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Spinal vs. Supraspinal Sites of Action of the α_2-Adrenergic Agonists Clonidine and ST-91 on the Acoustic Startle Reflex

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DAVIS, M., R. L. COMMISSARIS, S. YANG, K. R. WAGNER, J. H. KEHNE, J. V. CASSELLA AND N. M. BOULIS. *Spinal vs. supraspinal sites of action of the α_2-adrenergic agonists clonidine and ST-91 on the acoustic startle reflex.* PHARMACOL BIOCHEM BEHAV **33**(1) 233–240, 1989—Previous work has shown that stimulation of α_2-adrenergic receptors depresses the startle responses in rats. The present study suggests that this depressant effect involves supraspinal rather than spinal α_2-adrenergic receptors because intraventricular but not intrathecal infusion of the hydrophilic α_2-adrenergic agonist ST-91 depressed the acoustic startle reflex. To determine the point in the acoustic startle pathway where α_2-adrenergic receptor activation might ultimately alter neural transmission, startle responses were elicited electrically from different points along the acoustic startle pathway after systemic administration of clonidine. Clonidine depressed acoustically-elicited startle and startle elicited by electrical stimulation of the ventral cochlear nucleus to a comparable magnitude and over a similar time course. It also partially depressed startle elicited by electrical stimulation of the nucleus reticularis pontis caudalis (RPC). Taken together, these data suggest that α_2-adrenergic stimulation depresses startle by acting on supraspinal receptors, but that this effect is ultimately expressed, at least in part, by actions at both spinal and brainstem levels of the acoustic startle response pathway. The results are compared to other drugs known to affect the startle reflex.

Reticular formation Nucleus reticularis pontis caudalis Startle Clonidine Ventral cochlear nucleus ST-91 α_2-Adrenergic receptors Spinal cord

THE acoustic startle response is a rapid sequence of muscle contractions elicited by an intense auditory stimulus with a rapid onset. Several studies have suggested that stimulation of α_2-adrenergic receptors depresses the startle response. Systemic administration of the α_2-adrenergic agonist clonidine markedly depresses acoustic (6, 7, 11, 22) and tactile (13,14) startle. This effect is reversed by pretreatment with the central α_2-adrenergic antagonists piperoxane (7) or yohimbine (6), but not by the peripherally acting α_2-adrenergic antagonist phentolamine (7). The depressant effect of clonidine still occurs in acutely decerebrate rats (7), in rats with electrolytic lesions of the locus coeruleus (7), or in rats pretreated centrally with the catecholamine neurotoxin 6-hydroxydopamine (Commissaris, Kehne and Davis, unpublished observations), suggesting that postsynaptic rather than presynaptic α_2-adrenergic receptors are involved.

At the present time, the location of the α_2-adrenergic receptors that mediate the effects of clonidine on startle is not known. It was reported that intrathecal infusion of very low doses of clonidine markedly depressed acoustic startle (6). These data were consistent with the conclusion that α_2-adrenergic receptors in the spinal cord might mediate the depressant effects of clonidine on startle (6). More recently, however, we have found that small amounts of clonidine given intrathecally can have supraspinal actions as indicated by the depression of unit firing in the locus coeruleus (24). The depression of activity probably results by a rapid diffusion of highly-lipophilic clonidine from the subarachnoid space into the general circulation and then into the brain [e.g., (27,28)]. Because of this diffusion, it is necessary to use a hydrophilic α_2-adrenergic agonist given either intraventricularly or intrathecally to evaluate whether behavioral effects result from

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actions at spinal vs. supraspinal sties.

Exploratory work in our laboratory indicated that intraventricular, but not intrathecal administration of the hydrophilic mixed alpha-adrenergic agonist oxymetazoline depressed acoustic startle. Furthermore, ST-91, hydrophilic compound that exceeds oxymetazoline in selectivity for α_2- vs. α_1-adrenergic receptors, depressed acoustic startle following intraventricular administration (21). The first experiment of the present study further characterized the depressant effect of ST-91 by a) evaluating several doses given intraventricularly; b) comparing it to intrathecal ST-91 administration; and c) attempting to antagonize it with the selective α_2-adrenergic antagonist idazoxan.

