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Spinalization Unmasks Clonidine's α_1-Adrenergic Mediated Excitation of the Flexor Reflex in Rats[1]

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Abstract

Clonidine exerts α_2-adrenergic mediated depressant effects on most behaviors measured in a normal animal. However, in the spinal-transected (spinalized) animal, clonidine apparently facilitates the flexor reflex through a stimulation of spinal α_1-adrenoceptors. The purpose of the present study was to determine if spinalization *per se* causes the shift in clonidine's profile from an α_2- to an α_1-adrenergic agonist.

The hindlimb flexor reflex was elicited by electrical pulses delivered through electrodes implanted subcutaneously in the hindpaw and was measured with a force transducer and polygraph. In contrast to an α_2-adrenergic mediated inhibition of the flexor reflex in intact rats, clonidine produced an α_1-adrenergic mediated increase in flexor reflex amplitude in spinalized rats. Because decerebration did not alter the depression due to clonidine, and intraventricular (but not intrathecal) administration of oxymetazoline mimicked the effect of clonidine, the depressant effects of α_2-adrenergic agonists are mediated through α_2-adrenergic receptors localized in the brainstem.

Alternate methods for inducing a functional spinal transection (spinal block with intrathecal procaine; spinal ligation) indicated that the shift in clonidine's effect from inhibition of the flexor reflex to excitation occurred immediately following spinalization. Spinal ligation did not produce α_1-adrenergic supersensitivity at 15 min or 2 hr after transection, as measured by alterations in [^{3}H]prazosin receptor binding or behavioral responses to clonidine.

Thus, the shift in clonidine's effects from α_2-adrenergic mediated inhibition of the flexor reflex in intact rats to α_1-adrenergic mediated excitation in spinalized rats results because spinal transection unmasks clonidine's α_1-adrenergic stimulatory effect. Other conditions under which clonidine exerts α_1-adrenoceptor mediated excitatory effects on behavior are discussed.

It is generally believed that clonidine is an agonist at central α_2-adrenergic receptors. This conclusion has been supported by electrophysiological (Svensson et al., 1975; Cedarbaum and Aghajanian, 1976) and biochemical (Langer, 1977; Kobinger, 1978) studies. In addition, behavioral studies have also supported clonidine's α_2-adrenoceptor agonist property (for review, see Lal and Shearman, 1981). Clonidine exerts α_2-adrenoceptor-mediated depressant effects on locomotion, food and water intake, avoidance and operant behavior, bar pressing for electrical stimulation of the brain, acoustic startle, and shock-elicited aggression. Nevertheless, there are a number of psychopharmacological studies in which clonidine's profile does not correspond to α_2-adrenergic stimulation but rather conforms to that of an α_1-adrenergic agonist (Anden et al., 1970; Reinstein and Isaacson, 1977; Svensson and Strombom, 1977; Zebrowska-Lupina et al., 1977; Nomura et al., 1980; Nishikawa et al., 1983). The present study investigated one well-documented instance in which clonidine causes an α_1-adrenergic mediated activation of behavior.

In response to a noxious stimulus (i.e., toe pinch, electrical stimulation of the plantar region of the foot pad), reflexive withdrawal of the affected limb occurs. Because the neural circuitry for this polysynaptic flexor reflex is organized at the segmental level, appropriately placed transections of the spinal cord can leave the spinal circuitry intact yet neurally isolated from the brain. The spinal-transected (or "spinalized") animal is therefore used as a reduced CNS preparation to evaluate drug effects on spinal reflexes without the complicating influences from supraspinal areas.

In addition to their prominent ascending systems, monoaminergic cell bodies located in the caudal brainstem give rise to axons that terminate in the spinal cord. Descending noradrenergic neurons (particularly those originating in the locus coeruleus/subcoeruleus complex) innervate both the dorsal and ventral horns of the spinal cord (Nygren and Olson, 1977; Moore and Bloom, 1979). Furthermore, pharmacological studies indicate that stimulation of spinal, postsynaptic α_1-adrenergic receptors increases the force of contraction of the flexor reflex in the spinalized preparation (Martin and Eades, 1967; Vaupel and Martin, 1976; Nozaki et al., 1980).

In this paradigm, clonidine shows prominent adrenergic agonist activity which can be blocked by α_1-adrenergic antagonists (Anden et al., 1970; Bolme et al., 1974; Anden et al., 1975; Austin et al., 1976; Maj et al., 1976; Anden et al., 1978). In contrast to α_1-adrenergic antagonists, α_2-adrenergic antagonists were ineffective or weakly active in attenuating the clonidine-induced excitation of the flexor reflex. In addition, the stimulatory effect of clonidine was increased by chronic destruction of spinal norepinephrine terminals with the neurotoxin 6-hydroxydopamine (Henning et al., 1974; Nygren and Olson, 1976) but not by destruction of serotonin terminals with 5,6-dihydroxytryptamine (Nygren et al., 1974). These data are all consistent with an agonist action of clonidine at α_1-adrenergic receptors. It is important to note that clonidine's excitatory effect on the flexor reflex was seen at doses which are considered high (>100 μg/kg, i.p.) relative to doses that produced α_2-adrenergic depressant

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effects in other behavioral paradigms. However, clonidine generally does not show α_1-adrenergic agonist properties in other behavioral paradigms, even at high doses (see Lal and Shearman, 1984; Malick, 1984).

It is possible that clonidine would behave like an α_2-adrenergic agonist on the flexor reflex if an intact preparation were studied. Spinal transection might unmask a change in the profile of clonidine from an α_2- to an α_1-adrenergic agonist. The overall aim of the following series of experiments was to test this prediction and to further investigate the sites at which clonidine exerts its effects on the flexor reflex in the intact and spinalized rat.

Materials and Methods

Animals. A total of 219 Sprague-Dawley rats (Charles River) were used. Between-subjects designs were used exclusively; therefore, rats were tested once and then sacrificed. All rats were housed in group cages (four to five per cage) in a colony room with a light-dark cycle (12-hr-12-hr) and weighed 250 to 450 g at time of testing. Food and water were available *ad libitum*.

