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Serotonergic Mediation of Spinal Analgesia and Its Interaction with Noradrenergic Systems

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Serotonin was administered intrathecally onto cat spinal cords to evaluate the pharmacology by which it suppresses noxiously evoked activity of wide-dynamic-range (WDR) neurons in the spinal dorsal horn. Doses of 500, 1000 and 2000 μg serotonin produced significant suppression of the mean noxiously evoked activity of WDR neurons in the dorsal horn of the spinal cord (21, 44, and 69% at 30 min, respectively). The dose-dependent effects were partially reversed by the intravenous administration of the serotonin antagonist methysergide (1 or 2 mg). Intravenous administration of the alpha 2-adrenergic antagonist yohimbine (0.5 or 1.0 mg/kg) produced a significant antagonism of the effects of serotonin. In contrast to the effects of methysergide and yohimbine, intravenous administration of naloxone or the alpha 1-antagonist corynanthine had no effect upon the suppressive effects of serotonin. The combination of low-dose serotonin and low-dose clonidine produced a supraadditive effect (30% at 30 min). These data support the concept that noradrenergic systems, possibly through an alpha 2-adrenergic mechanism, are involved in the modulation of spinal WDR neurons by serotonin. (Key words: Neurotransmitters: serotonin. Spinal cord: dorsal horn. Sympathetic nervous system, catecholamines: norepinephrine.

THE COMPLEX PHARMACOLOGY of systems that modulate sensory processing within the spinal cord holds great promise for improved ways of providing spinal analgesia. Several lines of evidence demonstrate that descending serotonergic systems mediate inhibition of nociceptive dorsal horn neurons. Electric stimulation of brain stem sites known to have important serotonergic connections with the spinal cord produces a profound depression of nociceptive neurons in the dorsal horn.[1–3] These inhibitory effects have been reported to be reduced when serotonin levels are depleted.[3] Iontophoretically applied serotonin has been demonstrated to inhibit the responses of dorsal horn neurons.[4–6] It also has been shown in several behavioral studies that intrathecal injection of serotonin produces a definite elevation in nociceptive thresholds.[7–10]

There also is evidence implicating the involvement of adrenergic systems in the suppression of spinal pain transmission,[11,12] and the interaction of descending serotonergic and noradrenergic pathways has been suggested. A series of studies by Danysz et al.;[13] Minor *et al.*,[14,15] and Archer *et al.*[15,16] provided evidence of an important role for spinal noradrenergic systems in serotonergic modulation of spinal function. Hammond and Yaksh[17] reported that the antinociception produced by electric stimulation of raphe nuclei (an important source of descending serotonergic inhibition) was antagonized by intrathecal administration of phentolamine. These and other reports suggest an important interaction between descending noradrenergic and serotonergic pathways at a spinal level.

The purpose of the current study was to examine the effects of spinally administered serotonin in combination with adrenergic antagonists on noxiously evoked activity of wide-dynamic-range (WDR) neurons in the dorsal horn of the spinal cord of cats. We were particularly interested in determining the effects of intravenous yohimbine (alpha 2-adrenergic antagonist) and intravenous corynanthine (alpha 1-adrenergic antagonist) on the suppression of noxiously evoked activity produced by serotonin, in order to ascertain the interaction of serotonergic and noradrenergic pathways at the level of the spinal cord. These studies are directed at defining an optimum combination of substances capable of suppressing noxiously evoked activity at the level of the spinal cord.

Materials and Methods

This study was approved by the Yale Animal Care and Use Committee. Fifty-six cats of either sex weighing 2.7–4.5 kg were prepared for electrophysiologic experiments. Surgical preparation was carried out with halothane, nitrous oxide, and oxygen anesthesia until the point at which the animals were rendered decerebrate. A jugular vein and carotid artery were catheterized to provide routes for intravenous fluid and drug administration and for monitoring of arterial blood pressure. A tracheostomy was performed, and the animal's lungs were artificially ventilated after paralysis with pancuronium bromide (0.1–0.15 mg/kg/hr). After placement of the animals in a stereotaxic apparatus, the animals were rendered decerebrate by electrolytic lesions in the midbrain reticular formation. Decerebration allowed discontinuation of general anesthesia during the neurophysiologic recordings, and permitted us to study the drug effects in pain-free, drug-free (except for pancuronium) unconscious animals. The

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spinal cord was transected at T11 or T12 to remove supraspinal modulation, and to allow us to examine the effect of drugs in the absence of descending inhibitory systems. A laminectomy was performed from the L4 through the L6 spinous processes to expose the lumbar spinal cord. The dura was cut and reflected, and the spinal cord was bathed in 37° C physiologic saline. Blood pressure, body temperature, and end-tidal CO_2 were monitored and maintained within normal limits.

