistent picture has yet emerged. One thing that is needed is a reliable index of the functional effects of these neurons under normal physiological conditions. Therefore, the goals of the present series of studies are to establish such a functional model for investigating the effects of NE and to employ this model to examine whether NE influences sensorimotor processes in a modulatory fashion at the behavioral level, as has been suggested by several studies at the cellular level (e.g. Woodward et al., 1979).

There is indeed previous evidence to suggest that NE may modulate the reactivity of certain reflexive neuronal circuits. However, most of these studies have used lesioned [e.g., 6-hydroxydopamine (6-OHDA)] or reduced (e.g., spinal) preparations. The relevance of these results for demonstrating the normal physiological or behavioral role for NE, therefore, remains undetermined. In studies which have examined the effect of NE in intact animals, the complexity of the behavioral or physiological test system has precluded specification of the site and/or mechanism of action of NE. For instance, L-DOPA or clonidine enhanced the hindlimb flexor reflex in acutely spinalized rats that had been depleted of catecholamines previously (Anden et al., 1970) or in rats made supersensitive to NE by destruction of spinal noradrenergic terminals with 6-OHDA (Nygren and Olson, 1976). Hino et al. (1984), using chlorpromazine, suggested that descending NE exerts a tonic facilitatory effect on mono- and polysynaptic spinal reflexes elicited by stimulating the dorsal root and recording from the ventral root of rats. Also, locus coeruleus stimulation facilitated the monosynaptic reflex elicited by stimulating the exposed spinal dorsal root and recorded from the ventral root of the decerebrate cat (Strahlendorf et al., 1980). In addition, it has been shown that NE administered intrathecally enhances acoustic startle in rats (Astrachan and Davis, 1981).

The present study investigates the possible modulatory effects of noradrenergic manipulations on the responsivity of the masseteric jaw-closure reflex in the intact, anesthetized rat. The masseteric reflex is a monosynaptic brainstem reflex. Stretch receptors in the masseter muscle spindles with cell bodies in the mesencephalic nucleus of the trigeminal nerve (Me5) synapse upon motoneurons of the trigeminal motor nucleus (Mo5) which, in turn, directly innervate the masseter jaw muscle.

There are a number of reasons for examining the masseteric reflex as a possible indicator of noradrenergic function. First, it is a simple and well-understood circuit. Second, the response can easily be elicited and quantified in the intact animal. Third, there is a dense noradrenergic innervation of Mo5 arising from the lateral tegmental noradrenergic group (Levitt and Moore, 1979; Vornov and Sutin, 1983). Fourth, there is evidence that the masseteric reflex is modu-

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lated across the sleep-wake cycle (Chase and Babb, 1973) and by a variety of stimuli (Sauerland et al., 1967; Chase et al., 1970; Chase, 1980; Wyrwicka et al., 1982; Pettorossi, 1983). Finally, because the reflex can be elicited and recorded in the freely moving, unanesthetized cat (Chase and Babb, 1973) it can be used to examine modulation under physiological conditions. In these initial stages, however, we chose to use rats because it was felt that this would be less costly in terms of time, effort, and resources, while at the same time affording us a greater degree of freedom in terms of experimental manipulations than would the use of chronic cats. We observed, however, in pilot studies with freely moving, unanesthetized rats, that the high spontaneous background level of activity in the jaw muscles of these gnawing rodents made it impossible to observe and record single reflexively elicited responses. These observations, along with a desire to maintain a high degree of control, at least initially, over extraneous behavioral variables which may alter the level of noradrenergic transmission, such as spontaneous fluctuations in arousal, made the use of anesthesia advantageous. Thus, the present work was carried out on intact but anesthetized rats.

In this series of investigations, the masseteric reflex was elicited by direct electrical stimulation of the sensory neuronal cell bodies located in Me5. Systemically administered pharmacological agents were used to manipulate noradrenergic neurotransmission, and an attempt was made to distinguish between pre- and postsynaptic effects of these agents in order to clarify the role played by endogenous NE in their reflex-modulatory actions. An attempt was also made to specify the anatomical site of this effect as being the noradrenergic input to Mo5 by destroying that input with a local injection of a catecholamine-specific neurotoxin. We observed that agents which affect noradrenergic neurotransmission do indeed exert a modulatory influence on the masseteric reflex and that at least part of this influence can be localized to the noradrenergic input into Mo5.