Procedures. Surgical procedures were carried out in halothane-anesthetized rats that were held in a Kopf stereotaxic instrument. Following completion of surgery, routine postoperative care was taken.

For spinal transections, the vertebrae at the cervical-thoracic junction were exposed, and a laminectomy was performed with rongeurs. The spinal cord was severed at T_2 using surgical microscissors, Gelfoam was inserted into the site of the transection to prevent bleeding, and the incision was closed with sutures. Nonsurgical controls ("intact" rats) were anesthetized for an equivalent period of time (10 min). Following the operation, all rats were placed under a heat source until the time of testing (2 hr later, unless otherwise noted).

Previously described techniques were utilized for intrathecal (Yaksh and Rudy, 1976; Davis and Astrachan, 1981; Kehne et al., 1981) and intraventricular (Kehne et al., 1981) chronic implants. Rats were allowed at least 3 days for recovery following implants before testing began. Acute decerebrations were performed as described previously (Davis and Gendelman, 1977; Davis et al., 1977). Following testing, rats were sacrificed, and the brains were analyzed for completeness of the transection. Only data from rats with complete transections were used.

For spinal block experiments, rats were chronically implanted with intrathecal catheters as in Experiment 1, except that shorter catheters (5.5 cm) that terminated in the thoracic cord were used. Three to 4 days later, rats were placed in the test apparatus and given a 5-min base line. 2.0 mg/kg clonidine was then injected i.p., and the flexor reflex was monitored for 20 min (30-V stimulus intensity; 1-min interstimulus interval (ISI)). At this point (the time of near-maximal depression by clonidine), the contents of the catheter (4 μl of saline) were infused, followed by 1 to 2 μl of 20% procaine (in distilled water), and testing was carried out for an additional 15 min.

Rats were prepared for spinal ligation in the following manner. Under halothane anesthesia, the spinal cord was exposed using procedures described previously. However, instead of severing the spinal cord, a strand of surgical silk was tied loosely around the cord and externalized through the incision. Gelfoam and surgical jelly were packed around the cord, the muscle and incision were sewn shut, and the rat was allowed to recover from surgery for at least 5 hr before testing. At the time of testing, spinal ligation was accomplished by tying off the thread. This procedure produced a series of rapid, reflexive movements of the hindlimbs, qualitatively similar to postdecapitation kicking reflex (Mason and Fibiger, 1976), that lasted for about 30 sec, followed by hindlimb paralysis typical of spinal-transected animals. At no point did the rats exhibit any manifestations of discomfort. It should be noted that prior attempts had been made to evaluate the early consequences of spinal transection. However, it was determined that residual effects of exposure to halothane anesthetic during the spinal transection procedure significantly interfered with the analysis of post-transection events. Given the demonstrated limitations of prior attempts to evaluate the early consequences of spinal transection, the spinal ligation procedure was used to provide conclusive, important information that would otherwise be lacking. The test procedure was the same as that for spinal block (see above) except that spinal ligation was substituted for intrathecal procaine infusion. All rats were sacrificed immediately following the completion of testing and examined for completeness of transection.

[^{3}H]Prazosin and [^{3}H]clonidine assays were used to estimate dissociation constants (K_d) and receptor densities (B_{max}) in crude membrane homogenates of lumbar spinal cords (180 to 230 mg) as described previously (Hamburg and Tallman, 1981; Astrachan et al., 1983; Menkes et al., 1983). [^{3}H]Prazosin (Amersham-Searle; 0.5 to 1.0 nM specific activity, 20.2 Ci/mmol) was used, and nonspecific binding was assessed by the addition of 10 μM WB-4101 (Ward-Blenkinsop Ltd.). [^{3}H]Clonidine (New England Nuclear, 0.2 to 5.0 nM; specific activity, 23.8 Ci/mol) was used, and nonspecific binding was determined by the addition of 10 μM oxymetazoline.

The apparatus and testing parameters for measuring the flexor reflex were modified from descriptions in literature (Austin et al., 1976; Nygren and Olson, 1976; Anden et al., 1978; Pearson 1979). Prior to testing, rats were placed in a Bollman restraining cage that had been modified so that the hindlimbs were suspended through holes cut in the bottom of the cage. The left leg was taped to a support so that it remained stationary. The right leg was connected to a Grass force transducer, the output of which was amplified through a Grass Model 7P1-A low-level D. C. pre-amplifier (2 mV/cm sensitivity) and displayed on a polygraph chart-recorder. The stimulus for eliciting the hindlimb flexor was a 5-msec square wave pulse which was generated by a Grass S88 stimulator and fed through a Grass SIU5 Stimulus Isolation Unit. The stimulus was delivered through two insect pins implanted subcutaneously in the plantar region of the right hindpaw (3 mm separation between the tips of the needles). The electrode was taped securely to the hindpaw. The amplitude of the flexor reflex was quantified by measuring the pen displacement on the polygraph paper.

For the initial studies, the test session was divided into three phases: base line, saline test, drug test. During each phase, the rats were presented with 30 stimuli (10 at each of three different intensities—15, 30, or 60 V presented in a balanced, irregular order) at a 20-sec ISI. About 2 min separated each phase. Immediately before the saline test, all rats were injected i.p. with saline to determine if this would change their performance relative to the prior base line period. Immediately before the drug test, different groups of rats (n = three to six per group) were injected i.p. with various doses of clonidine (0.007 to 2.0 mg/kg) or its vehicle (saline).

Because qualitatively similar results were obtained at all three stimulus intensities, subsequent studies used a single intensity (30 V). An initial 5-min base line measurement was taken (five stimuli, 1-min ISI), after which the rats were injected with the drug or appropriate vehicle followed by a 30-min postinjection test period (30 stimuli, 1-min ISI).

Results

Effects of clonidine on the flexor reflex in intact and spinalized rats. Figure 1 shows the effects of vehicle injections (*right panels*) or 0.5 mg/kg clonidine injections (*left panels*) in spinalized (n = 18) (*upper panels*) or intact (n = 27) rats (*lower panels*) following the base line and saline phases. In the groups that received vehicle and saline injections, the amplitude of the flexor reflex remained relatively stable across the three test phases. In the rats that received saline followed by clonidine, a marked excitation occurred in the spinalized group after clonidine administration, whereas a depression occurred in the intact group.

