Noradrenergic Modulation of the Masseteric Reflex in Behaving Cats.

I. Pharmacological Studies

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The masseteric (jaw closure) reflex was utilized as a model system for assessing functional changes in central norepinephrine (NE) neurotransmission. This monosynaptic reflex was chosen because of its simple and well-defined circuitry, and because its motor component receives a dense NE innervation. Previous experiments in our laboratory described NE modulation of this reflex in the anesthetized rat. The present experiments examine the effects of NE on this response in the unanesthetized, behaving cat. The masseteric reflex was elicited by electrical stimulation of the mesencephalic trigeminal nucleus, and the response was recorded via electrodes permanently implanted in the masseter muscle. The amplitude of the reflex response was measured before and at various intervals following microinfusion (0.5 μ l) of NE or of various NE agonists directly into the motor trigeminal nucleus (MoV). Microinfusions of NE (0.125–5.0 μ g) produced dose-dependent increases in the amplitude of the elicited reflex response. These effects were evident within 1 min postinfusion and lasted up to 30 min; in all cases, the response amplitude returned to baseline levels. The increase seen in response to 0.5 μ g NE was blocked by pretreatment with the α -1-adrenergic antagonist prazosin, but not by pretreatment with the serotonin (5-HT) antagonist methysergide. Methysergide did, however, completely block the increase in the amplitude seen in response to microinfusion of 5-HT. Infusion of the α -1-adrenergic agonist phenylephrine also increased the amplitude of the reflex response. By contrast, infusion of the β -adrenergic agonist isoproterenol had no effect, whereas clonidine, a presynaptic α -2-adrenergic agonist, decreased its amplitude. Neither saline infusion nor infusion of NE outside of the motor nucleus had any effect on the amplitude of the reflex response. These data indicate that NE augments the masseteric reflex response by a direct action in the MoV, probably via α -1-adrenergic receptors. Studies in the following paper examine whether physiological activation of this NE input also facilitates the reflex response.

Initial biochemical and pharmacological studies demonstrated the existence of norepinephrine (NE) in the CNS and established its role as a central neurotransmitter (Euler, 1956). Later ana-

tomical studies localized NE-containing cell bodies, pathways, and axon terminals in the CNS (Dahlstrom and Fuxe, 1964; Moore and Bloom, 1979). More recent experiments have elucidated the effects of NE on its postsynaptic target neurons (see Rogawski, 1985, for a review). However, despite extensive research implicating NE in a wide variety of behavioral and physiological processes (Amaral and Sinnamon, 1977; Foote et al., 1983), a clear and consistent understanding of the role of central NE has remained elusive.

This may be attributable to several factors. First, many of the early methods used to study NE in intact animals were of necessity gross or imprecise, typically employing lesions or systemic pharmacology. Second, when more specific methods such as neurotoxins (e.g., 6-hydroxydopamine) are used, there is a lag between the time of the neural destruction and the time that the behavioral or physiological measurement is taken. This allows for regrowth and/or compensatory changes to occur, obscuring or altering the effects attributable to the lesion. Third, the results of many precise studies are confounded by the necessity of conducting them either under anesthesia or in reduced preparations. The generality of these results for determining the normal physiological or behavioral role for NE is, therefore, limited. Finally, NE may not serve an easily specifiable single role in the CNS, but may be involved in different functions in different systems (motor, sensory, hormonal, etc.).

Converging results from several different experimental paradigms suggest that central NE modulates motor output in a facilitatory fashion. At the cellular level, iontophoretic application of NE onto spinal (White and Neuman, 1980, 1983) or brain-stem (McCall and Aghajanian, 1979, 1980) motor neurons has been shown to facilitate the response to GABA or to afferent input without affecting spontaneous activity. These results are consistent with more general findings reported by Woodward and colleagues (Woodward et al., 1979) examining the effects of iontophoretic NE on forebrain and cerebellar target neurons. In these studies NE was shown to increase the response of target neurons to afferent input, both excitatory and inhibitory, by preferentially inhibiting background activity to a greater degree than evoked activity. This modulatory action has been interpreted as NE enhancement of the signal/noise ratio, thereby facilitating neuronal information processing.

Other studies at a greater level of neural complexity have more directly examined the functional effects of NE transmission. For example, Hino et al. (1984), recording from the ventral root of rats, report that chlorpromazine inhibits mono- and polysynaptic spinal reflexes elicited by stimulating the dorsal root, suggesting that descending NE input exerts a tonic facilitatory effect on this response. In addition, locus coeruleus stimulation has been shown to produce an NE-mediated increase in

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the amplitude of the monosynaptic spinal reflex (Strahlendorf et al., 1980; Fung and Barnes, 1981). More recent studies show that iontophoretic application of NE on spinal motoneurons results in a slow, long-lasting depolarizing potential which decreases the threshold for action potential production by intracellular depolarizing current (Fung et al., 1988). Although they make an important contribution, these types of studies are, nonetheless, somewhat limited because they were conducted either in anesthetized or spinalized animals and, therefore, cannot address the consequences of NE on functional output.