Materials and Methods

Subjects and apparatus

Male Sprague-Dawley albino rats bred in our colony and weighing between 250 to 350 gm were used as subjects. Masseter muscle activity was recorded with two platinum-alloy electrodes (Grass Instruments, model E2B) inserted directly into the muscle. The resulting signal was band-pass filtered (half-amplitude at 0.3 Hz and 3.0 KHz) and amplified (Grass, model P5 preamp) before being displayed on a storage oscilloscope for direct measurement.

The reflex was elicited by delivering a square wave pulse from a Grass S48 stimulator into Me5 through concentric bipolar electrodes (0.007-inch-diameter stainless steel wire within a 24 gauge stainless steel outer electrode; outer diameter 0.035 inch), insulated except at the tips. Stimulation was isolated from ground (Grass, model SIU5 stimulus isolation unit) and delivered as constant current (Grass, model CCU1A constant current unit). Optimal response was usually obtained with a stimulus duration of 0.10 to 0.40 msec. Current intensity was 50 to 500 μ A. A pulse triggering the oscilloscope sweep preceded stimulation by 4.0 msec.

Procedure

Reflex elicitation and measurement. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). Subjects in which drugs were to be administered intravenously were then prepared with a right jugular intravenous catheter. The animal was placed in a stereotaxic instrument, and coordinates for Me5 ($P = -1.1$ mm from lambda, $L = 1.5$ mm, $H = -6.8$ to -7.4 mm from skull surface) were determined from the atlas of Paxinos and Watson (1982). Stimulation was initially delivered at a rate of 1 Hz at 0.6 msec duration and 500 μ A beginning as the electrode entered the brain. The electrode was slowly lowered, and the muscle activity was continuously monitored as Me5 was approached until a response was obtained. The stimulus duration was then decreased, and electrode placement was adjusted to provide maximal response to minimal stimulation.

A base line current-response profile was obtained by administering stimuli at a rate of 0.1 Hz at five or six different current levels beginning, when possible, at a subthreshold level. Current was increased in steps of 50 μ A, and never exceeded 500 μ A. Three stimuli were delivered at each current

level in an ascending order and three in a descending order for a total of six stimuli at each current level.

After establishing the base line response profile, drug was administered, and the procedure was repeated 0 to 5 min after, and then at 10- to 15-min intervals following drug injection. In those studies in which a dose-response analysis was performed for a drug, multiple injections were made, and the procedure was repeated after each dose. Typically, when drugs were administered intravenously, effects were seen almost immediately; effects generally lasted the entire time of observation, which was, at most, 40 to 60 min, depending on the treatment. No attempt was made to follow drug responses to recovery, since the level of anesthesia often began to change at this point, thus confounding observations.

The elicited response was judged to be reflexive in nature, based on two criteria: the response amplitude, while increasing with increasing current, was nevertheless variable from trial to trial at any given current level; also, response latency was at least 2 msec. In those cases in which it was suspected that current spread caused direct stimulation of the motoneurons in Mo5, the response amplitude was constant from trial to trial, and the response latency was typically 1 msec or less. In such cases, an attempt was made to adjust electrode placement, current intensity, and/or stimulus duration so as to eliminate the direct motor stimulation. If that was not possible, the session was terminated for that subject. Electrode placement was histologically verified for a subset of animals.

In a pilot study, we observed that administration of the catecholamine precursor L-DOPA (50.0 mg/kg, i.p.) to animals previously depleted of monoamines by pretreatment with reserpine greatly enhanced the reflex response. Having demonstrated the viability of our procedure with this rather coarse treatment, the first study in the present series was an extension of this finding to intact subjects. Two groups of animals received either L-DOPA (50.0 mg/kg, i.p.) or an equivalent volume of saline with no pretreatment. Two lines of inquiry were then pursued to specify the mechanism of the observed effect. The first entailed use of several pharmacological means to verify the noradrenergic nature of the modulation, since L-DOPA is also a precursor for dopamine. The second was an attempt to anatomically specify the site of the effect as being the noradrenergic innervation of Mo5.