As seen in Figure 2, clonidine produced a dose-dependent excitation of the flexor reflex in spinalized rats, whereas the drug produced a dose-dependent depression in intact rats. Each *bar* represents the mean change score (flexor reflex amplitude after clonidine administration minus the response amplitude for the test period prior to clonidine administration) at each dose of clonidine or following vehicle administration.

The dose range for the excitatory effects of clonidine on the flexor reflex found in this experiment was similar to that reported by other investigators (Austin et al., 1976; Anden et al., 1978). In the spinalized group, the estimated ED_{50} was 0.125 to 0.25 mg/kg. In contrast, the estimated ED_{50} for the intact rats was between 0.03 to 0.125 mg/kg.

It is important to recognize the dramatic depression of base line level of the flexor reflex seen in the spinalized rats relative to the intact rats (see Figure 1). Without exception, spinalized rats showed weak flexor reflex responses to the same intensity stimuli that produced vigorous responses in the intact rats. This reduction in reflex excitability, attributable to spinal shock (Mountcastle, 1974), was seen even one day after transection (J. H. Kehne, D. W. Gallager, and M. Davis, unpublished observations).

Figure 3 shows the absolute postclonidine scores averaged across the three stimulus intensities following injection of saline or different doses of clonidine. It is apparent that, for the 0.5 and 2.0 mg/kg doses, the absolute levels of flexor reflex responding are

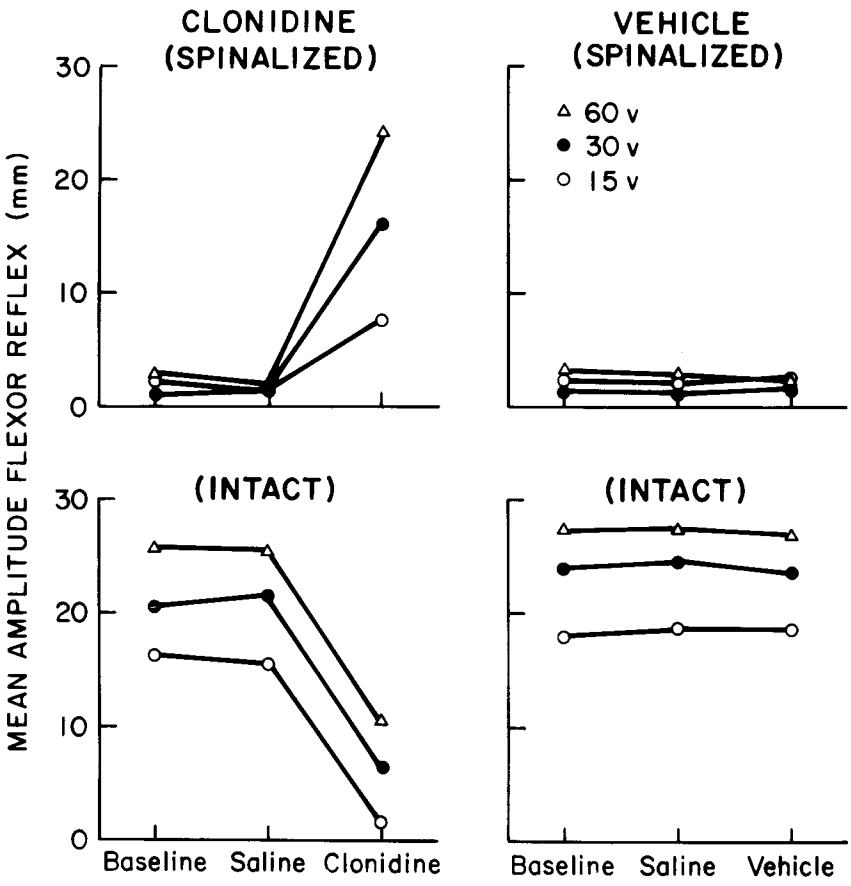

Figure 1. Mean amplitude flexor reflex following i.p. injection of 0.5 mg/kg clonidine (*left panels*) or vehicle (saline; *right panels*) in spinalized rats (n = 18; *top panels*) or intact rats (n = 27; *bottom panels*). Three eliciting stimulus intensities (60, 30, and 15 V) were used.

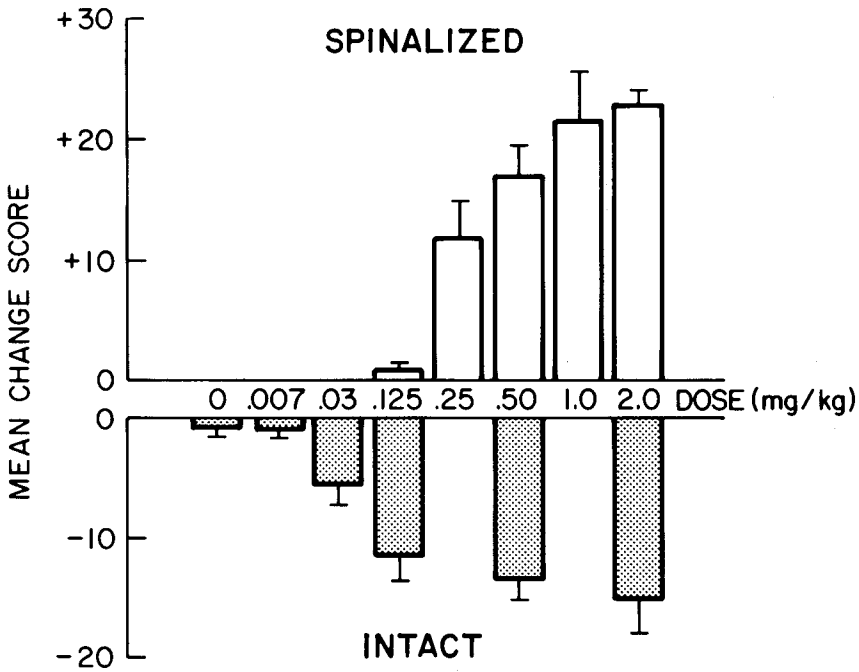

Figure 2. Mean flexor reflex change scores (clonidine minus vehicle) for rats treated with clonidine (.007 to 2.0 mg/kg i.p.) or vehicle in spinalized rats (*upper panel*) or intact rats (*lower panel*). A one-factor analysis of variance (ANOVA) of the change scores in the spinalized rats revealed a significant overall excitatory drug effect ($F(4,10) = 11.81$; $p < 0.001$) that was linearly related to dose (F-lin $(1,10) = 42.4$; $p < 0.001$). Analysis of the change scores for the intact rats revealed a significant overall depressant drug effect ($F(4,16) = 6.72$; $p < 0.005$) that was linearly related to dose (F-lin $(1,16) = 25.31$; $p < 0.001$).

lower in the intact rats relative to the spinalized rats. Therefore, in spite of changes in base line flexor reflex, clonidine depressed both absolutely and relatively the flexor in intact rats, while increasing the flexor reflex in spinalized rats.