Studies carried out in intact organisms by Davis and colleagues (reviewed in Davis, 1984) have come closest to understanding the functional role of NE in behavior. Their data suggest that NE facilitates the acoustic startle reflex in rats. They find that acoustic startle is increased by drugs that increase the availability of central NE by blocking its reuptake and is decreased by lesions of NE cell bodies in the locus coeruleus (Davis et al., 1977). In addition, they report that acoustic startle is increased following intrathecal administration of NE or the α -1-adrenergic agonist phenylephrine (Astrachan and Davis, 1981). In experiments such as these, however, the relative complexity of the neural circuitry underlying the behavior studied precludes determining the specific site of action of NE within the brain stem and spinal cord.

In summary, NE appears to facilitate sensorimotor processes, most likely via an α -1-adrenergic mechanism. This modulation has been fairly well described at a cellular level by numerous iontophoretic studies but is still not well understood at a behavioral level for the reasons outlined above. A fuller understanding of the functional role of brain NE systems would be achieved by the use of a simple neuronal system whose circuitry received dense NE innervation. In addition, it would be desirable if this system could be studied *in vivo*, in intact animals, in the absence of anesthesia or restraint, and under physiological conditions.

In a previous paper from this laboratory (Morilak and Jacobs, 1985), it was proposed that the masseteric, or jaw-closing, reflex provided a model system to study the functional effects of changes in NE synaptic transmission. In those studies, we reported that systemic administration of drugs that increased NE's synaptic action augmented the amplitude of the evoked reflex response; reciprocally, drugs that decreased the synaptic action of NE suppressed the response. Destruction of the NE input to the motor trigeminal nucleus (MoV), by means of the direct infusion of the neurotoxin 6-hydroxydopamine, significantly decreased the facilitatory effect produced by drugs that increase NE's synaptic action. These experiments demonstrated that NE modulated this simple behavioral response and specified the site of the modulation in intact, anesthetized rats. The present 2 groups of studies were designed to investigate the modulatory effects of NE on the masseteric reflex in the behaving cat and to go beyond these pharmacological studies to physiologically relevant conditions.

The masseteric reflex was chosen for several reasons. First, it is a simple monosynaptic reflex with a well-understood circuitry: stretch receptors in the masseter muscle, and with cell bodies in the mesencephalic trigeminal nucleus (MesV), synapse directly on α -motoneurons in MoV which innervate the masseter muscle. Second, there is a dense NE innervation of MoV from lateral tegmental NE neurons (Levitt and Moore, 1979; Vornov and Sutin, 1983; Grzanna et al., 1987). Third, the reflex has been shown to be modulated 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). Finally, the reflex can be easily and reliably elicited experimentally in behaving animals, allowing examination under physiological conditions.

In the first group of experiments, the masseteric reflex was elicited in behaving cats by means of electrical stimulation of the cell bodies of the sensory neurons located in MesV. Pharmacological agents, locally infused directly into MoV, were used to manipulate NE synaptic transmission. The results of these studies (1) characterize NE modulation of the masseteric reflex in the unanesthetized cat; (2) specify the NE receptor subtype mediating the effect; and (3) demonstrate neurochemical specificity by examining the effect of NE following pretreatment with systemic antagonist drugs. Studies described in the accompanying paper demonstrate that these pharmacological results can be generalized to physiological activation of NE neurons, produced by various environmental conditions. In addition, they establish that a causal relationship exists between increased NE activity and augmentation of the reflex response.

Materials and Methods

Surgical procedure. Adult cats weighing between 2.5 and 4.0 kg were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). Masseter muscle activity was recorded with the uninsulated tips of 2 flexible, Teflon-coated stainless steel electrodes (0.001 in. diameter), inserted 1 cm apart into the thickest portion of both masseter muscles, and soldered into place. The remaining insulated portions of the wires were led subcutaneously to a small incision in the scalp and externalized. After the cheek incisions were sutured, the animal was placed in a stereotaxic frame.

The scalp incision was lengthened along the midline and the temporalis muscle retracted. A stainless steel screw was placed in the frontal bone above the sinus to serve as an indifferent electrode. Another screw, placed in the skull over the parietal cortex and in contact with the overlying tissue, served as a ground electrode. Two additional screws were threaded into the skull to anchor the connector assembly. Insulated wires from all electrodes were later soldered to a standard 25-pin connector.

