end, the higher frequencies (600 to 1000 Hz) at the caudal end, and the intermediate frequencies in the intervening region. We found that seismic sensitivity is present over the entire saccular macula and over a narrow band in the center of the lagenar macula. Sensitivity to linear acceleration (for example, tonic gravitational sensitivity) was found over the large peripheral fields of the lagenar and utricular maculae. Sensitivity to changes in linear acceleration (for example, phasic gravitational sensitivity) was found within the narrow central fields of those maculae, directly beneath the striolae of the otoconial membranes.

Lewis and Li (*2*) lumped the hair cells of the bullfrog into six types based on surface topography and presented maps of the distributions of those types over the three maculae and two papillae. With those maps it was relatively easy to determine which hair-cell types were associated with each terminal arborization (Table 2). If we assume that the five seismic-vestibular lagenar units identified and traced so far owe their seismic sensitivity to the type E hair cells that they innervate and their vestibular sensitivity to the type C hair cells, the following correspondences between hair-cell type and function emerge from Table 2. (i) Seismic and auditory sensitivities are associated with hair-cell types A, D, and E. (ii) Phasic vestibular sensitivity is associated predominantly with hair-cell type F. (iii) Phasic-tonic vestibular sensitivity is associated predominantly with type C. (iv) Purely tonic vestibular sensitivity is associated with type B. Only the seismic or auditory hair cells (types D and E) possess kinociliary bulbs.

No tonotopic organization has been recognized so far in the basilar papilla. The clear tonotopic organization in the amphibian papilla is interesting inasmuch as that organ lacks a basilar membrane, the structure presumed to be at least partially responsible for tonotopy in the mammalian cochlea. The bullfrog is the third lower vertebrate shown to have auditory tonotopy; others are the alligator lizard and the granite spiny lizard (*17*). In the bullfrog amphibian papilla, the low-frequency region is directly adjacent to the thickest (and therefore presumably most massive) portion of the tectorium, while the high-frequency region is directly adjacent to the thinnest (presumably least massive) portion. These are the associations one would expect if the tectorium were serving as part of a distributed mechanical filter producing the tonotopy.

The seismic (vibratory) sensitivity of the saccular macula is especially acute (for example, in one saccular axon, responses could be seen to sinusoidal stimuli of $2 \times 10^{-5} g$ peak acceleration, or approximately 1 nm of substrate displacement, in the neighborhood of 100 Hz). This acuteness may account for the sensitivity observed in some saccular axons not only to seismic vibration but also to airborne sound, which inevitably is coupled to the substrate and thus produces some seismic vibration. To date, no axons have been traced to the center of the central field in the utricular macula. Therefore, the presence or absence of seismic sensitivity in that organ remains in doubt. We have also been unable to identify sensitivity variations corresponding to the mediolateral morphological variations in the auditory papillae or sensitivity differences between the central and peripheral fields in the sacculus.

EDWIN R. LEWIS
RICHARD A. BAIRD
ELLEN L. LEVERENZ
HIRONORI KOYAMA
Electronics Research Laboratory, University of California, Berkeley 94720

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Spinal Sympathetic Neurons: Possible Sites of Opiate-Withdrawal Suppression by Clonidine

Abstract. *Morphine, methadone, meperidine, fentanyl, and clonidine rapidly depressed transmission through sympathetic preganglionic neurons in cats with the spinal cord transected. Naloxone promptly antagonized this effect of the opiates but not that of clonidine which was reversed by α_2-adrenergic receptor antagonists. The independent depression of preganglionic neurons by clonidine may contribute to the ability of this drug to depress the symptoms of opiate withdrawal that are characterized by sympathetic hyperactivity.*

Clonidine, a potent centrally acting antihypertensive drug (*1*), is remarkably effective in reducing the symptoms of opiate withdrawal in man (*2*) and animals (*3, 4*). Although clonidine does not share the addiction liability of the opiates, both drugs produce sedation, analgesia, respiratory depression, bradycardia, and hypotension (*1, 4–7*). Furthermore, abrupt discontinuation of long-term therapy with clonidine can produce symptoms that closely resemble (*8*) but are milder than those precipitated by withdrawal from opiates (*2, 5*). Many features of these withdrawal syndromes indicate hyperactivity of the sympathetic nervous system, possibly because of a rebound hyperexcitability of some central neu-

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rons that control the sympathetic outflow.