Adrenergic receptors mediating clonidine's effects. Spinalized and intact rats received i.p. injections of either 10 mg/kg piperoxane (an α_2-adrenergic antagonist; e.g., Cedarbaum and Aghajanian, 1976), 1.0 mg/kg prazosin (an α_1-adrenergic antagonist; e.g., Davey, 1980; Cavero and Roach, 1980), or vehicle (distilled water) (n = 6 for each of the 6 groups) 30 min before being placed in the test apparatus. Five min later, half of the rats in each group were injected with 0.5 mg/kg clonidine and half with saline. The 0.5 mg/kg dose was chosen since it produced near-maximal effects on the flexor reflex.

Figure 4 shows the effects of pretreatment with vehicle (*left panels*), 10.0 mg/kg piperoxane (*middle panels*), or 1.0 mg/kg prazosin (*right panels*) on the flexor reflex in spinalized rats (*top row*) and in intact rats (*bottom row*) before and after treatment with

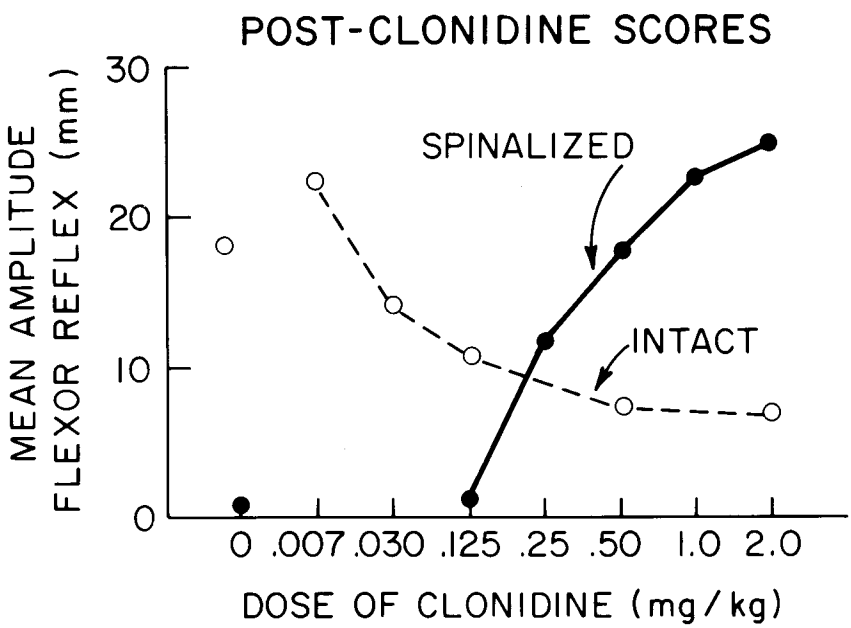

Figure 3. Mean amplitude flexor reflex following treatment with clonidine (.007 to 2.0 mg/kg i.p.) or vehicle in spinalized rats (*solid circles*) or intact rats (*open circles*). Each *point* represents the mean across the three stimulus intensities.