The second experiment sought to answer a different question regarding the modulation of the startle reflex by α_2-adrenergic receptors. In this case the question did not involve the location of the α_2-adrenergic receptors, but instead the location along the acoustic startle pathway where activation of α_2-adrenergic receptors ultimately alters neural transmission so as to affect the behavioral response. Considerable evidence now indicates that the neural pathway which mediates the startle reflex consists of the ventral cochlear nucleus (VCN), an area just medial to the ventral nucleus of the lateral lemniscus (the paralemniscal zone), the nucleus reticularis pontis caudalis (RPC), and spinal motoneurons (3,9). Startle-like responses in behaving rats can be elicited by single-pulse electrical stimulation at each of these loci (9). As argued elsewhere (1, 5, 8, 15), the technique for eliciting startle electrically from different points along the acoustic startle pathway can be used to localize the site(s) along that pathway where a drug ultimately influences transmission so as to affect the behavior. For example, a drug given systemically might depress acoustic startle by inhibiting, at the spinal level, the acitvation of motorneurons normally produced by the acoustic stimulus. In this case, startle responses elicited by electrical stimulation at any point along the startle pathway should be affected by the drug. In contrast, if the drug decreases transmission at a point in the reflex pathway prior to the spinal cord, for example, the paralemniscal zone, then startle elicited from the ventral cochlear nucleus should be depressed, whereas startle elicited from the nucleus reticularis pontis caudalis should not be depressed. This technique has been previously used to study the neural loci for the expression of several depressant and excitatory drug effects on startle, including diazepam (1), the hallucinogens N,N-dimethyltryptamine and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (5), or dopaminergic agonists (8,15).

In preliminary studies, systemically-administered clonidine appeared to depress startle elicited by electrical stimulation of either the VCN or RPC, suggesting that clonidine ultimately depresses startle at a spinal site. However, whereas VCN- and acoustically elicited startle were depressed to an equivalent extent, RPC-elicited startle appeared to be less depressed than acoustic startle, suggesting that clonidine might be decreasing transmission at several sites along the startle pathway. The second experiment of the present study formally assessed the effects of a single dose of clonidine on startle elicited from the VCN as well as a wide range of doses of clonidine on startle elicited from the RPC in an effort to determine possible sites where α_2-adrenergic agonists ultimately influence neural transmission so as to depress the acoustic startle reflex.

METHOD

Animals

Sprague-Dawley rats (Charles River Co.) were housed in group cages (four to five per cage) in a colony room with a light-dark cycle (12-hr–12-hr; lights on at 7:00 a.m.) and weighed 250 to 400 g at time of testing. Food and water were available ad lib.

Apparatus

The apparatus used to measure startle amplitude has been described previously (2). Briefly, a stabilimeter recorded the amplitude of the startle response. The stabilimeter consisted of an $8 \times 15 \times 15$ cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame. Cage movement resulted in displacement of an accelerometer which generated a voltage proportional to the velocity of displacement. Startle amplitude, defined as the maximum accelerometer voltage that occurred during the first 200 msec after the startle stimulus was delivered, was measured with a specially designed sample and hold circuit interfaced to a PDP-11 computer where startle amplitude could vary from 0 to 220 (ST-91 study) or an analogue-to-digital converter which printed out startle amplitudes ranging from 0 to 90 units (clonidine and electrically-elicited startle study). As described elsewhere, acoustically or electrically elicited startle has a latency of about 5 msec recorded electromyographically in the neck muscles, 8 msec in the hindleg (9). This very fast sequence of muscle contractions results in a cage movement having a frequency of about 7–10 Hz in this particular test cage (2). By sampling over a 200-msec period after the onset of the startle stimulus, the peak amplitude of cage movement initiated by the very short latency startle response can be reliably recorded (4). Thus, even though this 200-msec sample period is much longer than the actual duration of the startle response, it is an optimal time given the physical characteristics of the test cage system. Moreover, other studies have shown that sampling for 200-msec periods in the absence of a startle stimulus, or following a loud auditory stimulus which has a long rise time and hence does not elicit startle (Davis, unpublished observations), yields essentially a zero baseline, indicating that cage output measured over a 200-msec time period following presentation of a startle stimulus represents startle rather than nonstartle activity.

The stabilimeters were housed in a dimly-lighted, ventilated, sound-attenuating chamber, 10 cm from a high-frequency speaker (Radio Shack Supertweeter). For acoustically elicited startle studies the startle stimulus was a 50-msec, variable-intensity burst of white noise having a rise-decay time of 5 msec. Background white noise, provided by a Grason-Stadler white noise generator, was 55 dB. Sound level measurements were made with a General Radio Model 1551-C sound level meter (A-scale). Electrical elicitation of startle employed custom designed constant current stimulators connected to the rat through a mercury slip ring to permit relatively free movement of the animal. Current levels were continuously monitored on an oscilloscope during all phases of the experiment. Startle was elicited by a single cathodal, 1-msec pulse delivered through monopolar, bilateral electrodes.

Matching Procedure

Prior to implanting rats with intraventricular vs. intrathecal catheters all animals were given a brief test period in order to assign each rat into matched groups having equivalent startle levels. Naive rats were placed in the startle test cages and 5 min later presented with 30 noise bursts at a 20-sec interstimulus interval. Three different intensities were used (95, 105, and 115 dB), with 10 noise bursts at each intensity, presented in an irregular, balanced sequence across the session. Mean startle amplitudes across the 30 noise bursts were used to divide 20 rats into four groups of five rats each which had with similar means. This matching procedure is used because within-subjects variabil-

ity (test-retest variability) of startle is considerably less than between-subjects variability, thus improving the likelihood that baseline levels of startle amplitude will be roughly equivalent for different test groups.