After surgical preparation and a minimum of 2 h after the end of anesthesia, a tungsten microelectrode was advanced into the dorsal horn of the spinal cord in order to obtain extracellular recordings of electrical activity from single WDR neurons. Upon isolation of a single WDR neuron, light touching, pinching with forceps, cooling of the skin (ethyl chloride spray), and noxious radiant heat (51° C) were applied to the cell's receptive field on the hind paw. Skin temperature was measured by a thermocouple placed in the center of the neuron's receptive field. The thermocouple also provided feedback for control of stimulus temperature. A typical WDR neuron would demonstrate increased firing frequency in response to increasing intensity of stimulation, with maximum activity being elicited by noxious stimuli. This response profile suggests that WDR neurons are involved in the processing of information about noxious stimuli. After isolation and characterization of a single WDR neuron, control studies were performed in which noxiously evoked activity was elicited by the presentation of a 51° C radiant heat stimulus for 8 s to the cell's receptive field. Only one neuron was studied in each animal to avoid cumulative effects of the administered drugs.

After control studies, the saline that had been bathing the spinal cord was removed, and the serotonin–saline solution (0.5 ml, 37° C) was applied gently onto the spinal cord through a 27-G needle. The applied drugs were serotonin 250 μg (n = 5), 500 μg (n = 8), 1000 μg (n = 8), and 2000 μg (n = 24) and a combination of serotonin 250 μg and clonidine 5 μg (n = 5). After the application of serotonin, noxiously evoked activity was recorded every 3 min for a minimum of 30 min. In two cases after the administration of 1000 μg serotonin, and in five cases after 2000 μg serotonin, 1 or 2 mg methysergide (nonselective serotonin antagonist) was administered intravenously 31 min after serotonin application to evaluate the reversal of the serotonin effect. In addition, yohimbine (0.5 mg/kg or 1.0 mg/kg, n = 4), corynanthine (0.25 mg/kg or 0.5 mg/kg, n = 4), and naloxone (0.1 mg or 0.2 mg, n = 4) were administered intravenously 31 min after the 2000-μg serotonin application to determine their effect on the antinociceptive action produced by serotonin. Methysergide alone (n = 3) or yohimbine alone (n = 3) also were administered intravenously to examine the effects of these drugs by themselves on WDR neurons.

All data collected were analyzed on-line as well as off-line by a PDP 11/40 computer. Spontaneous activity was averaged for 30 s prior to stimulus onset. The evoked activity was observed during the period of time from stimulus onset until return of activity to within 10% of prestimulus control values. Student's paired and unpaired *t* test were used for statistical analysis. Differences were considered to be significant if *P* values were less than 0.05.

Results

Data were obtained from 56 neurons. All neurons from which noxiously evoked activity was recorded were classified as WDR neurons.

Serotonin Effects on the Evoked Activity of WDR Neurons

Figure 1 shows the effects of 2000 μg of spinally administered serotonin on a single WDR neuron. Fifteen minutes after spinal administration, the evoked activity was reduced to 75% of control, and at 30 min, it had been further reduced to 32% of control. Thirty minutes after drug administration, 1 mg methysergide was injected intravenously, and 3 min later the evoked activity had returned to 71% of the control value.

The effects of 500-, 1000-, and 2000-μg doses of spinally administered serotonin on the mean evoked activity are seen in figure 2. Five hundred micrograms serotonin produced a significant suppression of the mean evoked activity, to 79% of the control values at 30 min. Thirty minutes after the spinal administration of 1000 or 2000 μg serotonin, the mean noxiously evoked activity had been reduced to 56 and 31% of control values, respectively. A comparison of the amount of suppression produced by

