α_2-Adrenoceptor–Mediated Hyperpolarization of Locus Coeruleus Neurons: Intracellular Studies in vivo

Abstract. *Intracelluluar recordings in vivo from noradrenergic neurons of the rat locus coeruleus showed that membrane potential was hyperpolarized by the administration of clonidine (an α_2-adrenoceptor agonist) or after a burst of spikes evoked by intracellular pulses; both types of hyperpolarization were associated with a decrease in membrane input resistance, and both could be blocked by the α_2-adrenoceptor antagonist piperoxane. These results suggest that a hyperpolarization of membrane potential mediated by an α_2-adrenoceptor underlies both clonidine- and activation-induced inhibition of locus coeruleus cell firing.*

The locus coeruleus (LC) contains the largest clusters of norepinephrine-containing neurons in the central nervous system (*1*). Extracellular single-unit recordings indicate that α-adrenoceptors mediate various types of inhibitory responses in the LC. Systemically administered clonidine, an antihypertensive drug with selective "presynaptic" α_2-adrenoceptor agonist properties (*2*), inhibits the firing of LC neurons (*3*). The local (microiontophoretic) application of clonidine is also inhibitory, indicating the presence of α_2-adrenoceptors in the somatodendritic region of LC neurons. These results are in accord with autoradiographic studies showing a high density of α_2-adrenoceptor binding sites in the LC (*4*). Neurons in the LC are also inhibited by epinephrine and by norepinephrine (*3, 5*); the inhibition produced by these agonists as well as by clonidine can be blocked by the α_2-antagonist piperoxane but not the α_1-antagonist prazosin or the β-antagonist sotalol (*5, 6*). The rank order of potencies for the inhibition of LC neurons by adrenoceptor agonists (*7*) is characteristic of a "presynaptic" α_2-adrenoceptor (*8*). Inhibition is also seen after orthodromic (*9*) or antidromic (*10, 11*) activations of LC neurons. These postactivation inhibitions are presumed to result from recurrent collateral interactions between LC neurons (*10, 11*). Postactivation inhibition in the LC appears to be mediated by norepinephrine, the transmitter of LC neurons, acting upon somatodendritic α_2-adrenoceptors, as it can be blocked selectively by piperoxane applied locally (*11*). The intracellular events underlying α_2-adrenoceptor–mediated inhibitions in the LC are unknown. We now report on the first intracellular recordings from LC neurons in vivo and describe the occurrence of α_2-adrenoceptor–mediated hyperpolarizations after spikes produced by intracellular depolarization or the systemic administration of clonidine.

Experimental animals were 62 male albino rats (Charles River) weighing 275 to 325 g. Animals were anesthetized with chloral hydrate (400 mg per kilogram of body weight, injected intraperitoneally) and mounted in a stereotaxic frame. A burr hole was drilled in the skull over the vertical coordinates of the LC (1.2 mm posterior to lambda, 1.1 mm lateral to the midline). As described previously (*10*), practical aids in locating the LC during recordings include (i) depth of 5.5 to 6.0 mm below brain surface; (ii) mesencephalic V neurons just lateral to the LC, which are activated by mandible displacement; and (iii) in the LC per se, a cluster of slowly firing neurons all responding to a brief noxious stimulus by a burst of spikes followed by a long quiescent period (~ 1 second). Micropipettes for recording were filled with either potassium acetate (1.0*M*, containing 5 percent horseradish peroxidase, Sigma) or potassium chloride (3*M*). Electrodes were beveled by the thick slurry method (*12*) to a final resistance of 18 to 34 megohms (potassium acetate) or 10 to 20 megohms (potassium chloride). In some cases, LC neurons were injected with horseradish peroxidase and processed by the diaminobenzidine method (*13*) for histological localization of the injected cells. Because of highly unstable recording conditions in the LC, special measures had to be taken to allow for sustained intracellular impalements (*14*). Drugs were given by the intraperitoneal rather than the intravenous route to avoid sudden hemodynamic changes; the onset of drug effects was consistently within 4 to 6 minutes. Drug doses are given in terms of the weights of their salts as follows: clonidine hydrochloride (Boehringer-Ingelheim); piperoxane hydrochloride (Rhone-Poulenc).