Intraventricular and Intrathecal Implant Procedure

For implantation of intraventricular or intrathecal catheters all surgeries were carried out under halothane (Fluothane) anesthesia, with rats secured in a Kopf stereotaxic instrument. Following surgery, rats were housed individually and allowed to recover for at least one week before beginning behavioral testing. Fifty rats were implanted with chronic catheters in the cerebral ventricle and 20 rats were implanted with catheters in the subarachnoid space of the spinal cord. Previously described methods were used for intracerebroventricular [ICV; (18)] or intrathecal (20,29) implants. Briefly, for ICV implants, following a midline incision, a hole was drilled in the skull (1 mm caudal and 1.5 mm lateral to bregma) and the dura was punctured. The ICV cannula was constructed of 26-gauge hypodermic tubing partially inserted into a 4.5-cm length of PE-20 tubing, having a total void volume of approximately 8 μl. Approximately 3 mm of the 5 mm of hypodermic tubing extending from the PE tubing was stereotaxically lowered into the brain. A 50-cm section of PE-20 tubing filled with distilled water was attached to the cannula and the cannula was then slowly lowered into the brain until the water in the tube began to flow. At the point of maximum flow the cannula was attached by Loctite adhesive to a screw anchored in the skull. The incision was closed around the cannula with wound clips and a topical antibiotic was applied.

The standard intrathecal catheter used for drug infusion into the lumbar subarachnoid space was composed of an 8.5-cm length of PE-10 tubing fused to a 3-cm length of PE-20 tubing. The membranes overlying the cisterna magna were punctured, and the PE-10 portion of the catheter was inserted and snaked along the dorsal surface of the spinal cord such that the catheter terminated at the rostral margin of the lumbar enlargement. The catheter was rigidly attached to the skull and the rats were allowed to recover. These procedures resulted in implanted rats that were healthy and virtually indistinguishable from unoperated controls.

Electrode Implant Procedures

For electrically elicited startle studies, male rats (350–400 g) were anesthetized with chloral hydrate (400 mg/kg) and placed in a stereotaxic instrument. Bilateral, monopolar electrodes (0.25 mm diameter) insulated except for 0.5 mm at the tip were aimed for either the ventral cochlear nucleus (VCN—n = 5) at 11.3 mm posterior to bregma (P); 3.9 mm lateral to the midline (L); and 8.6 mm ventral to the surface of the skull (V) or the nucleus reticularis pontis caudalis (RPC—n = 5), 11.0 P, 1.5 L, 9.5 V. This location is actually about 1 mm posterior to the critical site in the RPC and was used to stimulate the reticulo-spinal tract which carries axons from the RPC to the spinal cord while minimizing the possibility of stimulating the ventral acoustic stria which passes ventral to the RPC proper and carries fibers from the VCN to the paralemniscal zone, afferent to the RPC. Stimulation electrodes and a skull-mounted indifferent electrode were enclosed in an Amphenol socket and mounted onto the skull with dental cement.

Testing Intraventricular vs. Intrathecal ST-91 on Startle

One week after surgery rats were placed into the startle test cages and 5 min later presented with the first of 42 noise bursts at a 20-sec interstimulus interval. They were then removed from the test cages and infused ICV with either artificial cerebrospinal fluid (CSF) or 1.6, 3.2, 6.25 or 25 μg ST-91 (n = 10 in each group). Immediately after infusion the rats were returned to the test cages and presented with the first of 90 noise bursts at a 20-sec interstimulus interval. These same procedures were repeated on the rats implanted with intrathecal catheters except that a single dose of 25 μg ST-91 was compared to artifical CSF (n = 10 in each group).

Testing Effects of Idazoxan on the Depressant Effect of ST-91 Given Intraventricularly

Another 40 rats were matched and all rats were implanted with ICV cannulas, as described above. One week later half the rats were injected intraperitoneally (IP) with the α_2-adrenergic antagonist idazoxan (1.0 mg/kg) and half with saline and 5 min later presented with the first of 42 noise bursts at a 20-sec interstimulus interval. They were then removed from the test cages and infused with either 6.25 μg ST-91 or artificial CSF. Immediately after infusion the rats were returned to the test cages and presented with the first of 90 noise bursts at a 20-sec interstimulus interval.

Testing Clonidine on Startle Elicited Electrically From the VCN

Three days following implantation of electrodes, the rats were placed in the startle chamber and 5 min later presented with bilateral, electrical pulses (25–200 μA per electrode, 1.0 msec duration) of the VCN alternating with acoustic noise bursts (105–115 dB) for 10 min. The interval between an acoustic and an electrical stimulus was 10 sec. Each rat exhibited intensity-dependent increases in startle for both acoustic and electrical stimulation. Current levels were adjusted from 30–80 μA per electrode for individual rats to produce a startle level roughly equivalent to the startle level elicited by a 115-dB acoustic stimulus in that particular animal. Thereafter, current levels were not changed.