Rhodes Nexus-200 bipolar electrodes, insulated except at the tips, were bilaterally implanted in MesV for eliciting the masseteric reflex according to the technique of Chase et al. (1968). One electrode at a time was lowered at a 30° angle off the vertical (anterior approach) to a starting point ~8 mm dorsal to MesV (P -2.5, L +2.5, V -0.5). Precise placement was achieved by mapping the boundaries of the nucleus through electrical stimulation while monitoring masseter muscle activity. As the electrode was slowly advanced toward the target, stimulation was applied (500 μ A double pulses, 0.5 msec duration, delivered at a frequency of 1 Hz) and masseter muscle activity simultaneously displayed on an oscilloscope. When a response (i.e., mass action EMG potential) was observed on the oscilloscope (as well as visually), stimulus duration and intensity were decreased and, if necessary, electrode placement was adjusted until the response was optimized. The response was judged to be reflexive in nature if it met the following criteria: latency greater than 3 msec following stimulation; slight variability in amplitude; and sensitivity to current level and duration.

A similar method was used to implant bilateral, 22-gauge stainless steel guide cannulae in the MoV nuclei (P -4.5, L +3.7, V -5.0). Each individual cannula was lowered at a 30° angle off the vertical (posterior approach) to an intermediate point dorsal to MoV. Final placement was determined by stimulating through a temporary electrode which extended 1 mm beyond the tip of the guide and making the appropriate adjustments until the best response to minimal current was obtained. The response was judged to be mediated via direct stimulation of motoneurons in MoV if it met the following criteria: constant latency less than 2 msec following stimulation; invariant amplitude; and insensitivity to changes in current intensity or duration once a threshold level was reached. The cannulae were cemented at a final point 0.5 mm below the coordinates determined to provide the best response since infused liquid spreads upward from the tip in an oval surrounding the cannula

barrel. The stimulating wire was removed and replaced with a 28-gauge stylet extending 1 mm beyond the tip of the cannula.

The leads from the masseteric recording and stimulating electrodes were soldered to the connector. The cannulae were housed inside a capped plastic tube and the entire assembly affixed to the skull with dental cement and covered with acrylic. At least 1 week of recovery was allowed prior to experimentation. During this time, the subjects were adapted to the experimental chamber, which was a sound-attenuated square wooden box (65 × 65 × 95 cm), fitted with a clear Plexiglas door.

Reflex elicitation in behaving animals. The reflex was elicited by delivering a square wave pulse to MesV through bipolar electrodes. Stimulation was isolated from ground (Grass model SIU5 stimulus isolation unit) and delivered as constant current (Grass model CCU1A constant current unit coupled to a Grass S48 stimulator). Optimal response was usually obtained with twin pulse stimulation, pulse duration of 0.05–0.10 msec, interpulse interval of 3–4 msec. Current intensity ranged from 50 to 300 μ A.

The signal from the masseter recording electrodes was band-pass filtered (half-amplitude at 0.3 Hz and 3.0 kHz) and amplified (Grass P5 preamp) before being displayed on a storage oscilloscope for direct measurement and stored on tape for subsequent analysis. A subset of studies was performed blind.

Baseline reflex determinations. Various pulse durations were first tested in order to determine that producing the most stable responses from trial to trial. Next, keeping the optimal duration constant, threshold and maximal response amplitudes were determined by adjusting current levels, beginning with a subthreshold level and gradually increasing until the maximal response was elicited. A baseline current-response curve was obtained by electrically stimulating MesV neurons at a rate of 0.1 Hz at each of 3 current levels, beginning with the lowest current that produced a consistent, suprathreshold response (usually 50 μ V amplitude). The middle and highest currents were ones which doubled and tripled, respectively, the amplitude of the lowest (if necessary, the current level was adjusted so as not to elicit maximal responses). In general, observable jaw closure was never elicited by the low current, occasionally elicited by the middle current, and always produced by the high current. Eight stimuli were delivered at each current level and the largest and smallest response for each level discarded. The mean response amplitude was then calculated for each current level, resulting in a baseline current-response curve.

This procedure was used to generate baseline profiles for each subject used in the following experiments. The criteria ensured that similar response amplitudes would be generated for each subject (~ 50 μ V, ~ 100 μ V, and ~ 150 μ V at the low, middle, and high current levels, respectively) although the specific values for pulse duration and current levels varied between subjects. A subject was first considered eligible for testing once current-response baselines were consistent for at least 3 consecutive days. From then on, whenever possible, the initial parameters established for a given subject continued to be used for all experiments involving that subject.