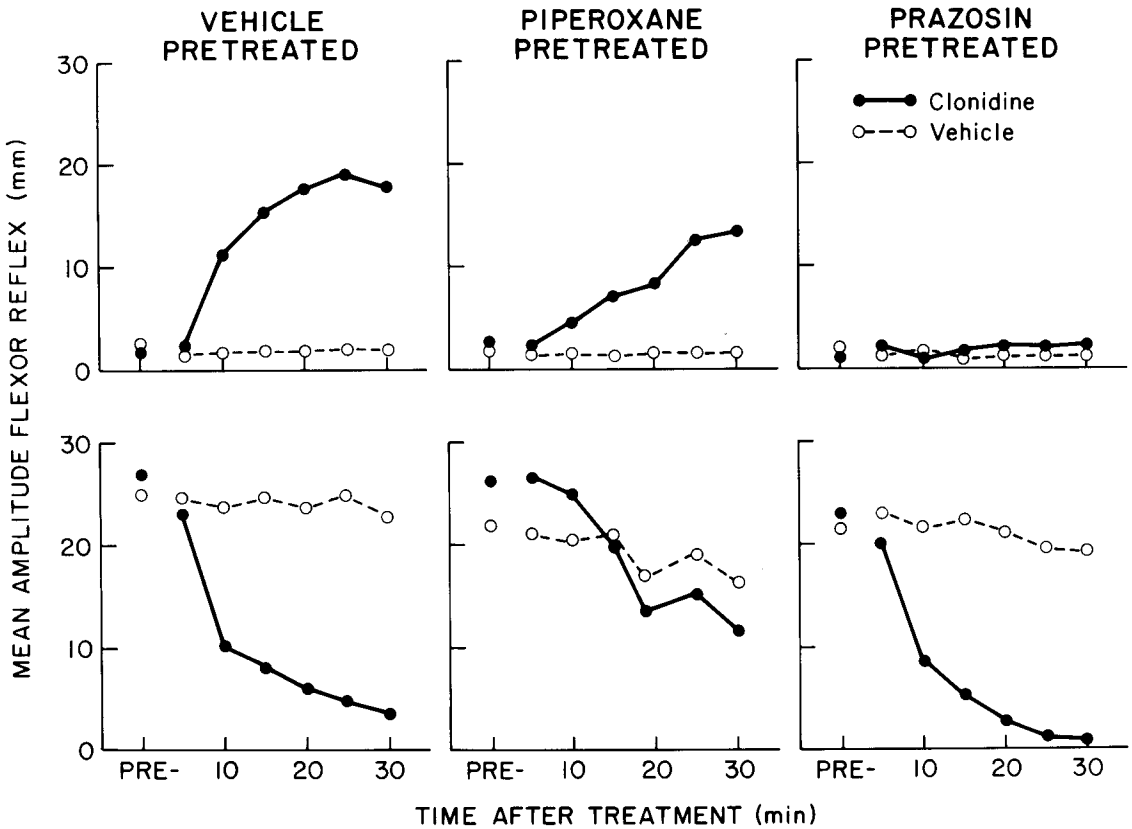

Figure 4. Mean amplitude flexor reflex following 30-min i.p. pretreatments with vehicle (distilled water; *left panels*), 10 mg/kg piperoxane (*middle panels*), or 1.0 mg/kg prazosin (*right panels*) followed by i.p. treatment with 0.5 mg/kg clonidine (●) or vehicle (○) in spinalized rats (*upper panels*) or intact rats (*lower panels*). An overall two-factor ANOVA revealed a significant clonidine effect ($F(1,12) = 82.21$; $p < 0.001$), a nonsignificant pretreatment effect ($F(2,12) = 2.73$; NS), and a significant interaction effect ($F(2,12) = 6.96$; $p < 0.01$). The clonidine depression was significant in all three pretreatment groups: water, $t(4) = 5.04$, $p < 0.01$; prazosin, $t(4) = 21.26$, $p < 0.001$; and piperoxane, $t(4) = 2.91$, $p < 0.05$. Although clonidine still depressed the flexor reflex in the piperoxane-pretreated rats, the magnitude of this depression was significantly less than that seen in the vehicle-pretreated group ($t(4) = 4.62$; $p < 0.05$). Thus, piperoxane attenuated clonidine depression in the intact rats. Analysis of the spinalized rats revealed significant pretreatment ($F(2,12) = 14.53$; $p < 0.01$), treatment ($F(1,12) = 63.24$; $p < 0.001$), and interaction ($F(2,12) = 15.5$; $p < 0.001$) effects. Clonidine enhanced the flexor reflex in rats pretreated with vehicle ($t(4) = 5.33$; $p < 0.01$) and piperoxane ($t(4) = 8.57$; $p < 0.002$) but not prazosin ($t(4) = 1.73$; NS).

either 0.5 mg/kg clonidine (*closed circles*) or vehicle (*open circles*). Each *point* represents the mean amplitude flexor reflex across five stimulus presentations. In all three pretreatment groups (for both spinalized and intact rats), saline treatment had little effect on the flexor reflex relative to the pre-injection base line. In spinalized rats, the excitatory effect of clonidine was completely blocked by prazosin, but not by piperoxane pretreatment. In intact rats, the depressant effect of clonidine was markedly attenuated by piperoxane (*middle panel*) but not by prazosin (*right panel*). Data analysis (see legend) indicated that the α_2-adrenergic antagonist piperoxane preferentially blocked the depressant effect of clonidine in intact rats, whereas the α_1-adrenergic antagonist prazosin blocked the excitatory effect in spinalized rats.

Local infusion and decerebration studies. Local infusion of oxymetazoline, an α_2-adrenergic agonist, was used to evaluate whether stimulation of supraspinal (i.c.v. infusion) or spinal (intrathecal infusion) α_2-adrenergic receptors depress the flexor reflex. Oxymetazoline was chosen since it is hydrophilic and therefore more likely to remain localized to the site of infusion than is clonidine (Marwaha et al., 1983). Figure 5 shows the mean change scores (30 min postinfusion minus 5 min pre-infusion) following i.c.v. (*closed circles*) and intrathecal (*open circles*) administration of the lipophobic α_2-adrenergic agonist oxymetazoline or water. Intrathecal infusion of oxymetazoline into the lumbar subarachnoid space did not differ from water in its effects on the flexor reflex. In contrast, i.c.v. infusion of oxymetazoline markedly depressed the reflex. Furthermore, pretreatment with piperoxane (10 mg/kg) 30 min before i.c.v. infusion of oxymetazoline (3.3 μg) significantly antagonized the oxymetazoline depression ($F(1,8) = 20.07$; $p < 0.005$) (data not shown). As seen in Figure 6, 2.0 mg/kg clonidine (relative to saline) depressed the flexor reflex in rats that were decerebrated 2 hr before testing. Decerebration would remove connections to the forebrain, leaving brainstem to spinal cord pathways intact. Taken together, these data indicate that the depressant effect of clonidine is mediated by an action in the caudal brainstem.

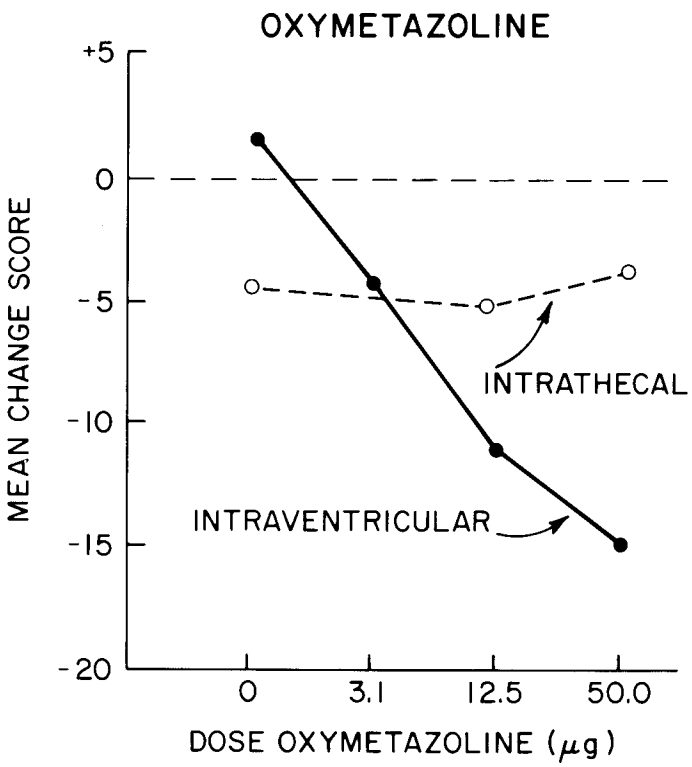

Figure 5. Mean flexor reflex change scores (30-min post- minus 5-min pre-injection) following i.c.v. (●) or lumbar intrathecal (O) infusion of oxymetazoline (0.25 to 50 μg) or saline in intact rats. $n = 3$ at each dose. ANOVA revealed an overall depressant drug effect following i.c.v. infusion ($F(3,9) = 10.29$; $p < 0.005$) but not following intrathecal infusion ($F < 1$). The i.c.v. depressant effect was linearly related to dose (F-lin $(1,9) = 29.54$; $p < 0.001$).