Two days later, each rat was placed into the startle test cage and 5 min later presented with the first of 40 startle-eliciting stimuli (20 acoustic and 20 electric stimuli presented at a 15-sec interstimulus interval, i.e., 30 sec between stimuli of the same modality). Immediately after this 10-min pretest the rats were injected IP with either saline or 40 μg/kg clonidine and then tested immediately with the first of 120 startle-eliciting stimuli (60 acoustic and 60 electric) presented at a 15-sec interstimulus interval. Two days later these same procedures were repeated except that rats previously injected with clonidine were now injected with saline and vice versa.

Testing Clonidine on Startle Elicited Electrically From the RPC

Three days following implantation of electrodes, the rats were placed in the startle chamber and 5 min later average startle levels elicited electrically or acoustically were established at different currents, as described above for startle elicited through the VCN. Thereafter, testing was conducted using 100 μA per electrode as the electrical intensity and 115 dB as the acoustic intensity, since these stimulus values produced nearly equal control startle levels. Two days later, each rat was injected with various doses of clonidine (10–80 μg/kg) or saline and then immediately placed in the startle cages and tested for 40 min (i.e., 120 occurrences of each of the two modes of stimulation). A total of five test sessions separated by 2–3 days were given to every rat so that each of the five treatments (saline or 4 doses of clonidine) was administered to each animal. The order of treatments was determined from a Latin square design. An additional test session was carried out with a

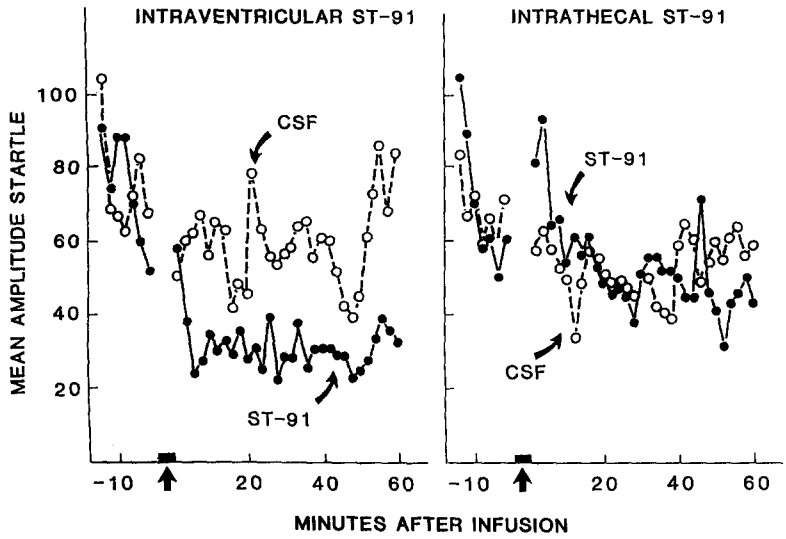

FIG. 1. Mean amplitude startle response prior and following infusion of either 25 μg ST-91 or artifical CSF given intraventricularly (left panel) or intrathecally (right panel).

high dose of clonidine (320 μg/kg). Data from this dose were presented graphically, but were not included in the overall ANOVA, since they were not part of the randomized design.

Histology

After testing, the animals were sacrificed by chloral hydrate overdose and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were stored for at least 2 days in 30% sucrose formalin and subsequently, 40-μ frozen coronal sections were cut through the areas containing the electrodes. Every fourth section was mounted and stained with cresyl violet cell stain. Electrode locations were transcribed onto atlas sections (26).

RESULTS

The left panel of Fig. 1 shows the mean amplitude startle response combined across 6 stimuli (i.e., 2-min blocks of testing) prior to and following infusion of 25 μg ST-91 ICV. Figure 2 shows the mean percent change in startle [(mean change in startle after infusion/mean amplitude of startle before infusion) × 100] after artificial CSF or various amounts of ST-91 given intraventricularly. Figures 1 and 2 show that ST-91 depressed startle in a time- and dose-dependent manner. An overall analysis of variance using the mean startle amplitude levels across the 14 min prior to infusion (Pre) vs. the mean startle amplitude levels across the 60 min after infusion (Post) found a highly significant depressant effect of ST-91, $F(1,45)=46.48$, $p<0.01$, that was linearly-related to the amount of ST-91 infused, $F(1,45)=6.89$, $p<0.01$. A similar analysis of the percent change scores found a significant difference among the various doses of ST-91, $F(4,45)=5.82$, $p<0.001$, that was also linearly-related to the amount of ST-91 infused ICV, $F(1,45)=21.51$, $p<0.001$.

In contrast, the right panel of Fig. 1 shows that even the highest dose of ST-91 given intrathecally did not appear to alter startle and no statistically significant difference was found between artificial CSF and ST-91 given intrathecally. Taken together, these data strongly suggest that the depressant effect of ST-91 results from activation of supraspinal receptors.