Intracellular recordings from LC neurons of acceptable quality (that is, action potentials > 60 mV) were obtained in 16 animals (of 62) for periods ranging from 12 minutes to 1.5 hours. In 5 of these 16, cells injected with horseradish peroxidase were recovered for histological examination; in all cases the marked cells were located within the LC (Fig. 1A) The general characteristics of intracellularly recorded LC neurons were the same as found previously in extracellular studies (for example, an activation-inhibition cycle induced by noxious stimulation). When LC neurons were excited by intracellular depolarizing pulses, they exhibited an activation-inhibition cycle associated with a prolonged hyperpolarization (Fig. 1B). In general, the magnitude of the after-hyperpolarization and the duration of the inhibitory period were proportional to the number of spikes evoked. To measure changes in membrane conductance accompanying the hyperpolarizations, a constant-current hyperpolarizing test pulse was applied before and after the depolarizing pulse. During the period of after-hyperpolarization, the amplitude of the deflection caused by the second hyperpolarizing pulse declined (Fig. 1B). By Ohm's law, this decrease in voltage response indicates an increase in membrane conductance. The two types of electrodes that is, containing potassium acetate or potassium chloride) recorded after-hyperpolarizations of similar magnitude.

Extracellular studies have shown that the postactivation inhibition of LC neuronal firing induced by orthodromic or antidromic stimuli can be blocked by piperoxane. Accordingly, we tested the ability of this α_2-adrenoceptor antagonist to block hyperpolarizations after intracellularly induced activations. Once baseline conditions were established, piperoxane (2 to 3 mg/kg, intraperitoneal) was administered to six animals. In all of these experiments, postactivation hyperpolarizations were markedly attenuated within 4 to 6 minutes after the injection (Fig. 2A). During the initial 100 to 200 msec after the end of the depolarizing pulse, the blocking effect of piperoxane was less complete than at later periods (200 to 1000 msec). Nevertheless, piperoxane reduced even the initial period of after-hyperpolarization by at least 30 percent in all of the cells tested (Fig. 2A).

In view of the above evidence that postactivation hyperpolarizations in the LC may be mediated in part by α_2-adrenoceptors, we investigated the ability of clonidine, an α_2-agonist, to hyperpolarize LC neurons. The administration of clonidine (50 to 200 μg/kg, intraperitoneal) caused a hyperpolarization ranging from 5 to 15 mV and a suppression of evoked and spontaneous spikes (seven cells); the hyperpolarization was accompanied by a reduction in membrane input resistance (Fig. 2B). In five of these experiments, piperoxane (2 to 4 mg/kg) was administered after the clonidine; both the hyperpolarization and the de-

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crease in resistance were invariably reversed by piperoxane (Fig. 2B). If piperoxane was not given (two cells) no reversal occurred within a similar period of time (8 to 12 minutes).

Our results show that membrane hyperpolarizations underlie the inhibition of LC neurons produced by the α_2-adrenoceptor agonist clonidine or induced by a burst of spikes. Both of these effects seem to be mediated by α_2-adrenoceptors, as they can be attenuated by piperoxane, an α_2-antagonist. The clonidine-induced hyperpolarization and the spike-induced after-hyperpolarization were accompanied by decreased membrane input resistance (that is, increased membrane conductance), which suggests that the hyperpolarizations could result from an increase in either K^+ or Cl^- conductance (G_K or G_{Cl}). The fact that the hyperpolarizing potentials were not reversed when Cl^--containing electrodes were used (which would increase intracellular Cl^-) argues against an increase in G_{Cl} as the basis for the hyperpolarization. An increase in G_K remains as the most likely mechanism. Our findings are consistent with the long-standing observation that catecholamines hyperpolarize peripheral noradrenergic (sympathetic) neurons by means of an α-adrenoceptor (*15*). More recent work has shown that the hyperpolarizing somatodendritic receptors of sympathetic neurons are of the α_2 variety (*16*). Moreover, it has been suggested that an increase in G_K may cause an α-adrenoceptor–mediated hyperpolarization of sympathetic nerve terminals (*17*). Thus, there are a number of striking physiological and pharmacological similarities between the properties of peripheral and central noradrenergic neurons. It has also been reported that norepinephrine suppresses voltage-dependent Ca^{2+} potentials in sympathetic neurons (*18*); our present data do not permit any conclusion about whether this may also occur in LC neurons.