Observations that both clonidine and opiates depress the excitability of noradrenergic, locus coeruleus neurons in animals (*9*, *10*) prompted the original clinical trials demonstrating the efficacy of clonidine in opiate withdrawal (*2*). These findings have focused attention on the possible role of central noradrenergic neurons, especially those of the locus coeruleus, in the withdrawal reactions of both drugs. However, the role of the locus coeruleus in regulation of sympathetic activity remains obscure (*11*), and actions at other central sites that are directly involved in sympathetic control may be more relevant to suppression of opiate withdrawal symptoms by clonidine.

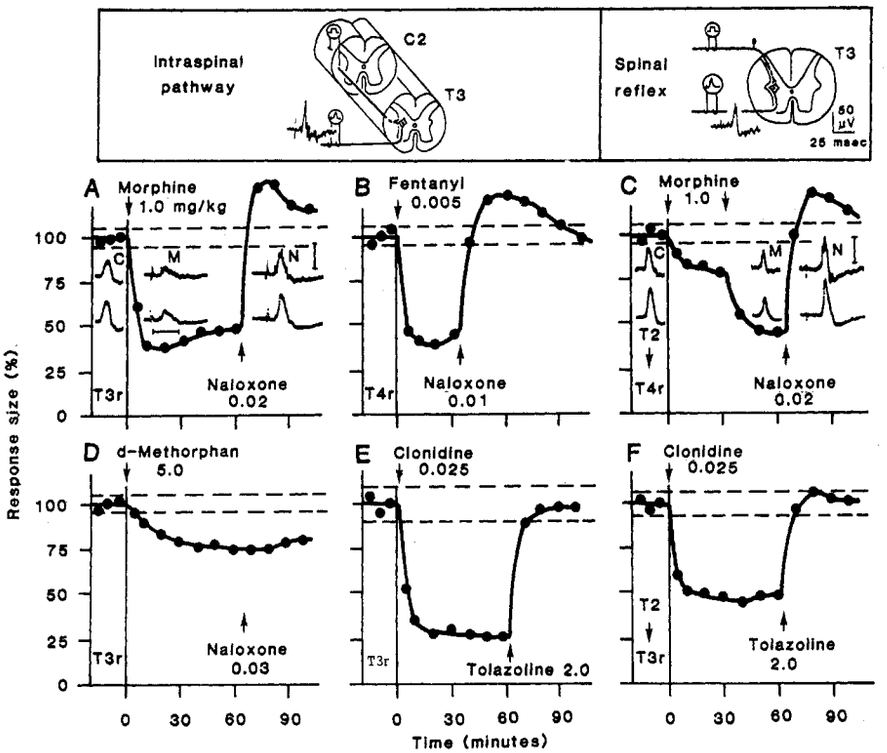

Fig. 1. Depression of transmission through descending intraspinal excitatory pathways (A, B, D, and E) and spinal reflex pathways (C and F) to sympathetic preganglionic neurons by opiates or clonidine. Each graph illustrates the time course of a single experiment before and after intravenous drug administration and after injection of an antagonist (all doses are in milligrams per kilogram of body weight). Each point represents the integrated size of computer averages of 16 consecutive responses as a percentage of the mean of six to eight individual control values obtained during the hour before drug administration; horizontal dashed lines designate ± 2 standard deviations from mean control values. Preganglionic rami from which evoked responses were recorded are indicated at lower left (*T3r* indicates the third thoracic ramus). Reflexes in (C) and (F) were evoked by stimulation of the second thoracic (intercostal) nerve. Single traces (upper) and their computer averages (lower) in (A) and (C) show evoked responses during control periods (*C*), after morphine (*M*), and after naloxone (*N*). Horizontal calibration in (A) indicates 20 msec for all traces in (A) and (C); vertical calibrations for upper traces indicate 100 μV in (A) and 40 μV in (C).