Figure 3 shows startle amplitude during the pretest period and posttest period after ICV infusion of 6.25 μg ST-91 or artificial CSF following pretreatment with saline (left panel) or the α_2-adrenergic antagonist idazoxan (right panel). Figure 3 indicates that idazoxan completely blocked the depressant effect of ST-91, indicating that the depressant effects of ST-91 given ICV resulted from activation of an α_2-adrenergic receptor. Consistent with this conclusion an overall analysis of variance found a significant Pretreatment (saline vs. idazoxan) vs. Test drug (ST-91 vs. artificial CSF) interaction, $F(3,36)=5.39$, $p<0.01$. Subsequent t-tests showed significant depression of startle by ST-91 after saline pretreatment, $t(18)=4.86$, $p<0.001$, but not after idazoxan

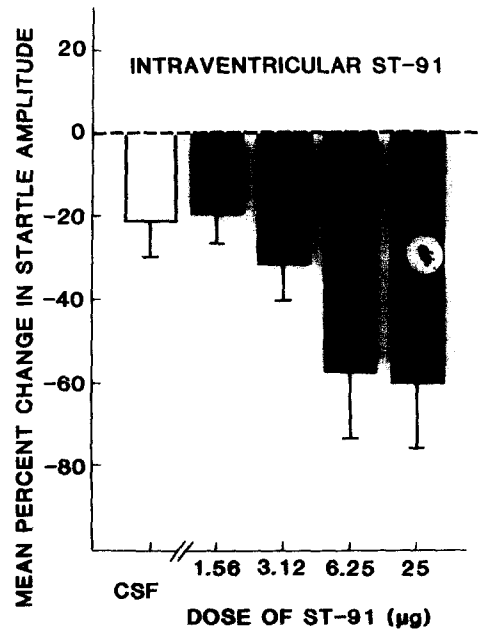

FIG. 2. Mean percent change in startle following intraventricular infusion of either artificial CSF or various amounts of ST-91.

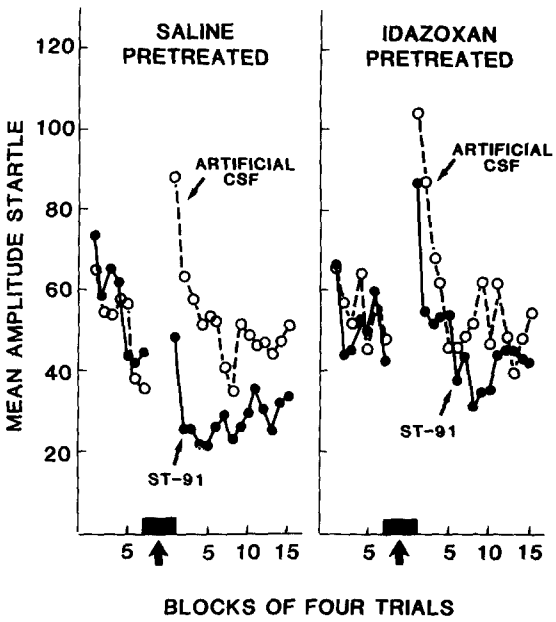

FIG. 3. Mean amplitude startle response prior to and following intraventricular infusion of either 6 μg ST-91 or artificial CSF given after pretreatment 15 min earlier with either saline (left panel) or the α_2-adrenergic antagonist idazoxan (1 mg/kg—right panel).

pretreatment, $t(18)<1.0$.

Figure 4 shows the mean amplitude startle response elicited acoustically (left panel) or electrically through the VCN (right panel) before and after IP injection of 40 μg/kg clonidine. Figure 4 indicates that both the magnitude and time course of the depressant effect of clonidine were highly similar when startle was elicited either acoustically or electrically. An overall analysis of variance found a highly significant depressant effect of clonidine, $F(1,4)=36.01$, $p<0.01$; and a significant Drug by Trials interaction, $F(11,44)=4.41$, $p<0.001$, indicating that the depressant effect of clonidine was greater toward the end of the session indicative of either a progressive absorption of the drug or an increased rate of startle habituation, as described previously (7). Importantly, there was no overall effect of Mode of Stimulation (i.e., acoustic vs. electric elicitation of startle), no Drug by Mode of Stimulation interaction, and no Drug by Mode by Time interaction (all F's<1.0). Thus, the statistical results are consistent with the conclusion that both the magnitude and the time course of the depressant effect of clonidine were the same when startle was elicited acoustically or electrically through the VCN.

Figures 5 and 6 show that clonidine injected IP depressed the amplitude of startle responses elicited either by acoustic stimulation (Fig. 5) or by electrical stimulation of the RPC (Fig. 6). The left panels show the time course of the effects of 80 μg/kg clonidine for each mode of stimulation, where each point represents the mean startle amplitude of 5 rats blocked over 6 trials (i.e., 2 min of testing). The right panels show the effects of various doses of clonidine on acoustically or electrically elicited startle, where each bar represents the mean startle amplitude during the 40-min clonidine test session minus the mean startle amplitude during the saline test session. Data for the 10–80 μg/kg doses were from the same rats tested repeatedly in a counterbalanced design, whereas the data from the 320 μg dose were from a separate test run.