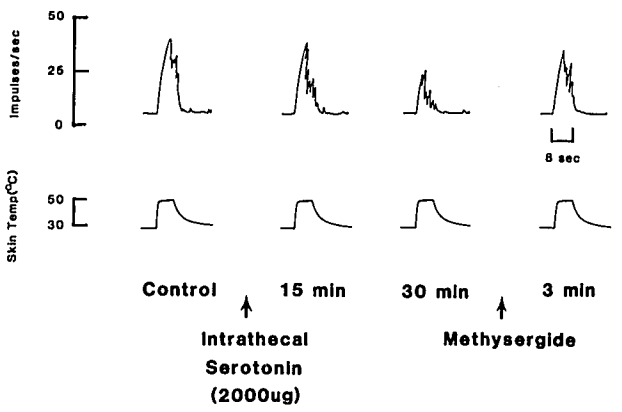

FIG. 1. Effects of 2,000 μg intrathecal serotonin on a single WDR neuron and reversal by intravenous administration of 1 mg of methysergide. The bottom traces represent the skin temperature at the center of the receptive field. The top traces represent the evoked activity expressed as impulses per second.

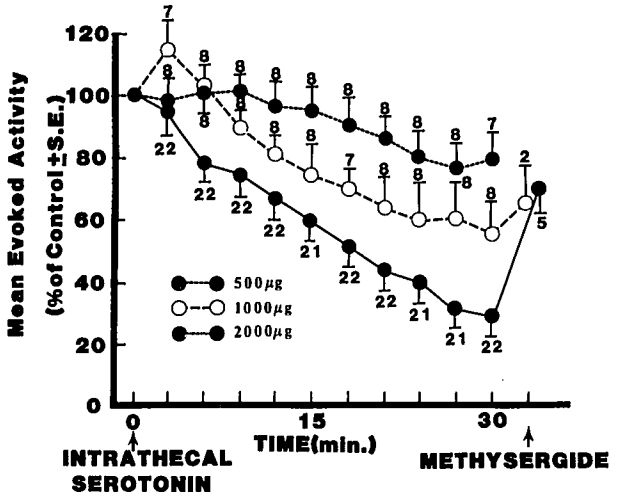

FIG. 2. Effects of 500 (n = 8), 1,000 (n = 8), and 2,000 μg (n = 22) of spinally administered serotonin on the mean noxiously evoked activity of WDR neurons. Abscissa: time in minutes after spinal serotonin administration. Ordinate: mean evoked activity expressed as a percent of control. The bars indicate ± SEM. Numbers near each point indicate sample size. Suppression at 30 minutes by all doses was statistically significantly different from control. The suppression at 30 minutes by 2,000 μg also was statistically significantly different from that produced by 500 μg.

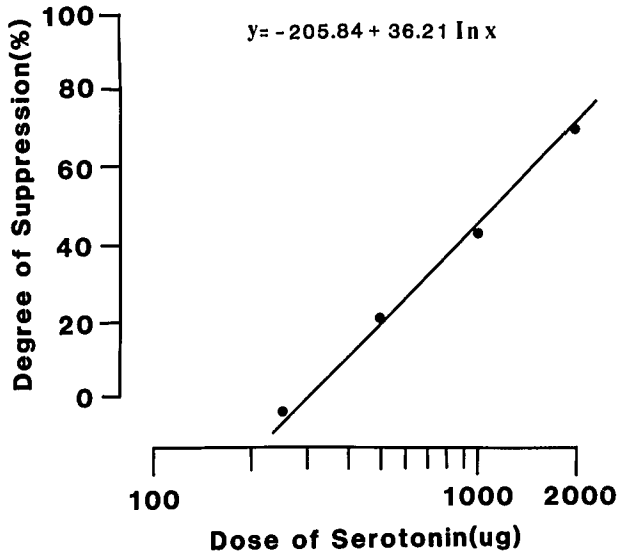

FIG. 3. The dose–response curve presented for the effects of intrathecally administered serotonin on the mean evoked activity of WDR neurons at 30 min after administration. The applied doses were 2,000 (n = 22), 1,000 (n = 8), 500 (n = 8), and 250 μg (n = 5).

the 2000-μg dose of spinally administered serotonin with that produced by the 500-μg dose indicates that the 2000-μg dose produced a significantly greater suppression of the noxiously evoked activity. These dose-dependent effects were partially reversed by the intravenous administration of methysergide: the evoked activity of neurons administered 1000 and 2000 μg serotonin was returned to 65 and 69% of control value, n = 2 and 5, respectively. With two neurons, 2000 μg serotonin produced a significant and long-lasting (>30 min) excitant effect, and in one of these neurons, the excitant effect was reversed by methysergide administration.