Drug infusions. On the day of an experiment, the subject was placed in the experimental chamber and prepared for a local infusion. The stylet was removed and replaced with a preloaded 28-gauge stainless steel injector connected to a Hamilton 10- μ l syringe via PE 20 tubing. In order to leave the subject undisturbed, the syringe was driven by a remotely controlled syringe pump set to deliver 0.5 μ l over a 1-min period. Previous studies indicated that slow infusions of this amount of dye, NE, or serotonin (5-HT) via small-gauge cannulae diffuse 0.3–0.8 mm from the injection site (Meyers et al., 1971), thus restricting most, if not all, of the drug to MoV, which in the cat is larger than 1 mm in diameter in all dimensions. Since level of arousal has been shown to affect the amplitude of the reflex, all testing was conducted during quiet waking (QW) behavioral states. These were defined as periods in which there were no gross bodily movements; infrequent or no large eye movements; and a stable tonic EMG (as indicated by the presence of spontaneous background masseter activity). This issue is also addressed in the Discussion section of the accompanying paper.

Once the subject was judged to be in a QW state, baseline current-response profiles were determined. The baseline was considered to be stable if 2 consecutive runs produced similar response amplitudes within each current (i.e., < 10% variability); the mean amplitude at each current was taken as the baseline value. At this point, the drug infusion was begun and, allowing 1 min for diffusion, the reflex test procedure repeated 1, 5, 15, 30, and 60 min later. Once again, in order not to disturb

the subject, the injector was left in place for the duration of the experimental session (we found that any drug leakage or diffusion was below threshold for producing a physiological effect). The test drugs (various doses of NE, phenylephrine, isoproterenol, clonidine, or 5-HT) were administered in a random order; successive infusions were separated by at least 3 d. In subjects with bilateral cannulae, the contralateral side could be tested the next day.

Five subjects were pretreated either with the α -1-adrenergic antagonist prazosin (5 mg/kg, i.p.) or with the serotonergic antagonist methysergide (0.5 mg/kg, i.p.) 45 min prior to local infusions of 0.5 μ g NE. These drugs and doses were chosen because previous studies of NE modulation of the masseteric reflex in the anesthetized rat (Morilak and Jacobs, 1985) showed that prazosin blocked the increase in the masseteric reflex normally produced by yohimbine, which increases NE release via inhibition of presynaptic NE autoreceptors (Starke and Altman, 1973); conversely, studies in the facial motor nucleus show that methysergide is effective in blocking the facilitating action of 5-HT, but not that of NE (McCall and Aghajanian, 1980). We chose to utilize the 0.5- μ g dose of NE because initial results showed that this dose reliably augmented the masseteric reflex in all subjects and was still on the rising phase of the dose-response curve. For this series of experiments, the last preinfusion values were taken as the baseline and tests were conducted only at the low current level (since current level did not seem to differentially affect the response to microinfusion of NE). All subjects were also tested with 0.5 μ g NE alone in order to provide for within-subject analysis. Again, the order of treatments was randomly determined.

Saline infusions (0.5 μ l of 0.9% physiological saline) were tested in 6 subjects in order to control for possible nonspecific effects. In addition, the pH of the saline solution was adjusted to match that of the 1.0- μ g solution of NE (which produced the largest increase in the reflex response) in order to control for possible pH-related effects. In 4 animals, an injection cannula deliberately placed outside of MoV during surgery allowed us to test for site specificity; this was accomplished by first localizing MoV and then moving 2 mm above that stereotaxic coordinate, making the final placement rostral and dorsal to MoV, above the supratrigeminal nucleus. The effects of infusions of 1.0 μ g NE were then tested in this control site as described above.

Histology. At the conclusion of all experiments, the animals were overdosed with Nembutal and perfused with normal saline followed by 10% formalin. The brains were extracted and blocked; coronal sections (40 μ m thick) were cut and counterstained with cresyl violet. MoV cannulae positions were determined by examination of the cannula tracks. Only those subjects with cannulae located in MoV were included in data analysis.

Drugs and doses. Drugs used were NE hydrochloride (Sigma); clonidine hydrochloride (Sigma); phenylephrine hydrochloride (Sigma); isoproterenol hydrochloride (Sigma); prazosin hydrochloride (Pfizer); 5-hydroxytryptamine bimalate (Sigma); and methysergide maleate (Sandoz). All drugs, with the exception of prazosin, were dissolved in physiological saline; prazosin was dissolved in heated propylene glycol. Doses are expressed as the salt. Solutions were made fresh prior to each session.

Data analysis. Effects of a single dose were first analyzed using an analysis of variance (ANOVA) with current and time as repeated measures; post-hoc comparisons between individual time points were then analyzed with Newman-Keuls tests. For the antagonism tests, the difference score between the amplitude following NE alone and that following NE with antagonist pretreatment was calculated for each subject. These scores were then entered into a within-subject ANOVA with 3 levels of the independent variable.