An overall analysis of variance using the saline minus clonidine change scores at each dose, collapsed over the entire test session, revealed a significant effect of clonidine Dose, $F(3,24)=7.35$, $p<0.001$, indicating a greater depressant effect of clonidine, the higher the dose administered. In addition, there was a significant effect of Mode of Stimulation (acoustic vs. electric), $F(1,24)=5.32$, $p<0.05$, indicating that the depressant effect of clonidine was somewhat greater for acoustic versus RPC-elicited startle. Subsequent individual paired t-tests revealed a statistically significant ($p<0.05$) depression of acoustically elicited startle by doses of clonidine 20 μg/kg and higher and of electrically elicited startle by doses of clonidine 40 μg/kg and higher (significance denoted by the stars in each figure).

Figure 7 shows a histological reconstruction of the location of electrode tips aimed for either the ventral cochlear nucleus or the nucleus reticularis pontis caudalis. Electrodes were localized in the dorsal or ventral cochlear nucleus in each of the five rats. However, because of current spread and the variability of the

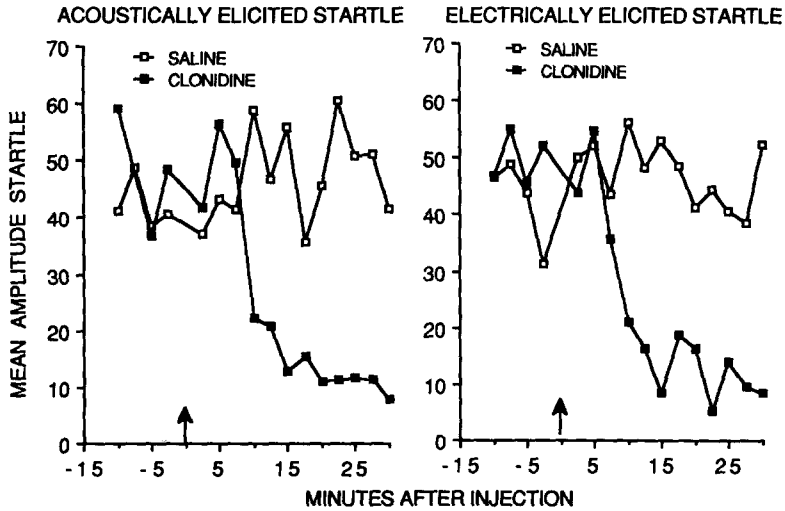

FIG. 4. Mean amplitude startle prior to and following IP injection of saline or clonidine (40 μg/kg) when startle was elicited acoustically (left panel) or electrically from the ventral cochlear nucleus (right panel).

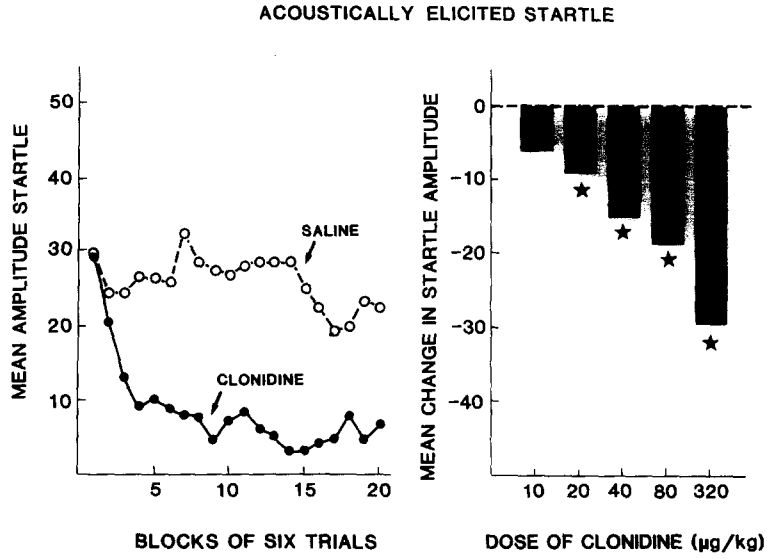

FIG. 5. Effects of IP clonidine on startle elicited by acoustic stimulation. The left panel shows the time course of 80 µg/kg clonidine, whereas the right panel shows the change scores for various doses of clonidine. A star denotes a significant depressant effect of clonidine relative to saline injection, $p<0.05$, paired t-test.

electrode placements, it cannot be determined whether startle-like responses elicited in the vicinity of the VCN result from activation of the VCN, DCN or both. Because other data indicate that startle is not eliminated by lesions of the DCN, but only by lesions of the VCN (9), it is probable that startle elicited from these sites results from actions in the VCN. In the RPC group, electrodes were located in posterior regions of the RPC, in an area which carries axons down to the spinal cord from cell bodies located more anteriorally in the RPC. This was done purposely to activate the spinal component of the startle reflex and to minimize activation of the acoustic stria which carries fibers from the VCN to the ventral nucleus of the lateral lemniscus.