Figure 3 is a dose–response curve of the effects of intrathecally administered serotonin on the mean evoked activity of WDR neurons. There exists a definite correlation between dose and the inhibitory effects of serotonin (r = 0.99925).

Interaction of Serotonin and Noradrenergic Systems at the Spinal Level

In the four neurons in which it was tested, yohimbine produced a significant, though incomplete, antagonism of the effect of spinally administered serotonin.

Figure 4 contains a summary of the effects of the systemic administration of methysergide, yohimbine, corynanthine, and naloxone on the serotonin suppression of WDR neurons. Both methysergide and yohimbine produced partial antagonism of the suppression of evoked activity that was produced by 2000 μg serotonin. In contrast to the effects of methysergide and yohimbine, intravenous administration of corynanthine or naloxone had no effect upon the nociceptive effects of serotonin. Intravenous administration of methysergide or yohimbine alone produced no change in the evoked activity of WDR neurons (data not shown; n = 3 for each drug).

Table 1 indicates the effects of 250 μg of spinally ad-

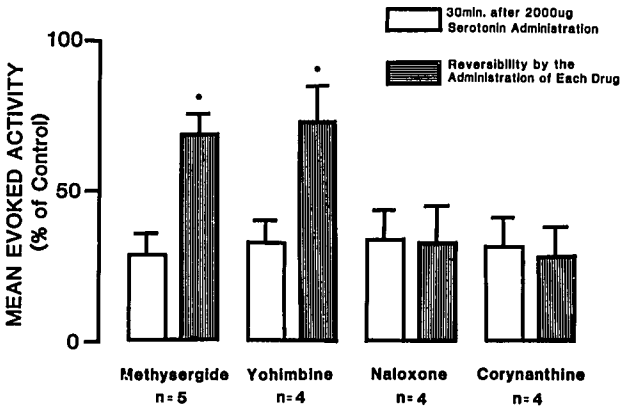

FIG. 4. The effects of intravenous administration of methysergide (1 or 2 mg, n = 5), yohimbine (0.5 or 1.0 mg/kg), corynanthine (0.25 or 0.5 mg/kg, n = 4), and naloxone (0.1 or 0.2 mg, n = 4) on the serotonin suppression of WDR neurons. The bars indicate ± SEM. Methysergide or yohimbine produced a significant antagonism of the effect of serotonin as is indicated by the asterisk.

TABLE 1. Effects of Low-Dose Serotonin, Low-Dose Clonidine, and the Combination of Serotonin and Clonidine on the Mean Evoked Activity of WDR Neurons

	Control (%)	15 min (%)	30 min (%)
Serotonin 250 μg (n = 5)	100	106.1 ± 9.01	107.4 ± 10.05
Clonidine 5 μg* (n = 8)	100	90.4 ± 4.56	97.4 ± 8.49
Serotonin 250 μg + clonidine 5 μg (n = 5)	100	88.9 ± 3.54	70.4 ± 4.40†

Mean ± SE. *$P < 0.05$ compared with control. †From Murata *et al.*[18]

ministered serotonin alone and 5 μg of spinally administered clonidine alone, and the combination of 250 μg serotonin and 5 μg clonidine, on the neuronal activity of WDR neurons. Data for 5 μg clonidine were cited from the study of Murata *et al.*[18] In contrast to the ineffectiveness of spinally administered serotonin or clonidine alone at these doses, the combination of serotonin and clonidine produced a significant suppression of the evoked activity of WDR neurons within 30 min (70% of control). This effect was partially reversed by yohimbine applications (to 86% of control).

Any drug administered intrathecally or intravenously produced no discernible alteration of the mean blood pressure during the time that neuronal activity was evaluated.

Discussion

Both serotonergic and noradrenergic systems are capable of suppressing noxiously evoked activity of spinal dorsal horn neurons.[4–6,12,19] Behaviorally, there is evidence that serotonergic inhibition of noxiously evoked activity at the level of the spinal cord is dependent on a noradrenergic system, probably an alpha-2 system.[20] The results of this study confirm the ability of spinally administered serotonin to inhibit the noxiously evoked activity of neurons in the spinal dorsal horn. They also demonstrate, as evidenced in behavioral studies, the interactions between adrenergic (specifically, alpha-2) systems and spinal serotonergic systems.