Neurochemical identification. In order to identify the neurochemical nature of the observed effect, the noradrenergic agonist clonidine (CLO) was used. CLO acts as an agonist at presynaptic α -2 adrenoreceptors, thought to be involved in the autoinhibition of noradrenergic neuronal transmission (Starke and Altmann, 1973; Svensson et al., 1975; Andrade and Aghajanian, 1982). There is an advantage in using a presynaptic agonist such as CLO in that, rather than activating postsynaptic effects with an exogenous agent, the endogenous NE innervation is what is manipulated. At high doses, CLO also tends to exhibit only partial postsynaptic α -agonist activity (Roach et al., 1983). To determine the effective dose of CLO in this paradigm, cumulative intravenous doses of 10, 30, 130, and 230 μ g/kg were administered, and the reflex response pattern was measured after each dose. Saline was injected as a control. Clonidine was also administered in single doses of 30 or 130 μ g/kg to control for any effects peculiar to cumulative dose injections. The ability of a single injection of CLO (130 μ g/kg, the most effective dose as determined above) to be blocked by pretreatment with the preferential presynaptic α -2 antagonist yohimbine (YOH; 0.50 mg/kg, i.v., given 30 min prior to CLO) was then assessed.

While YOH is preferential for presynaptic α -2 receptors, it does demonstrate postsynaptic α -antagonist activity at higher doses (Starke et al., 1975). Therefore, both to specify further the noradrenergic nature of this modulation and to clarify and parcel out the pre- versus postsynaptic effects of this system on the reflex modulation, a dose-response analysis of YOH was performed. YOH was administered in cumulative intravenous doses of 0.25, 0.50, 0.75, and 1.00 mg/kg, and the reflex response pattern was determined 5 min after each injection. It was expected that the lowest dose of YOH would stimulate release of NE by blocking presynaptic autoreceptors and that the highest dose would act directly as a postsynaptic antagonist, thereby decreasing effective noradrenergic transmission. YOH was also administered in a single dose of 1.0 mg/kg.

To verify further the noradrenergic nature of the effect seen with YOH, as well as to investigate the receptor subtype mediating the effect, the YOH dose-response analysis was carried out in animals that had been pretreated 40 min before testing with prazosin (PRAZ; 10 mg/kg, i.p.), an α -1 noradrenergic antagonist. An α -antagonist was studied because previous studies of motoneurons in the facial nucleus (McCall and Aghajanian, 1979, 1980) and spinal cord (White and Neuman, 1980, 1983) have suggested that the facilitatory effects of NE may be mediated postsynaptically by an α -receptor.

Anatomical localization. To determine whether the site of action of the manipulations described above was the noradrenergic innervation of Mo5,

the YOH dose-response analysis was performed on animals pretreated 6 to 8 days prior with a unilateral injection of the catecholamine-specific neurotoxin 6-OHDA into Mo5. Animals were anesthetized with chloral hydrate (400 mg/kg) and placed in a stereotaxic instrument. The 6-OHDA was injected in a volume of 1.0 μ l at a concentration of 4.0 μ g/ μ l, measured as the free base. The toxin was mixed fresh before the injections into a vehicle of 0.2% ascorbate in saline. A cannula, loaded with toxin just before entering the brain, was lowered to coordinates P -0.6 mm from lambda, L 2.0 mm, H -8.8 mm from the skull surface (Paxinos and Watson, 1982). Toxin was injected at a rate of 0.5 μ l/min, after which the cannula remained in place for 3 min to allow for diffusion. Controls were injected with 1.0 μ l of vehicle.

Immediately after testing, the animals were decapitated, and the brains were rapidly removed and stored in liquid nitrogen. A small portion of brainstem containing Mo5 was punched out while still frozen. Assays were performed by high pressure liquid chromatography with electrochemical detection (Mayer and Shoup, 1983) to determine percentage depletion of NE as compared to vehicle-injection controls.

Drugs

Drugs used were L-DOPA (Sigma Chemical Co.), clonidine hydrochloride (Boehringer Ingelheim Ltd.), yohimbine hydrochloride (Sigma); prazosin hydrochloride (Pfizer); and 6-hydroxydopamine hydrobromide (Sigma). Doses are expressed as the salt, except for 6-OHDA, which is expressed as the free base. All drugs with the exception of prazosin were dissolved in physiological saline. Prazosin was first dissolved in a small quantity of hot *N,N*-dimethylacetamide (Eastman Kodak Co.) and then diluted to 5.0 mg/ml with hot 5% dextrose in distilled water.