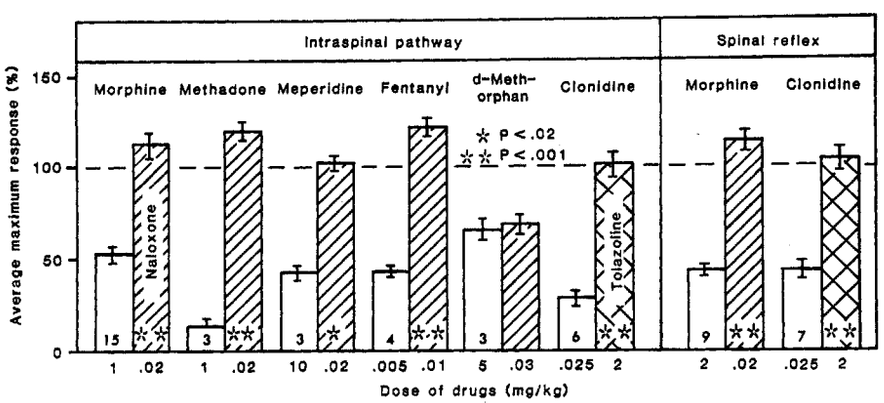

Fig. 2. Average depression of transmission through spinal sympathetic pathways by opiates and clonidine and reversal by their antagonists. The number of experiments is indicated in the open bars. All single-hatched bars are for naloxone, and both the crosshatched bars are for tolazoline. Brackets represent standard errors of the mean. Significance values were calculated by Student's paired *t*-tests.

We (*12*) and others (*13*) have shown that clonidine depresses preganglionic sympathetic discharges evoked by stimulation of two separate spinal pathways in the isolated spinal cord, presumably by actions on sympathetic preganglionic neurons. We now report that opiates also depress transmission through these neurons and propose that clonidine may prevent the symptoms of opiate withdrawal in part by depressing sympathetic preganglionic neurons through actions on nonopiate receptors.

Cats with the spinal cord transected at the first cervical segment and the brain rendered ischemic were used for these experiments. Sympathetic discharges, recorded from upper thoracic preganglionic rami, were evoked from the unanesthetized animals either by biphasic, microelectrode stimulation (0.1 Hz) of intraspinal excitatory pathways in the dorsolateral funiculus of the cervical spinal cord or by activation (0.2 Hz) of spinal reflex pathways (Fig. 1) as described previously (*12*). Sizes of evoked discharges (Fig. 1, A and C) were analyzed on-line every 5 or 10 minutes by signal averaging (Nicolet 1072) for at least 1 hour before and for several hours after intravenous drug administration. Blood pressure, body temperature, and expired CO_2 were monitored throughout surgical and experimental procedures and were maintained at optimum levels.

Intraspinally evoked discharges were rapidly and markedly depressed by, per kilogram of body weight, 1 mg of morphine sulfate (Fig. 1A) or 5 μg of fentanyl citrate (Fig. 1B). The effects of both morphine (0.5 to 2 mg/kg) and fentanyl (2.5 to 10 μg/kg) were dose-related. The depression produced by morphine was maintained for more than 3 hours with little recovery whereas that produced by fentanyl subsided within about 1 hour, reflecting its brief duration of action. Likewise, methadone hydrochloride (1 mg/kg) and meperidine hydrochloride (10 mg/kg) rapidly depressed transmission through intraspinal sympathetic pathways (Fig. 2). Morphine also rapidly depressed transmission through spinal sympathetic reflex pathways (Figs. 1C and 2); these were somewhat less sensitive than the intraspinal pathways.

Depression of transmission through both pathways by the opiates was

promptly antagonized by small doses of naloxone hydrochloride (5 to 20 μg/kg; Figs. 1, A to C, and 2). Antagonism by naloxone was dose-dependent and competitive, was nearly complete within 2 minutes, was frequently characterized by an initial overshoot above control levels, and was equally effective before or after opiate administration. Nalorphine hydrochloride (0.2 mg/kg) was also an effective antagonist.

Although the nonnarcotic *d* isomer of an active opiate, dextromethorphan hydrobromide (5 to 10 mg/kg), produced a moderate depression of intraspinally evoked responses, this depression was slow in onset and was not antagonized by naloxone (Figs. 1D and 2). Therefore, this effect was not attributed to an action on opiate receptors.