A recent series of studies that used transmitter depletion to demonstrate the interactions between noradrenergic and serotonergic systems emphasized, as a possible mechanism of action, changes in receptor sensitivity.[15,16,20] The short time course of this study in acute, spinally transected animals makes those types of receptor changes unlikely. Our results suggest that serotonin suppression of noxiously evoked activity in the spinal dorsal horn depends, in part, on a final common pathway that involves a noradrenergic system. If this is the case (*i.e.*, if serotonin suppresses noxiously evoked activity only by causing the release of norepinephrine), it may be more appropriate to consider adrenergic (specifically, alpha-2) rather than serotonin administration for spinal analgesia. However, the supraadditive affects of clonidine and serotonin seen in this study suggest that the spinal interaction of serotonin and norepinephrine is not simply at the final common pathway with norepinephrine. Clearly, additional studies are required in order better to understand the interactions of these two transmitter systems in spinal analgesia.

Additional studies also should take into consideration the likely serotonin receptor type or types involved. There are currently proposed to be at least seven serotonin (5-hydroxytryptamine [5-HT]) receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1P}, 5-HT_2, and 5-HT_3).[21] Methysergide appears to be relatively selective for the 5-HT_2 receptor subtypes, but it is not at all clear how important spinal 5-HT_2 sites are in the production of analgesia.[22,23] The methysergide reversal of serotonin suppression of noxiously evoked activity in the current study suggests a possible role for 5-HT_2 sites in spinal analgesia. The transection of the spinal cords eliminated possible supraspinal effects, but not the possibility that the action was peripheral to the spinal cord.

Behavioral studies using spinal serotonin-induced analgesia have shown effects to be of shorter duration and possibly greater intensity than those seen in this study. In rats, Minor *et al.*[20] demonstrated behavioral analgesia (tail-flick, hot-plate, shock titration) at 5 min after intrathecal doses of 200 and 250 μg serotonin. No analgesia was apparent at 30 min. Yaksh and Wilson[10] demonstrated that spinally administered serotonin could increase the time it takes a physiologically intact, awake, drug-free cat to withdraw (skin-twitch and escape) from a noxious thermal stimulus (70° C) that was applied to the depilated skin. In that study 500 μg of spinally administered serotonin produced a maximum effect on escape latencies (approximately doubling them) 15 min after drug administration, with recovery observed within 30 min after drug administration.

The differences between peak effect and duration in the above behavioral studies and in the current neurophysiologic study point out an important consideration for the evaluation of drug-induced changes in neurophysiology. It is very apparent that there is a close correspondence between the ability of various drugs to suppress noxiously evoked activity of WDR neurons in the spinal dorsal horn and the ability of those same drugs to produce behavioral analgesia in many animals. That correspondence, however, does not tell us how much suppression of noxiously evoked activity must occur in order for analgesia to be present. A still unresolved question for all parts of the nervous system is how much change

in firing frequency is necessary to produce a change in behavior. Although that question has not been answered, the correspondence between behavioral changes and neurophysiologic changes clearly demonstrates an important role for WDR neurons in the processing of sensory information within the spinal cord.

An additional important difference between the doses of serotonin used in this study and in behavioral studies[10,20] is the higher dose ranges used in this study. Serotonin is capable of causing constriction of vascular smooth muscle. Because we did not observe "blanching" of surface vessels on the cord, we do not believe that a reversible depression of cord blood flow was the cause of the observed neuronal suppression.

The question of a possible ischemic effect of serotonin is important. We must not assume that because a substance naturally occurs in the spinal cord, it may be administered there with impunity. In the absence of data to the contrary, any substance that is administered perispinally, even if it occurs naturally in the cord, must be assumed to be potentially toxic. Although we used some high concentrations of serotonin in this study, our ultimate goal is to define combinations that avoid side effects, by using extremely low doses of appropriate agonists.

Intravenous naloxone did not reduce the inhibition of evoked activity caused by the serotonin administration. This result agrees with another study in which serotonin effects on spinal dorsal horn neurons were evaluated.[19] The inability of naloxone to antagonize the serotonin effect implies that the spinal effect of this amine is not mediated by the activation of a spinal opiate system.

In summary, the current experiments have confirmed that intrathecally administered serotonin suppresses the noxiously evoked activity of WDR neurons in the dorsal horn. The reversibility of the inhibitory effect of serotonin by yohimbine suggest an important interaction between noradrenergic and serotonergic pathways, possibly at the spinal level. The possibility of an adrenergic final common pathway suggests that adrenergic agonists may be more appropriate than serotonergic agonists for spinal analgesia.

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