DISCUSSION

The present study showed that intraventricular administration of the α_2-adrenergic agonist ST-91 depressed the acoustic startle reflex in a dose-dependent fashion. This depressant effect could be

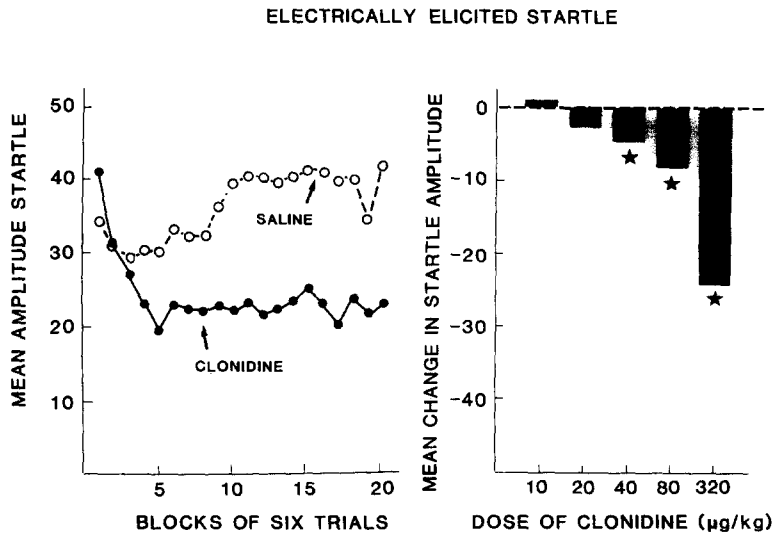

FIG. 6. Effects of IP clonidine on startle elicited by electric stimulation of the nucleus reticularis pontis caudalis (RPC). The left panel shows the time course of 80 µg/kg clonidine, whereas the right panel shows the change scores for various doses of clonidine. A star denotes a significant depressant effect of clonidine relative to saline injection, $p<0.05$, paried t-test.

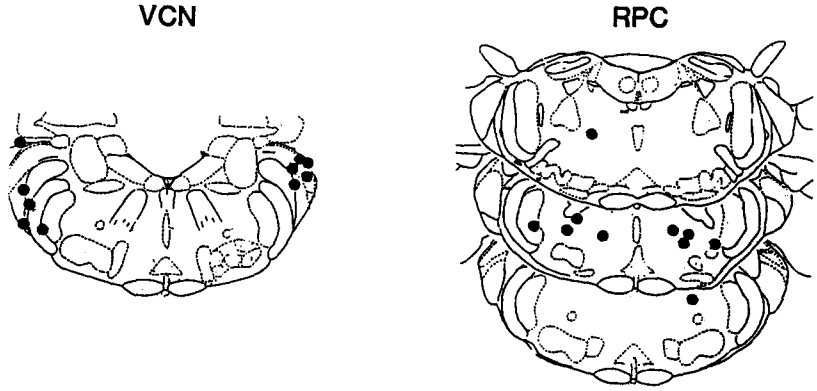

FIG. 7. Histological reconstruction of location of electrode tips aimed for either the ventral cochlear nucleus (left panel) or the posterior region of the nucleus reticularis pontis caudalis. The VCN plate is 11.3 mm posterior to bregma and for the RPC 10.3, 11.3 and 11.8 posterior to bregma according to the atlas of Paxinos and Watson (26).

completely blocked by pretreatment with the α_2-adrenergic antagonist idazoxan. In contrast, intrathecal administration of even the highest dose of ST-91 did not depress startle, leading to the conclusion that activation of supraspinal rather than spinal α_2-adrenergic receptors is responsible for the depressant effects of α_2-adrenergic agonists on startle. Previously, we concluded that spinal α_2-adrenergic receptors might also be involved based on the finding that intrathecal administration of the α_2-adrenergic agonist clonidine markedly depressed the startle response (6). Later work showed, however, that even very low doses of clondine given intrathecally can have supraspinal actions (24), probably because the drug rapidly diffuses into the general circulation and then into the brain after intrathecal administration [e.g., (27,28)].