Statistical analyses

Effects of single dose of drug over time or cumulative-dose effects were first analyzed by a repeated-measures analysis of variance; posthoc analyses were then performed with the Newman-Keuls test. Significance in all tests was set at the $p < 0.05$ level.

Results

Neurochemical identification

Figure 1 is an example of a typical masseteric reflex response displayed on an oscilloscope.

Saline. Neither intraperitoneal ($n = 3$) nor intravenous ($n = 6$) administrations of saline alone in volumes comparable to those of the other drugs used in these studies produced any significant effects on the reflex responses ($F = 0.31$ and $F = 0.37$, respectively).

L-DOPA. Administration of L-DOPA (50.0 mg/kg, i.p.; $n = 6$) to

Figure 1. Oscilloscope display of a typical response recorded from the masseter muscle after stimulation in Me5 (arrow). The measure of response amplitude is the peak-to-peak value of the response, expressed in microvolts. Stimulus parameters were 270- μ A, 0.15-msec-duration square wave pulse.

intact animals produced significant enhancement of the reflex ($F = 3.64$; Table I). This, then, provided a basis for further investigation of noradrenergic modulation of this system by demonstrating that catecholaminergic manipulations can have effects on the reflex.

Clonidine. Analysis of the cumulative dose-response ($n = 4$) revealed that CLO significantly affected the reflex ($F = 5.70$). Table II shows that the most effective inhibitory cumulative dose was 130 μ g/kg, i.v.

A single dose of 130 μ g/kg ($n = 5$) also produced immediate, profound, and long-lasting depression of the elicited reflex ($F = 15.96$; Figs. 2 and 3). Interestingly, CLO did not produce a biphasic dose-response effect. That is, we never observed reflex-enhancement, even at the highest dose, where it may be expected that CLO might begin to act postsynaptically. This may be due to its action as only a partial agonist at postsynaptic α -1 receptors (Roach et al., 1983).

The lower doses of CLO produced a moderate but significant depression of the reflex but only at the most intense current levels; that is, only at the levels which produced the highest base line responses and may, therefore, be more sensitive in revealing weak modulation. In addition, the effects of the low doses of CLO may be not only mild but also too short-lived to be detected with our standard procedure, which requires approximately 8 to 10 min (see "Discussion"). Therefore, CLO was administered in a single dose of 30 μ g/kg, and 10 stimuli were repeated at 2-sec intervals at only one intermediate current level, in order to amplify and facilitate rapid demonstration of any moderate effects. Under these conditions, CLO at 30 μ g/kg ($n = 4$) had a significant inhibitory influence on the reflex ($F = 8.05$).

Yohimbine + clonidine. The reflex suppression produced by 130 μ g/kg of CLO was effectively blocked by pretreatment 30 min before with YOH (0.5 mg/kg, i.v.; $n = 7$; $F = 0.32$; Fig. 3). There was an apparent reversal of the CLO effect at the lowest current level, though this was not seen at any other level. This probably does not represent a real effect, since most scores at this level were subthreshold, and only a few scores contributed to the apparent excitation, which represents only a small increase in the actual mean response amplitude (5 to 16 μ V).

Yohimbine. It was expected that at low doses, at which YOH would act preferentially at presynaptic autoreceptors, YOH would affect the reflex by inducing release of endogenous NE. When a high dose was administered, at which YOH displays postsynaptic antagonist activity, the effect should reverse. In the dose-response study of YOH ($n = 5$), it was found that YOH administration significantly affected the reflex amplitude ($F = 10.68$). Subsequent analyses revealed that the lowest dose (0.25 mg/kg) produced significant enhancement of the reflex within 5 min of administration (Table III, Fig. 4). At the highest dose administered (1.0 mg/kg), the reflex was significantly suppressed, in comparison both to the low dose and to base line. A single-dose injection of 1.0 mg/kg ($n = 3$) produced a similar suppression ($F = 21.35$).