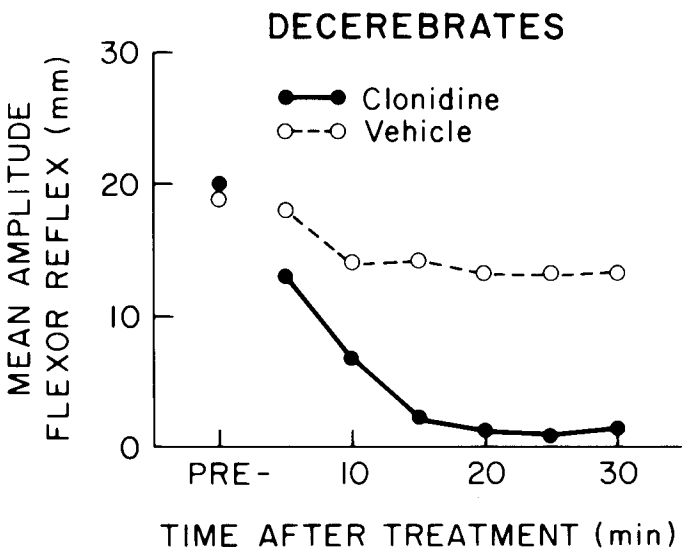

Figure 6. Mean amplitude flexor reflex following treatment with 2.0 mg/kg clonidine (●) or vehicle (O) in rats that were decerebrated at the midcollicular level 2 hr before testing ($n = 3$ in each group). Analysis of the change scores (30-min post- minus 5-min pre-infusion) revealed that clonidine significantly depressed flexor reflex amplitude relative to saline ($t(4) = 2.71$; $p < 0.05$).

Effects of intrathecal procaine-induced spinal block. An attempt was made to mimic the effect of spinal transection by inducing a spinal block with intrathecal administration of the local anesthetic procaine into the thoracic cord. As seen in the *bottom panel* of Figure 7, prior to procaine infusion, rats injected with 2.0 mg/kg clonidine showed depressed flexor reflex responses relative to rats that had been injected with saline (*top panel*). However, when the clonidine-injected rats were infused with procaine, within minutes there was a sudden reversal of the flexor reflex from inhibition to excitation, concomitant with paralysis of the hindlimbs. The excitatory effect was transient, lasting 4 to 5 min before the response levels returned to their original low levels. The decay of the excitation was accompanied by a loss of complete paralysis (that is, a return of muscle tone and/or some spontaneous leg movements). In contrast, vehicle-pretreated rats showed a rapid, transection-like decline in response amplitude following procaine infusion (*top panel* of Figure 7), concomitant with hindlimb paralysis. However, complete reversibility of the anesthetic was not demonstrated in that a return of muscle tone and some spontaneous movement was not necessarily accompanied by a return to base line.

Effect of spinal ligation. Because spinal block is not strictly equivalent to the mechanical process of spinal transection, a surgical technique was developed in order to eliminate the potentially confounding interference from local or general anesthetics. Figure 8 shows the effects of spinal cord ligation in rats that had been treated with either vehicle (*open circles*) or 2.0 mg/kg clonidine (*closed circles*). Prior to ligation, clonidine again produced a flexor reflex depression relative to the pre-injection base line, whereas saline injection had little effect. Immediately following spinal ligation, saline-pretreated rats showed a transient excitation of the flexor reflex, followed by a rapid and precipitous decline to near zero levels of responding (typical of spinal-transected or procaine-treated rats). In marked contrast, flexor reflex levels of clonidine-treated rats also were immediately elevated but continued to remain elevated throughout the postligation test period. It is important to emphasize that this elevation was relative to both the immediate pretransection level and also relative to the original base line. Thus, an immediate and lasting

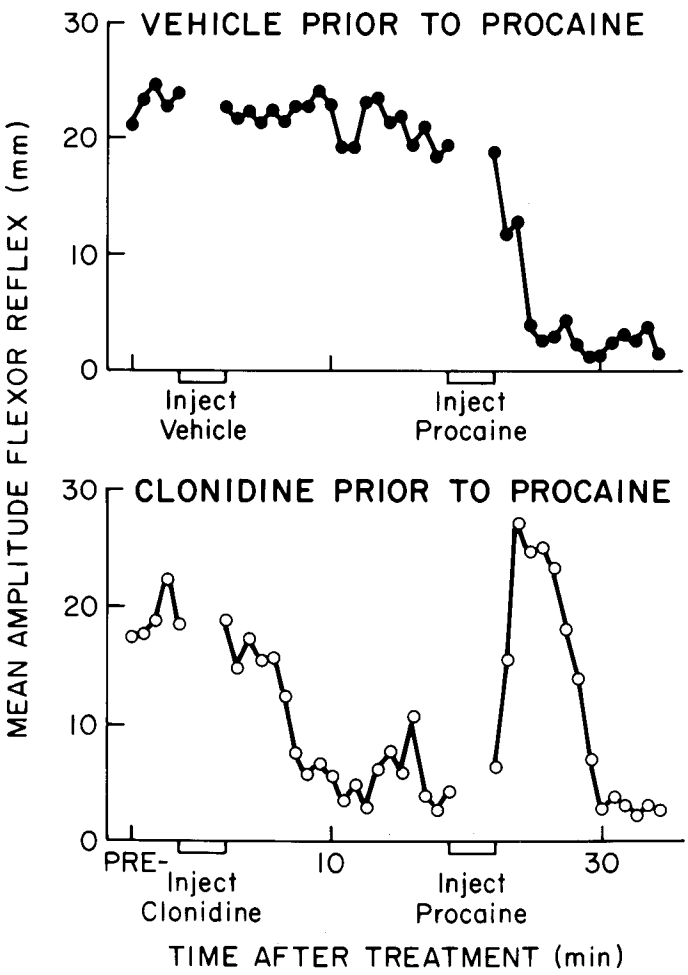

Figure 7. Mean amplitude flexor reflex following thoracic intrathecal procaine infusion in rats pretreated with 2.0 mg/kg clonidine (i.p.; *lower panel*) or vehicle (*upper panel*; $n = 3$ in each group).