Using a different technique which sought to determine where α_2-adrenergic agonists might ultimately alter neural transmission along the acoustic startle pathway, it was found that clonidine depressed startle elicited electrically from the VCN very similarly to the way it depressed acoustically-elicited startle. Thus, both the magnitude and time course of the effects of an intermediate dose of clonidine were essentially identical for both modes of stimulation. Because elicitation of startle through the VCN activates most of the startle pathway, it might be expected that clonidine would affect it the same way that it affects acoustic startle. In fact, previous studies have shown that habituation, sensitization by prior background noise, and conditioned fear alter acoustic startle and startle elicited from the VCN in a similar manner (1,10). Based on these data one can conclude that the effects of clonidine ultimately alter startle at or beyond the VCN, as opposed to actions in the cochlea or perhaps via α_2-adrenergic receptors on the auditory nerve. In contrast, depressant effects of benzodiazepines that occur with acoustic startle are not reproduced when startle is elicited electrically from the VCN (1). The marked difference between the effects of clonidine in the present study and benzodiazepines in our other work reinforces the conclusion that benzodiazepines depress acoustic startle by actions very early in the acoustic startle pathway.

Perhaps surprisingly, clonidine also depressed startle elicited from the RPC even though ST-91 did not depress acoustic startle when infused intrathecally. Because elicitation of startle through posterior regions of the RPC should primarily activate only spinal elements of the startle pathway, these data suggest that clonidine alters acoustic startle, at least partially, by actions ultimately in the spinal cord, even though this effect is initiated at supraspinal receptors. It was the case, however, that clonidine was less potent in depressing RPC-elicited startle compared to VCN-elicited or acoustic startle. This potency difference is evidenced by a shift to the right in the dose-response effects of clonidine on RPC-elicited vs. acoustic startle. At the present time sufficient data are not available to interpret quantitative differences in the effects of drugs on acoustically and electrically elicited startle. However, because of the close similarity between the effects of clonidine on acoustic startle and startle elicited from the VCN, it is clear that electrical elicitation of startle can behave very similarly to acoustic startle. Moreover, drugs like strychnine and 5-methoxy-N,N-dimethyltryptamine affect acoustic- and RPC-elicited startle in a very similar manner (5,8). Hence, it could be postulated that systemically administererd clonidine inhibits acoustic startle by decreasing neural transmission at sites within the startle circuit in or prior to the RPC as well as by actions in the spinal cord. If systemically administered clonidine acted at both early and later nuclei within the startle circuit, then startle responses elicited from later portions or the circuit (e.g., RPC) would be only partially affected as was found. Nonetheless, one has to be cautious about asserting that clonidine acts partially before the RPC since the pattern of activation set up by direct electrical stimulation of the RPC might be different from the pattern of activation of the RPC initiated by an acoustic stimulus or electric stimulation of the VCN and that the magnitude of clondine's effects might somehow be dependent on the exact pattern of neural activation of the RPC and spinal cord.

Clonidine's depression of RPC-elicited startle can be contrasted to the effects of other drugs known to depress acoustic startle. Systemic administration of the hallucinogen N,N,-dimethyltryptamine (DMT) depresses acoustic startle but does not alter RPC-elicited startle (5). DMT does depress startle elicited electrically from the VCN (R. Commissaris and M. Davis, unpublished findings), suggesting that DMT affects transmission at some point between the VCN and the RPC. As mentioned earlier, the benzodiazepine diazepam differs from either clonidine or DMT in that systemic diazepam administration depresses acoustic startle, but does not alter either RPC- (M. Davis, unpublished findings) or VCN-elicited startle (1).

The present study cannot distinguish between the RPC or the spinal cord as the site where the inhibitory effect of clonidine influences transmission. It is noteworthy that an area immediately adajacent to the part of the RPC critical for startle contains high densities of adrenergic (A5) cell bodies (17,25). Hence, local infusion of α_2-adrenergic agonists into this area might be especially effective in depressing acoustic startle. However, because the RPC is a long way from the lateral ventricle and because ST-91

would not be expected to diffuse widely through the brain, it seems unlikely that the depressant effects of ST-91 given ICV could be explained by actions on α_2-adrenergic receptors in the RPC. Local infusion of a hydrophilic α_2-adrenergic antagonist into the RPC after ICV infusion of ST-91 or systemic injection of clonidine could be used to test this possibility.

It has been reported that activation of supraspinal receptors can ultimately modulate behavior by influencing transmission at the spinal level. For example, the hindlimb withdrawal reflex (the neural circuit of which is localized at the spinal level) can be depressed by intraventricular infusion of oxymetazoline (19). There is a substantial literature indicating that analgesic drugs (e.g., morphine) can act in the brain to ultimately inhibit nociceptive signal transmission at the spinal level [see (12) for review]. The characteristic of the acoustic startle reflex that distinguishes it from these other system is that the afferent (sensory) limb of the reflex is located at a supraspinal level (VCN; nucleus of the lateral lemniscus), distinctively separated from the motor neurons in the ventral horn of the spinal cord. The electrically elicited startle technique makes it possible to distinguish drug effects on sensory activity from drug effects on motor responses. The fact that α_2-adrenergic receptor stimulation ultimately affects transmission, at least in part, on the motor side of the startle reflex arc suggests a common site of drug action on other behaviors which would share the motoneurons used in the startle response pathway.

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