Prazosin + yohimbine. The dose-response test with YOH was repeated following pretreatment with PRAZ (10 mg/kg, i.p.; $n = 5$). Such pretreatment abolished the ability of YOH to significantly affect the reflex ($F = 0.91$; Table III, Fig. 4).

Anatomical localization

NE depletion by 6-OHDA. Injections of the noradrenergic neurotoxin 6-OHDA into Mo5 were effective in reducing the NE content in the vicinity of Mo5 to a mean of 61% of the vehicle-injected controls. While this indicates that the lesions produced only a partial denervation, this is most likely an underestimate of the true extent of depletion. The motor nucleus was extremely difficult to discern in the unfixed brains; therefore, the punched samples which were assayed certainly included more tissue than Mo5 alone, and thus probably contained some amount of unlesioned tissue. Bearing this in mind, we conclude that the lesions were at least partially effective.

YOH after NE denervation. The same dose-response test of the

TABLE I
Effects of L-DOPA on the amplitude of the masseteric reflex response

Condition	Current Level ^a					
	1	2	3	4	5	6
Base line	21 ± 12 ^b	31 ± 16	52 ± 18	78 ± 23	125 ± 26	208 ± 44
L-DOPA (50 mg/kg, i.p.) ^c	58 ± 27	78 ± 31	170 ± 102 ^d	191 ± 78 ^d	298 ± 118 ^d	346 ± 98 ^d

^a Current levels represent steps of 50 μ A in all cases, although the initial level may have been different for each subject.

^b Values are means $\pm$ SEM (μ V).

^c Thirty minutes postinjection.

^d Significantly different from base line; $p < 0.05$.

TABLE II
Effects of cumulative doses of clonidine on the amplitude of the masseteric reflex response

Dose (mg/kg, i.v.)	Current Level ^a				
	1	2	3	4	5
0	20 ± 13 ^b	33 ± 16	83 ± 29	160 ± 32	349 ± 97
10	29 ± 31	35 ± 22	68 ± 27	132 ± 45	300 ± 124 ^c
30	26 ± 20	38 ± 25	85 ± 41	167 ± 59	272 ± 120 ^c
130	19 ± 16	19 ± 6	41 ± 19	59 ± 24 ^c	125 ± 63 ^c
230	21 ± 16	31 ± 21	40 ± 22	84 ± 37 ^c	141 ± 55 ^c

^a Current levels represent steps of 50 μ A in all cases, although the initial level may have been different for each subject.

^b Values are means $\pm$ SEM (μ V).

^c Significantly different from base line; $p < 0.05$.

effects of YOH was repeated 6 to 8 days following local noradrenergic denervation of Mo5 by 6-OHDA ($n = 8$) or injection of vehicle ($n = 5$). Base line elicitation of the reflex was unaffected by the lesions. That is, the stimulus parameters, current range, and response amplitudes observed in the denervated animals were comparable to those for the intact subjects. This precludes any alternate interpretations based on nonspecific toxic effects of 6-OHDA. Vehicle controls were not significantly different from intact subjects (Dose $\times$ Group Interaction, $F = 1.85$). There was a significant Dose $\times$ Group Interaction, however, when the lesioned group was compared to either the intact group alone ($F = 7.39$) or to the pooled intact + vehicle-injection subjects ($F = 4.51$). In the denervated animals, YOH no longer had a significant effect on the reflex response ($F = 2.16$). However, it should be noted that both the low and high doses of YOH tended to produce a slight, though nonsignificant, increase in the reflex response (Table III, Fig. 4).

Discussion

The present series of studies sought to establish the masseteric reflex as a model system with which to gauge the functional level of activity of the CNS noradrenergic neurons which innervate it. An attempt was also made to generalize from observations at the cellular level, which have demonstrated a modulatory role for NE (see below) to a demonstration of the same type of modulatory action at the behavioral level.

The present studies have shown that agents which increase the level of transmission of endogenous NE also increase the amplitude of the masseteric reflex response and that agents which decrease the functional level of noradrenergic transmission decrease the amplitude of the reflex. L-DOPA, a NE precursor, and a low dose of YOH, which should antagonize autoinhibition of noradrenergic transmission, both enhanced the reflex response. The high dose of YOH, which should directly antagonize postsynaptic receptor activation, as well as CLO, a presynaptic autoreceptor agonist, both act to decrease functional noradrenergic transmission, and both attenuated the reflex.