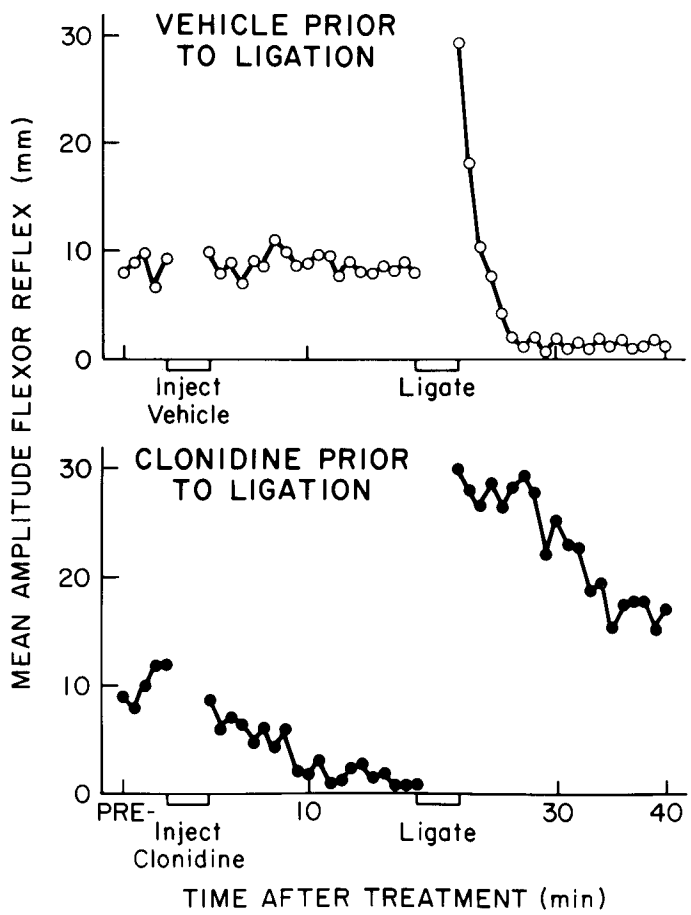

Figure 8. Mean amplitude flexor reflex following spinal ligation in rats pretreated with 2.0 mg/kg clonidine (i.p.; *lower panel*) or vehicle (*upper panel*; $n = 3$ in each group).

shift in the effect of clonidine from inhibition to excitation was obtained.

It is possible that the shift in clonidine's adrenergic profile might be attributed to a rapid development of α_1-adrenergic supersensitivity (e.g., Corr et al., 1981). Scatchard analysis of [^{3}H]prazosin binding in pooled lumbar spinal cord crude homogenates indicated that, at 2 hr following spinal transection (performed under halothane anesthesia), B_{max} was slightly elevated in spinalized rats relative to anesthetized controls (7 to 14%; data not shown). Following spinal ligation (in which residual effects of the halothane anesthetic could be ruled out), there was no indication of an increased number of α_1-adrenergic receptors (Table I). Lumbar cords were removed from rats either 15 min or 2 hr after spinal ligation and compared to cords taken from sham-operated or untreated rats ($n = 3$ in each group). Specific binding was measured in homogenates from individual cord in the presence of a single concentration of either [^{3}H]prazosin (1.0 nM) or [^{3}H]clonidine (4.0 nM). As seen in Table I, no overall group differences in receptor density were found for either [^{3}H]prazosin or [^{3}H]clonidine binding. A trend toward a decreased number of [^{3}H]prazosin binding sites was seen 2 hr following ligation. Furthermore, when spinal ligation was performed and the flexor reflex was evaluated using 2.0 mg/kg clonidine either 15 min or 2 hr later, no difference in the magnitude of the clonidine effect was seen (data not shown).

TABLE I

Mean specific [^{3}H]prazosin and [^{3}H]clonidine receptor binding (fmol/mg protein) in lumbar spinal cords taken from individual rats that were untreated, sham-operated but not ligated, ligated 15 min before sacrifice, or ligated 2 hr before sacrifice ($n = 3$ in each group)

Specific binding was measured in the presence of [^{3}H]prazosin (1.0 nM) or [^{3}H]clonidine (4.0 nM). One-factor ANOVAs revealed no significant change in [^{3}H]prazosin binding ($F(3,8) = 1.02$, NS) or in [^{3}H]clonidine binding ($F(3,8) < 1$).

Experimental Group	Lumbar Spinal Cord Specific Binding (fmol/mg protein)	
	[^{3}H]Prazosin	[^{3}H]Clonidine
Untreated	63.1 ± 6.1[a]	91.0 ± 8.6
Sham-operated	57.4 ± 8.4	89.1 ± 7.0
Ligated + 15 min	57.1 ± 7.1	98.7 ± 5.1
Ligated + 2 hr	49.2 ± 10.4	105.6 ± 9.8

[a] Mean ± SE.

Discussion

The overall aim of the proposed studies was to gain an understanding of why clonidine acts like an α_1-adrenergic agonist to excite the flexor reflex in spinalized rats when, in other behavioral paradigms, clonidine exerts α_2-adrenergic receptor-mediated depressant effects. The first experiment showed that spinal transection was, in fact, a necessary condition for the expression of clonidine's excitatory effect. When tested in an intact rat, clonidine markedly depressed the flexor reflex through a stimulation of α_2-adrenergic receptors. Spinalization resulted in a shift of clonidine's pharmacological profile from an α_2- to an α_1-adrenergic agonist. Although spinalization markedly depressed the flexor reflex base line, this depression could not account for the opposite effects of clonidine seen in the two preparations.