Results

Long-term stability

The evoked response amplitude was variable for the first week postsurgery and then remained fairly stable from session to session throughout the duration of the experimental procedure. In agreement with a previous report (Soja et al., 1987), we found that the variability of individual responses within a given run of 8 trials was minimal (approximately 10%). In general, once stimulation parameters were determined for a given subject prior to the start of experimentation, they continued to produce the same magnitude of response. In some cases, however, it was

Figure 1. Mean masseteric reflex response amplitude ($\mu\text{V} \pm \text{SEM}$) measured before (baseline, BL) and at various time points following infusion of $0.125 \mu\text{g}$ NE into MoV on 2 separate test sessions, 4 weeks apart, in the same subject. There was no significant difference in response between the first NE test (open circles) and the second (closed circles).

necessary to adjust the parameters used (usually increasing current or duration) in order to maintain a consistent baseline amplitude. This change could reflect decreases in the efficacy of the stimulating electrode over time due, for example, to metal deposits or gliosis.

In addition to reliability of baseline responses, the response seen following a given microinfused drug dose did not differ across repeated tests within a given subject [$F(5,10) = 0.249$, $p > 0.05$]. That is, the same dose produced equivalent increases in response amplitude on different sessions. Representative data from one subject are shown in Figure 1. First, note that the baseline amplitudes did not differ across sessions, which were 4 weeks apart: baseline was $55.3 \mu\text{V}$ on the first test and $48.9 \mu\text{V}$ on the second; the same stimulation parameters were used during both tests. The first NE infusion ($0.125 \mu\text{g}$) produced a 50.6% increase in the amplitude of the reflex in min 1 (from $55.3 \mu\text{V}$ to $83.3 \mu\text{V}$); the second time the same dose was tested, a comparable increase in the amplitude of the reflex was produced (from $48.9 \mu\text{V}$ to $105 \mu\text{V}$ in min 1). Similar results were seen at the other time points: 55% increase in min 5 on test 1 vs 68% increase on test 2; 19% increase in min 15 on test 1 vs 21.0% on test 2. In both instances, the masseteric reflex amplitude returned to baseline by min 30, indicating that the time course was also unchanged from the first test.

NE dose-response relationships

Infusions of NE into MoV resulted in a dose-dependent facilitation of the reflex amplitude which was manifested as an increase in the evoked masseter muscle response, as illustrated in Figure 2. This graph presents the facilitation (expressed as mean percent baseline) observed 5 min postinfusion for all the NE doses tested at the 3 current levels. Inspection of the figure reveals, first, that the magnitude of the increase was inversely related to current; that is, the largest facilitation was observed at the lowest current level. For example, at the low current, the evoked amplitude of the reflex was increased to 643.9% of baseline by infusions of $1.0 \mu\text{g}$ NE; at the midcurrent, the increase was 393.7% of baseline, and at the highest current level, 235.9%

Figure 2. Mean percent of baseline (BL) amplitude of the masseteric reflex 5 min postinfusion for all the NE doses tested, at the low (open circles), middle (closed circles), and high (closed squares) current levels. The reflex response amplitude was significantly increased by $0.25 \mu\text{g}$ NE at the low current ($n = 6$ subjects); the $0.5 \mu\text{g}$ ($n = 6$) and $1.0 \mu\text{g}$ ($n = 7$) doses significantly increased the reflex response amplitude at all current levels. The $0.125 \mu\text{g}$ ($n = 7$) and $5.0 \mu\text{g}$ ($n = 6$) doses of NE had no significant effect on the masseteric response amplitude.

of baseline amplitude. This current effect pattern was evident across all doses tested [$F(2,46) = 66.3$, $p < 0.001$].

Second, magnitude of the facilitation was positively correlated to dose of NE, with lower doses producing significantly less of an increase in the response [$F(4,23) = 3.6$, $p < 0.01$]. Looking at results from the low current, the net increase in amplitude 5 min after infusions of the lowest dose tested ($0.125 \mu\text{g}$ NE) was 24.9% (± 13.8 SEM); $0.25 \mu\text{g}$ NE produced a 53.2% (± 20.9) increase; $0.5 \mu\text{g}$ NE increased the response by 209% (± 129.1); the peak effect was seen following infusions of $1.0 \mu\text{g}$ NE (543.9% increase, ± 336). Similar results were produced at the mid- and high currents, although, as described above, the magnitude of the increases was smaller. However, the highest dose tested, $5.0 \mu\text{g}$ NE, had no effect on the amplitude of the reflex response, producing only a nonsignificant 20.8% (± 21.8) change. This lack of effect at a high NE dose is consistent with results reported for iontophoretic NE experiments (Rogawski and Aghajanian, 1980) and may reflect, as those results suggest, depolarization block.