The effects of the opiates on spinal sympathetic pathways therefore satisfy the accepted criteria for demonstration of specific actions on opiate receptors (*5*): (i) all four opiates acted rapidly and consistently produced depression; (ii) the relative potencies of these opiates approximated their analgesic and opiate-receptor binding potencies; (iii) the depression was rapidly and completely reversed by small doses of specific antagonists; and (iv) the dextromethorphan data suggested that the effect was stereospecific. These findings correlate with the presence of abundant spinal opiate receptors (*14*) that may provide normal substrates for endogenous enkephalins localized in the spinal cord (*15*). Although no sympathoinhibitory effect of opiates at the spinal level was found in a previous study (*16*), the marked and consistent depression of spinal sympathetic centers by small doses of opiates in our experiments suggests that a significant part of their hypotensive effect is exerted at this level.

Small doses of clonidine (25 μg/kg) also produced a rapid depression of transmission through both intraspinal (Figs. 1E and 2) and spinal reflex pathways (Figs. 1F and 2). As noted previously (*12*), these effects were dose-dependent, and the intraspinal pathways were more sensitive. Depression of either pathway by clonidine showed little recovery for up to 5 hours but could be rapidly reversed at any stage by the α_2-adrenergic receptor antagonists tolazoline hydrochloride (Figs. 1, E and F, and 2) or yohimbine hydrochloride (0.5 mg/kg).

The possibility that the depressant effects of the opiates and clonidine involved a common receptor was assessed by testing for cross-antagonism. No such cross-antagonism could be demonstrated on spinal reflex pathways, and prior administration of naloxone or tolazoline did not block the depressant effects of clonidine or opiates, respectively, on either pathway. Although large doses of tolazoline (15 to 30 mg/kg) appeared to reverse depression of intraspinal transmission by morphine in two of six experiments, and naloxone (20 to 40 μg/kg) appeared to reverse that by clonidine in two of four, such reversals were slow in onset (10 to 20 minutes) and were incomplete by 1 hour; similar gradual increases in intraspinal transmission by the antagonists alone have been noted in some experiments. Therefore, these results indicate that the opiates and clonidine act independently on opiate receptors and α_2-adrenergic receptors, respectively. This conclusion is consistent with the lack of cross-antagonism found in the locus coeruleus (*10*), in analgesia (*5, 7*), and in competitive binding studies (*17*).

The similar depressant effects of clonidine and opiates on both intraspinal and spinal reflex pathways suggest that their respective receptors are located in close proximity to sympathetic preganglionic neurons. Furthermore, the common depression of these cholinergic neurons by independent activation of opiate or α_2-adrenergic receptors is strikingly similar to that of the noradrenergic, locus coeruleus neurons which are also depressed by activation of either receptor (*9, 10*).

Previous proposals concerning the antiwithdrawal effect of clonidine (*2, 10*) have suggested that clonidine might act by depressing central noradrenergic neurons, such as those of the locus coeruleus, which are regulated by both opiate and α_2-adrenergic receptors and which become hyperactive upon withdrawal from their chronic depression by opiates. Inhibition by clonidine would thereby replace inhibition by opiates at such sites to prevent hyperactivity upon withdrawal. The present results suggest that a significant part of clonidine's ability to block opiate withdrawal and its attendant sympathetic hyperactivity may be exerted at the level of sympathetic preganglionic neurons. These spinal neurons appear to be similarly regulated by both opiate and α_2-receptors and are strategically located to control sympathetic activity or hyperactivity regardless of their origins.

Whether the increased activity of either locus coeruleus (*10*) or preganglionic neurons that emerges upon withdrawal is intrinsic or reflects enhanced excitability at other central sites is unknown. However, since all central sympathetic activity must necessarily be routed through the preganglionic neurons, the marked depressant effect of clonidine at this site would suppress it. Considering the complexity of the opiate withdrawal syndrome (*2, 5*), it seems probable that other central sites as well as locus coeruleus and sympathetic preganglionic neurons are involved in the antiwithdrawal effect of clonidine. It would be of interest to determine whether opiates would prevent the withdrawal symptoms of long-term therapy with clonidine.

DONALD N. FRANZ
BRADFORD D. HARE
KEVIN L. MCCLOSKEY
Departments of Pharmacology and *Anesthesiology, University of Utah School of Medicine, Salt Lake City 84132*

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