It should be noted here that, in electrophysiological studies of noradrenergic neurons, it has been found that CLO in doses of 5 to 20 μ g/kg, i.v., was sufficient to suppress the firing of these neurons (Svensson et al., 1975; Andrade and Aghajanian, 1982). However, the suppression which occurred at these doses was short-lived, and

firing could be restored, albeit at reduced levels, by peripheral stimulation such as a toe-pinch (see Andrade and Aghajanian, 1982). Since the procedure used in our studies requires a minimum of approximately 8 to 10 min to complete, the brief suppression caused by a very low dose of CLO as described above may be insufficient to produce as profound a depression of the reflex in the present paradigm. In addition, the presence of continuous stimulation of Me5 may in itself bring about a more rapid restoration of noradrenergic unit activity when such low doses are employed. Thus, while 130 μ g/kg was the most effective inhibitory dose in our standard procedure, we also found a significant reflex-suppression with 30 μ g/kg by stimulating at only one current level and administering several stimuli within a shorter time in order to amplify and facilitate observation of any short-lived or mild effects.

The neurochemical specificity of the observed effects was further verified by blocking the inhibition produced by CLO by pretreatment with YOH and also by blocking the effects seen in the dose-response studies of YOH by prior administration of PRAZ. These findings lend support to the hypothesis that NE acts to facilitate transmission through the reflex circuitry, thus facilitating the response mediated by that circuitry. Furthermore, it is suggested that this modulation may be mediated by receptors of the α -1 subtype, although a role for other receptors cannot be ruled out.

The site of this modulation was then localized to the noradrenergic innervation of Mo5 by locally destroying the noradrenergic terminals there with 6-OHDA. This manipulation prevented the effect exerted by YOH on the reflex. There was, however, still a slight, though nonsignificant, tendency for YOH to enhance the reflex in the lesioned subjects. This probably indicates that some number of noradrenergic terminals persisted after denervation, a possibility which is supported by the neurochemical data.

Thus, it appears that changes in the responsiveness of the masseteric reflex may serve as a reliable index of functional changes in noradrenergic transmission. It also appears that NE is capable of modulating, in a facilitatory manner, a simple response in intact animals. We have recently obtained similar results in a pilot study of the masseteric reflex in an awake, freely moving cat, in which CLO had a depressant effect on the reflex at doses of 10 to 20 μ g/kg, i.v., and YOH enhanced the reflex at a dose of 0.5 mg/kg. This indicates that NE exerts an observable effect on at least a simple behavior in an intact animal and that this effect may have relevance to the

Figure 2. Oscilloscope display of an example of depression of the masseteric reflex response amplitude by intravenous administration of 130 $\mu\text{g/kg}$ of CLO. A, Preinjection base line; B, 3 min postinjection; C, 15 min postinjection. Note the variability of the response amplitude, as discussed in the text. Stimulus rate in all traces is 1 Hz; stimulus parameters are 270- μA , 0.15-msec-duration square wave pulse.

functioning of that animal under physiological (i.e., unanesthetized) conditions.

The importance of these findings is best examined in the context of a broader hypothesis of the role of NE in the normal functioning of an organism (see Amaral and Sinnamon, 1977 and Foote et al., 1983 for reviews). NE has been implicated in a wide variety of physiological and behavioral functions ranging from attention, learning, and anxiety to cardiovascular control, cerebral blood flow, and brain metabolism. However, a preponderance of negative and conflicting results, as well as a number of difficulties inherent in the techniques used, have made it difficult to state with certainty precisely what role NE plays in any of these functions. In light of these observations, a new view has been taken of the possible role of NE in modulating physiological and behavioral processes in general, rather than mediating any one specifically (Woodward et al., 1979). One can think of a modulator as not directly eliciting excitation or inhibition but rather as altering the response characteristics of its

Figure 3. Effects on the masseteric reflex amplitude of a single injection of CLO (130 $\mu\text{g/kg}$, i.v.) either alone (open bars) or following pretreatment with 0.5 mg/kg of YOH (solid bars). Current levels represent steps of 50 μA in all cases, though the initial level may have been different for each subject.

target to excitation or inhibition. That is, it may act more as a gain-set mechanism than as an on-off switch.