In addition to magnitude, the time course of the effects was also correlated with dose. That is, the shortest-lasting increase was the smallest in amplitude (i.e., following infusions of $0.125 \mu\text{g}$ NE), whereas higher doses produced correspondingly larger and longer-lasting increases. The results of ANOVAs with time as the repeated measure revealed that the amplitude of the response was significantly increased above baseline following infusions of $0.125 \mu\text{g}$ NE [$F(5,30) = 4.4$, $p < 0.005$]; $0.25 \mu\text{g}$ NE [$F(5,25) = 2.6$, $p < 0.05$]; $0.5 \mu\text{g}$ NE [$F(5,25) = 3.8$, $p < 0.01$]; and $1.0 \mu\text{g}$ NE [$F(5,30) = 6.6$, $p < 0.001$]. The highest dose tested, $5.0 \mu\text{g}$ NE, had no significant effect [$F(5,15) = 1.7$, $p > 0.05$]. Post hoc comparisons between individual time points using Newman-Keuls tests revealed that, at all current levels, $0.125 \mu\text{g}$ NE significantly increased the amplitude of the response for only 1 min postinfusion; the response had returned to baseline by 5 min after the infusion and remained so for the duration of the test. The next higher dose, $0.25 \mu\text{g}$, significantly

Figure 3. Mean percent of baseline (BL) amplitude in masseteric reflex measured at various time points following infusion of 1.0 µg NE into MoV. Mean baseline values were 31.8 µV (± 3.8 SEM) at the low current, 105.5 ± 14.9 µV at the middle current, and 266.3 ± 57.8 µV at the high current ($n = 7$ subjects). NE significantly increased the reflex response amplitude at the 1-, 5-, and 15-min time points at all current levels; in addition, at the high current, the reflex response amplitude was significantly increased at the 30-min time point.

increased the response at the 1- and 5-min time points, but only at the low current level; no significant change was seen at the other current levels. After infusion of 0.5 µg NE, the response remained significantly elevated for 5 min at all currents. The longest-lasting increase was seen with 1.0 µg NE; at this dose, the response remained significantly above baseline for 15 min at the low and middle currents and for 30 min at the high current (Fig. 3).

Agonist tests

NE agonists were tested in order to pharmacologically classify the receptor subtype mediating the NE facilitation of the reflex. The results at the low current level are presented in Figure 4. Infusions of the α -1-adrenergic agonist phenylephrine produced a moderate, but significant, increase in the reflex amplitude at the 2 doses tested, 0.5 µg [$F(5,25) = 3.6$, $p < 0.01$] and 1.0 µg [$F(4,24) = 8.1$, $p < 0.003$]. The increase was evident at all current levels by 1 min postinfusion and remained elevated for up to 60 min. Phenylephrine seemed to be less potent than NE in that the increase seen after phenylephrine infusions was smaller than that after NE, particularly at the low current: 266.4% of baseline 5 min postphenylephrine vs 643.4% of baseline 5 min post-NE.

Infusions of the α -2-adrenergic agonist clonidine (0.25 µg) produced a long-lasting, significant depression of the masseteric reflex [$F(5,15) = 3.79$, $p < 0.02$]. By the first minute, the amplitude was reduced to 70.7% of baseline at the low current, 89.9% at the midcurrent, and 84.1% at the high current. The decrease was seen 1–5 min postinfusion and lasted for 60–90 min. The peak suppression was seen 15 min after clonidine, 54% of baseline at the low current level, 60.2% at the midcurrent level, and 63.3% of baseline at the high current level.

Infusions of the β -adrenergic agonist isoproterenol, by contrast, had no effect on the amplitude of the masseteric reflex at either of the 2 doses tested, either 0.5 µg [$F(4,20) = 1.02$, $p > 0.05$] or 1.0 µg [$F(4,20) = 0.94$, $p > 0.05$].

Figure 4. Comparison of mean percent of baseline (BL) amplitude of the masseteric reflex response at various time points following infusion of either the α -1-adrenergic agonist phenylephrine (PE, 1.0 µg; $n = 7$ subjects), the α -2-adrenergic agonist clonidine (CLON, 0.25 µg; $n = 6$), or the β -adrenergic agonist isoproterenol (ISO, 1.0 µg; $n = 6$). Baseline amplitude values were 47.3 µV (± 4.63 SEM) for the PE group, 41.9 ± 6.52 µV for CLON, and 52.7 ± 6.15 µV for the ISO group. PE infusions significantly increased the reflex response amplitude at all time points, CLON significantly decreased the amplitude.