In the intact rat, local infusion of the hydrophilic α_2-adrenergic agonist oxymetazoline into the lateral ventricle depressed the flexor reflex, whereas local infusion into the subarachnoid space of the lumbar spinal cord did not. These results suggest that, in the intact rat, the depression of the flexor reflex results from stimulation of supraspinal and not spinal α_2-adrenergic receptors. Furthermore, these receptors are located in the caudal brainstem, since clonidine still depressed the flexor reflex in acutely decerebrate rats. At the present time, it is not clear if these α_2-adrenergic receptors are located presynaptically on noradrenergic terminals or cell bodies or if they are postsynaptic (U'Pritchard et al., 1979; Bylund and Martinez, 1981; Dooley et al., 1983).

Several techniques were used to evaluate two hypotheses for the shift in the α-adrenergic properties of clonidine following spinalization. The first hypothesis suggested that denervation supersensitivity might "bring out" or accentuate the α_1-adrenergic agonist properties of clonidine, whereas the second hypothesis was that an α_2-adrenergic inhibitory influence from the brainstem in the intact preparation might prevent the expression of an α_1-adrenergic receptor-mediated spinal excitatory effect of clonidine. Spinal block and spinal ligation were used to evaluate the immediate consequences of spinalization without interference from residual surgical anesthetic. Both techniques clearly demonstrated that spinal transection caused clonidine's effect on the flexor reflex to *immediately* shift from inhibition to excitation. Thus, no evidence for a rapid development of spinal α_1-adrenoceptor supersensitivity was found. Therefore, these data support the conclusion that spinal transection "unmasks" the α_1-adrenergic excitatory property of clonidine, which is normally overridden in the intact preparation by a supraspinal inhibitory effect.

What is the nature of this "masking" influence? One possibility is that clonidine's stimulation of supraspinal α_2-adrenergic receptors (pre- or postsynaptic) potently overrides the effect of spinal α_1-adrenergic receptor stimulation. The fact that pretreatment with the α_2-adrenergic antagonist piperoxane did not "unmask" an underlying excitatory effect of clonidine argues against this possibility. A variant of this explanation is that some other pharmacological action of clonidine, such as an effect on histamine receptors (Sastry and Phyllis, 1977), actively prevents the expression of the α_1-adrenergic excitation of the flexor reflex. Finally, it is possible that activity in some neural system that is not influenced by clonidine tonically prevents the expression of clonidine's spinal α_1-adrenergic agonist properties in the intact rat. In these last two cases, it would be predicted that an appropriate pretreatment (pharmacological antagonist or lesion) would mimic the effect of spinal transection and unmask the excitatory effect of clonidine in the intact rat.

In the introduction, it was noted that clonidine generally exerts depressant effects on behavior that are mediated by a stimulation of α_2-adrenergic receptors. Nevertheless, there are reports that, under special circumstances (other than spinal transection), clonidine's effects can be shifted from α_2-adrenergic inhibition to α_1-adrenergic excitation. One of these circumstances is combining clonidine with other pharmacological treatments. Thus, the following effects of clonidine were reported to be α_1-adrenergic receptor-mediated: (*1*) potentiation of apomorphine-stimulated locomotor behavior in reserpine pretreated mice (Anden et al., 1970); (*2*) enhanced stimulatory effect of clonidine on apomorphine-potentiated locomotor activity in reserpinized mice during withdrawal from chronic clonidine treatment (Svensson and Strombom, 1977); and (*3*) stimulation of locomotor behavior in rats given the following combinations: 6-OHDA + reserpine; 6-OHDA + α-methyl-para-tyrosine + para-chloro-phenylalanine (Zebrowska-Lupina et al., 1977). Zebrowska-Lupina et al. (1977) noted that no single factor (i.e., serotonin depletion or norepinephrine receptor supersensitivity) could account for their findings, but rather the unmasking of clonidine's α_1-adrenoceptor-mediated excitation in the intact animal may require a combination of these circumstances. In the Anden et al. (1970) study, two factors (reserpine depletion of serotonin and apomorphine stimulation of dopamine receptors) may interact to unmask clonidine's α_1-adrenergic excitatory effect on locomotion.

Nonpharmacological factors can interact with clonidine to cause a shift in its α-adrenergic effects on behavior. For example, clonidine exerts a depressant effect on shock-elicited jumping through an α_2-adrenergic action when low-intensity footshock is used, whereas a high intensity reveals α_1-adrenoceptor-mediated excitation (Nishikawa et al., 1983). Finally, a developmental shift in the pharmacological profile of clonidine has been reported. Thus, clonidine exerted α_1-adrenergic excitatory effects on locomotor activity in rat pups tested at an early age (i.e., postnatal day 7), whereas clonidine's profile shifts to α_2-adrenergic inhibition by postnatal day 20 (Reinstein and Isaacson, 1977; Nomura et al., 1980). For locomotor behavior, developing forebrain neurochemical systems (e.g., serotonin) may mask the α_1-adrenoceptor agonist property of clonidine in the adult animal.

The studies on locomotor behavior provide future directions for the study of clonidine's effects on the flexor reflex in intact rats. Pharmacological studies could determine if clonidine's excitatory effect on the flexor reflex is unmasked in the intact (nonspinalized) rat by similar drug pretreatments (i.e., reserpine + 6-OHDA). Developmental studies could determine if there is a shift in clonidine's effect as a function of age. In addition, local infusion of α_2-adrenergic agonists into specific areas of the caudal brainstem could evaluate the location of the specific receptors that mediate clonidine's depressant effects on the flexor reflex.

In summary, the present experiments indicate that, in the intact rat, some supraspinal influence (either an inhibitory system activated by clonidine or another, independent system that is tonically active) prevents the expression of clonidine's α_1-adrenergic activation of the flexor reflex. Spinal transection produces a release from this inhibitory influence, allowing the excitatory effects to occur.

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