In an elegant series of experiments, Woodward and colleagues have demonstrated this type of action for NE in cerebellar cortex (e.g., Freedman et al., 1976, 1977; Moises et al., 1981, 1983). These studies show that NE, at levels with little or no direct inhibitory effect on Purkinje cell spontaneous activity, acts to enhance synaptic transmission through the cerebellar circuitry. The interesting point is that, while NE alone may be inhibitory, it enhances both excitatory and inhibitory synaptic actions. The combined effects of reducing spontaneous activity and enhancing both excitatory and inhibitory synaptically evoked activity result in an overall amplification of the signal-to-noise ratio for information being transmitted through the cerebellar circuitry. Similar effects have since been demonstrated in many other sensory and motor areas of the CNS innervated by NE (e.g., McCall and Aghajanian, 1979, 1980; Waterhouse and Woodward, 1980; White and Neuman, 1980, 1983).

From this, a functional role for brain NE may be postulated. The noradrenergic systems of the CNS may act to enhance an organism's reactivity to internal or external stimuli; that is, the processing of sensory information and of the motor responses to it. This enhancement may occur at times of heightened general arousal—when interaction with environmental stimuli is most likely to occur—or, alternatively, in response to a specific alerting stimulus, since these are the conditions under which noradrenergic neurons are activated (Foote et al., 1980; Aston-Jones and Bloom, 1981a, 1981b; Grant and Redmond, 1983; Jacobs et al., 1984). What has been lacking is a study generalizing the cellular modulation discussed above to the level of behavior.

The results of the present study provide such a generalization and support the above hypothesis by demonstrating that an increase in the functional level of noradrenergic transmission increases the responsivity of a simple reflexive behavior. Such simple behavioral systems, like the masseteric reflex, which can be easily studied, quantified, and manipulated in behaving animals, may be invaluable tools in the investigation of the role of NE and other monoamine neurotransmitters in physiology and behavior. In future experiments, we hope to combine measurement of the masseteric reflex with single unit studies of monoaminergic neurons in freely moving animals, thus observing changes in both the reflex response and single unit activity under normal behavioral and physiological conditions.

TABLE III

Effects of cumulative doses of yohimbine on the amplitude of the masseteric reflex response when administered alone, after pretreatment with prazosin, or after denervation of Mo5 with 6-OHDA

Group	Dose (mg/kg, i.v.)	Current Level ^a				
		1	2	3	4	5
Yohimbine	0	16 ± 12 ^b	58 ± 23	117 ± 34	301 ± 70	630 ± 98
	0.25	49 ± 24	104 ± 31	222 ± 58	574 ± 127 ^c	978 ± 175 ^c
	1.00	19 ± 8	45 ± 14	61 ± 19	145 ± 27 ^c	387 ± 149 ^c
Prazosin (10 mg/kg, i.p.) + yohimbine	0	59 ± 15	96 ± 13	178 ± 27	349 ± 54	589 ± 105
	0.25	55 ± 16	91 ± 16	175 ± 34	344 ± 84	548 ± 111
	1.00	56 ± 19	84 ± 21	196 ± 57	302 ± 53	537 ± 113
6-OHDA + yohimbine	0	18 ± 9	32 ± 14	110 ± 37	229 ± 65	500 ± 95
	0.25	18 ± 13	55 ± 21	197 ± 66	304 ± 78	699 ± 159
	1.00	35 ± 22	81 ± 34	170 ± 58	359 ± 73	619 ± 132

^a Current levels represent steps of 50 μ A in all cases, although the initial level may have been different for each subject.

^b Values are means $\pm$ SEM (μ V).

^c Significantly different from base line; $p < 0.05$.

Figure 4. The effects on the masseteric reflex amplitude of a low dose (0.25 mg/kg, i.v.; open bars) or a high dose (1.00 mg/kg, i.v.; solid bars) of YOH. The top panel shows the effects of YOH alone in cumulative doses; the middle panel shows the effects of YOH following pretreatment with PRAZ (10.0 mg/kg, i.p.); the bottom panel shows the effects of YOH following local denervation of noradrenergic terminals in Mo5 with 6-OHDA. See the legend to Figure 3 for further details.

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