Antagonist tests

Systemic administration of both prazosin, an α -1-noradrenergic antagonist (5 mg/kg, i.p.) and the serotonergic antagonist methysergide (0.5 mg/kg, i.p.) decreased the baseline amplitude of the masseteric reflex. Neither antagonist produced any dramatic drug-induced state changes. However, methysergide pretreatment did not prevent the increase normally seen following infusions of 1.0 µg NE, whereas prazosin pretreatment completely blocked the increase (Fig. 5). One minute after infusion of 1.0 µg NE, the amplitude of the masseteric reflex was increased to 582% of baseline in untreated subjects and by 594% in the same subjects when pretreated with methysergide; it decreased to 90.5% of baseline following prazosin pretreatment. An ANOVA comparing the response to 1.0 µg NE following prazosin or methysergide to that following NE alone shows that the response to NE was significantly different following prazosin, but not methysergide, pretreatment [$F(2,8) = 4.72$, $p < 0.05$]. This same dose of methysergide was, however, effective in blocking an increase seen in response to infusions of 5-HT. In another group of subjects, 0.125 µg 5-HT increased the amplitude of the reflex to 300.6% of baseline values. When the same dose of 5-HT was infused following methysergide (0.5 mg/kg, i.p.), the increase in the amplitude of the reflex was markedly attenuated (116% of baseline). This demonstrates that the inability of methysergide to block an increase to NE was pharmacologically specific.

Controls

Consistent with a previous report (Soja et al., 1987), infusion of 0.5 µl of saline, same pH (5.5) as a 1.0-µg NE solution, did not change the amplitude of the masseteric reflex at any time point tested [$F(4,20) = 1.02$, $p > 0.05$; Fig. 6, top]. In addition, infusion of 1.0 µg NE in control sites 2 mm above MoV had no significant effect on the reflex response [$F(5,15) = 0.94$, $p > 0.05$; Fig. 6, bottom].

Figure 5. Within-subject comparison of mean percent of baseline (BL) amplitude of the masseteric reflex response following infusion of $0.5 \mu\text{g}$ NE alone or following pretreatment 45 min earlier either with the α -adrenergic antagonist prazosin (PRAZ; 5.0 mg/kg , i.p.) or with the 5-HT antagonist methysergide (0.5 mg/kg , i.p.). Baseline amplitudes for the 3 conditions were $45.5 \mu\text{V}$ ($\pm 5.7 \text{ SEM}$) for NE alone, $26.8 \pm 5.1 \mu\text{V}$ for NE/METHY, and $35.3 \pm 10.1 \mu\text{V}$ for NE/PRAZ. Tests were conducted at the low current level; $n = 5$ subjects for each condition. The response to NE following methysergide pretreatment did not differ from that following NE alone. The response to NE following prazosin pretreatment differed significantly from that in the other conditions.

Discussion

The results of the present study show that microinfusion of NE into the MoV increases the amplitude of the evoked masseteric reflex response in the intact, unanesthetized cat in a dose-dependent fashion. The facilitation observed was evident within 1 min of NE infusion and, at the higher doses, lasted for up to 30 min. This long duration most likely reflects an overwhelming of the NE uptake system by the presence of artificially high NE levels produced by the infusions.

Although the facilitatory effect was observed at each of the 3 current levels used, the degree of NE facilitation was inversely related to current strength (i.e., the largest percent increase was evident at the low current, the next larger at the middle current, and the smallest at the high current). In addition, the highest NE dose tested ($5 \mu\text{g}$) had no effect on the amplitude of the reflex response at any current level. Consistent with these findings, Rogawski and Aghajanian (1980) report that NE facilitation of lateral geniculate neurons was produced with low iontophoretic currents. Although increasing the ejection current within a limited range strengthened the response, currents in excess of that range resulted in a depression or lack of effect, similar to what we observed here with the highest NE dose. The diminished response seen at high doses or currents could reflect depolarization block or tachyphylaxis. Although this was not tested directly in the present experiment, Rogawski and Aghajanian (1980) report that lateral geniculate neurons did exhibit a widening and a decreased amplitude of the extracellular action potential, suggesting that overdepolarization can occur in response to the application of NE with supramaximal currents.

The increase normally seen following NE infusion was blocked by pretreatment with the specific α -1 receptor antagonist prazosin, but not by pretreatment with the serotonergic antagonist methysergide. Consistent with this, the NE facilitation was mimicked by the α -1-adrenergic agonist phenylephrine, but not

Figure 6. *Top*, Mean percent of baseline (BL) amplitude of the masseteric reflex measured at various time points following infusion of $0.5 \mu\text{l}$ of 0.9% saline, pH 5.5, into MoV. Mean baseline values ($n = 6$ subjects) were $35.6 (\pm 3.7 \text{ SEM})$ at the low current, $102.6 \pm 7.6 \mu\text{V}$ at the middle current, and $229 \pm 30.8 \mu\text{V}$ at the high current. Saline did not significantly affect the reflex amplitude at any time point. *Bottom*, Mean percent of baseline amplitude of the masseteric reflex amplitude measured at various timepoints following infusion of $0.5 \mu\text{l}$ of $1.0 \mu\text{g}$ NE in a control site 2 mm above MoV. Tests were conducted at the low current, and mean baseline values ($n = 4$ subjects) were $63.1 (\pm 4.4 \text{ SEM})$. There was no significant effect at any time point.