That excitation of single LC neurons by intracellular pulses causes a marked autoinhibitory after-hyperpolarization is unexpected in view of the current belief that postactivation inhibition results from collateral interactions between multiple LC neurons (*10, 11*). However, inhibitions induced by orthodromic or antidromic activations may be explained, at least in part, by an after-hyperpolarization independent of any interneuronal collateral interactions. After-hyperpolarizations mediated by a Ca^{2+}-dependent G_K have been reported for motoneurons (*19*) and hippocampal

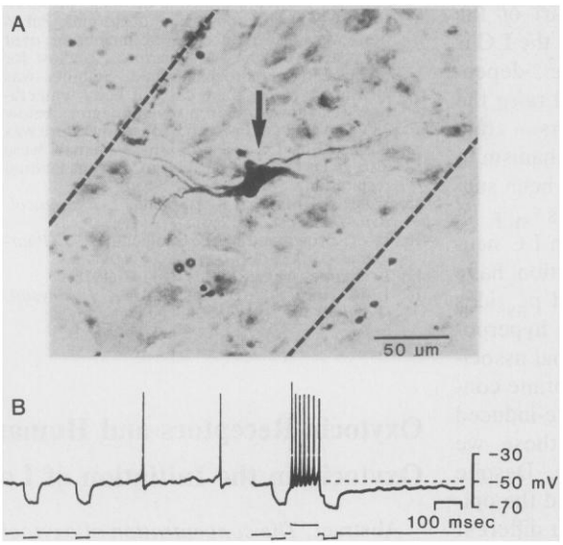

Fig. 1. (A) Frontal section through the anterior pons showing a LC neuron (arrow) marked with intracellular horseradish peroxidase. The enzyme was ejected into the cell with depolarizing pulses (4 nA, 1 Hz, 900 msec in duration) applied for 4 minutes. The medial (left) and lateral (right) boundaries of the LC are indicated by dotted lines. Vibratome sections (100 μm); cresyl violet counterstain. (B) Postactivation inhibition of an LC neuron induced by intracellular depolarizing pulses. Left trace: When no depolarizing pulse was applied, spontaneous activity was present (two spikes). Initial voltage deflections were caused by double constant-current hyperpolarizing pulses (50 msec, 0.5 nA). The current monitor is shown beneath the membrane potential trace. Right trace: Suppression of spontaneous activity in conjunction with an after-hyperpolarization in same cell following a depolarizing pulse (100 msec, 1.0 nA). Initially, the after-hyperpolarization was approximately 10 mV in magnitude; note the gradual return toward resting potential (dotted line). A 38 percent reduction in the voltage deflection produced by the second hyperpolarizing pulse can be seen during the initial period of after-hyperpolarization (that is, a deflection of 10 mV rather than 16 mV when no depolarizing pulse was given). Intracellular injections of current were made through the recording electrode by means of a balanced bridge circuit (WPI M707 MicroProbe System, WPI Instruments). Data were stored on a frequency-modulated tape recorder (Hewlett-Packard 3960 Instrumentation Recorder) and later retrieved through the use of a storage oscilloscope.