by the β -agonist isoproterenol, suggesting that the facilitation was mediated via postsynaptic α -1-adrenergic receptors. Infusions of the α -2-adrenergic agonist clonidine, which decreases NE release via autoreceptor activation (Starke and Altman, 1973), decreased the amplitude of the masseteric reflex. This finding, together with the observation that prazosin pretreatment also decreases the baseline amplitude, is consistent with this general hypothesis and further suggests that tonic NE release may play a role in modulating the reflex. Thus, these results demonstrate that pharmacologically induced changes in NE transmission in MoV increase the amplitude of the masseteric reflex in behaving cats and extend the results we previously reported in the anesthetized rat (Morilak and Jacobs, 1985).

The mechanism by which NE increases the amplitude of the masseteric reflex was not directly tested in the present experiment. However, the relative lack of interneurons in MoV (Sessle, 1977; Card et al., 1986) suggests that the facilitatory effects of NE are caused either by an action on the terminals of afferent

fibers or by a direct action on MoV neurons. A presynaptic action is unlikely, however, since ultrastructural studies of MoV have, to date, failed to reveal axoaxonic connections involving NE neurons (Card et al., 1986; Card, personal communication). Our results are consistent with a postsynaptic action on MoV motoneurons since the NE effect was mimicked by phenylephrine, an α -1 receptor agonist, and blocked by pretreatment with prazosin, an α -1 antagonist. Moreover, infusions of NE in a control site outside of MoV had no effect on the amplitude of the reflex response, indicating that the facilitation was a result of the action of NE in MoV.

Indeed, there is much evidence that NE facilitation of neuronal activity, in a number of other target areas, is due to a postsynaptic action at α receptors. Electrical stimulation of NE neurons in the locus coeruleus of decerebrate cats facilitates the monosynaptic response of lumbar motoneurons to afferent (dorsal root) inputs; this effect is partially blocked by systemic administration of the α -adrenergic antagonist phenoxybenzamine (Strahlendorf et al., 1980). Intracellular studies show that this spinal motoneuron facilitation is associated with a small membrane depolarization, which is also partially blocked by phenoxybenzamine (Fung and Barnes, 1981). Similarly, in anesthetized rats, the facilitation of the masseteric reflex produced by electrical stimulation of the area of the lateral lemniscus (the source of the NE input to MoV in the rat) is also blocked by α -adrenergic antagonists (Vornov and Sutin, 1986). Iontophoretic application of NE on facial motor neurons (McCall and Aghajanian, 1979; VanderMaelen and Aghajanian, 1980), spinal motor neurons (White and Neuman, 1980), and lateral geniculate neurons (Rogawski and Aghajanian, 1980) also produces a small membrane depolarization which is blocked by α -adrenergic antagonists. The NE effect in these previous studies was considered modulatory in that the small depolarization did not produce neuronal activation but, rather, decreased the threshold for activation by other afferent inputs.

In other target areas, NE has been shown to produce enhancement of inhibitory inputs. Iontophoretic application of NE, at doses that have no effect on spontaneous activity, augments GABA or synaptically induced inhibition in cerebellar Purkinje cells (Yeh and Woodward, 1983) and in somatosensory cortex cells (Waterhouse and Woodward, 1980). Thus, NE application appears capable of enhancing both excitatory and inhibitory afferent inputs. The net effect of NE enhancement combined with the reported inhibition of background activity is to increase the "signal-to-noise" ratio, thereby facilitating information transfer through the circuitry.

Our results support previous neurophysiological studies indicating that NE facilitates the motoneuron response to excitatory inputs and extend this observation from a cellular to a behavioral level. We demonstrated that pharmacological increases in the level of NE transmission increase the responsivity of this simple behavioral response in the unanesthetized, behaving cat. The present results are consistent with the hypothesis that NE acts to enhance transmission through reflex circuits, thereby facilitating the motor response mediated by that circuit. Moreover, they validate the utility of the masseteric reflex as a model for studying NE transmission in the CNS of behaving animals.

These findings are relevant not only within the context of motor control, but to a more general hypothesis concerning the role of NE in the normal functioning of an organism. A current view posits a role for NE in modulating an organism's reactivity to stimuli. Thus, a response is likely to be enhanced at conditions

under which NE neurons are activated—such as stress, arousal, or interaction with novel environmental stimuli (Foote et al., 1983; Jacobs, 1986; Abercrombie and Jacobs, 1987). This hypothesis predicts that conditions which produce physiological release of NE should also enhance behavioral responses such as the masseteric reflex, and that this enhancement would be correlated to the strength and duration of activation. This hypothesis was tested in the experiments presented in the following paper.

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