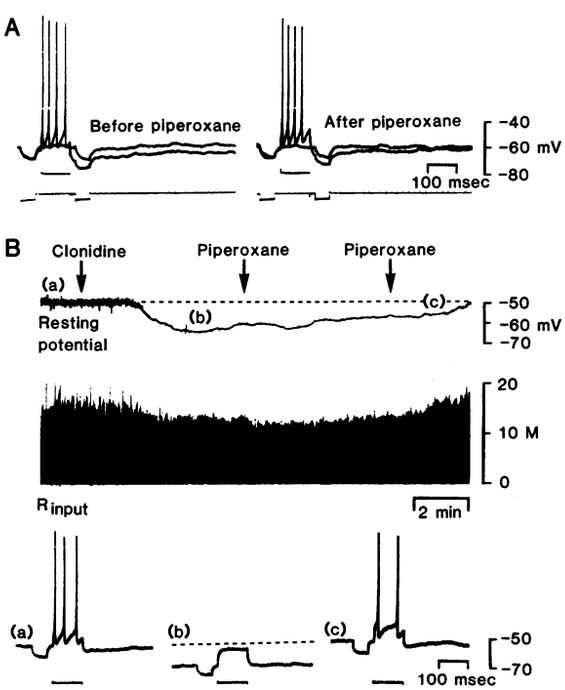

Fig. 2. (A) Before the administration of piperoxane, a depolarizing pulse (100 msec, 2.0 nA) induced a persistent after-hyperpolarization unlike a superimposed sweep in the absence of a depolarizing pulse (left trace); in the right trace, 4 minutes after the administration of piperoxane (3 mg/kg, intraperitoneal) both the amplitude and duration of the after-hyperpolarization were reduced. In six LC cells tested before piperoxane was administered, the average hyperpolarization just after the end of the depolarizing pulse was 10 mV (standard deviation, 1.7); 4 to 6 minutes after piperoxane, the hyperpolarization was reduced to 6 mV (± 1.3). A quantitative analysis at later times was not attempted because the depolarizing pulses tended to evoke an increased number of spikes when the full effect of the piperoxane was attained. (B) Injection of clonidine (200 μg/kg, intraperitoneal) induced a 10- to 15-mV hyperpolarization of this LC neuron; piperoxane (2 mg/kg, intraperitoneal × 2) reversed the effect. The upper trace shows a low-pass filtered record of membrane potential; the middle trace represents input resistance taken from isolated voltage deflections induced by hyperpolarizing pulses (0.5 nA, 0.5 Hz); the lower traces are sample oscilloscope sweeps at points a, b, and c. At point a, a depolarizing pulse (1.0 nA) evoked three spikes; at point b, because of the marked hyperpolarization and decrease in input resistance after clonidine injection, the same depolarizing pulse failed to induce spiking; at point c, after piperoxane, nearly complete reversal of the clonidine effect is seen. The reversal of clonidine-induced hyperpolarization by piperoxane was studied in five LC cells. Membrane potential was hyperpolarized by clonidine to 9.8 mV (± 3.8) below resting potential; within 8 to 12 minutes after the administration of piperoxane, the hyperpolarization was reversed and membrane potential became slightly depolarized in relation to resting potential (+ 1.0 mV ± 1.6).

pyramidal cells (*20*). It remains to be determined whether all or part of the after-hyperpolarization seen in the LC is also due to an increase in Ca^{2+}-dependent G_K. Such a finding would raise the possibility that α_2-adrenoceptors operate through a Ca^{2+}-dependent mechanism to hyperpolarize LC cells, as has been suggested for sympathetic neurons (*18*).

Intracellular recordings from LC neurons in a brain slice preparation have shown that opiates and opioid peptides produce a naloxone-reversible hyperpolarization of membrane potential associated with an increase in membrane conductance (*21*). These opiate-induced membrane effects resemble those we have observed with clonidine. Despite these similarities, clonidine and the opiates have been shown to act at different receptors in the LC (*22*). Nevertheless, it is possible that α_2-agonists and opiates hyperpolarize LC neurons through a common final mechanism (such as an increase in G_K). Such similarities between the effects of α_2-agonists and opiates on LC neurons may provide a basis for the proposal that clonidine suppresses symptoms of opiate withdrawal by a functionally parallel action on central noradrenergic neurons (*22, 23*).

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14. To increase the stability of recording, a cluster of four No. 0 insect pins (Clay Adams) (set 2 mm apart in a square pattern and held in a slotted plate) was placed vertically into the brain surrounding the recording site. The pins seemed to improve stability by damping the transmission of cardiac and pulmonary pulsations within the brain near the recording site: high-gain d-c recordings showed a dramatic decrease in pulsatile potentials after placement of the pins. Pulsations were also reduced if the membrane over the cisterna magna was punctured to allow for drainage of cerebrospinal fluid. Stability was also improved in some cases if body temperature was allowed to drop several degrees below 36°C; no effect of lowered body temperature was observed on LC cell properties. Animals were also given O_2 by nasal tube to prevent labored respiration.
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Oxytocin Receptors and Human Parturition: A Dual Role for Oxytocin in the Initiation of Labor

Abstract. *The concentration of oxytocin receptors increased in the myometrium of pregnant women and reached maximum levels in early labor. Concentrations of oxytocin receptors were also high in the decidua and reached a maximum at parturition. In vitro, prostaglandin production by the decidua, but not by the myometrium, was increased by the addition of oxytocin. Oxytocin may therefore stimulate uterine contractions by acting both directly on the myometrium and indirectly on decidual prostaglandin production. Oxytocin receptors are probably crucial for the onset of human labor, and the stimulus for the increase in uterine prostaglandins may be oxytocin originating from the fetus.*

The mechanism of the initiation of human parturition remains an enigma. The concentrations of estrogen and progesterone, the main regulatory hormones in the maternal circulation, do not appear to change at the onset of parturition (*1*). Activation of the fetal adrenals, an important factor in the onset of parturition in sheep and goat, does not seem to be of critical importance for the timing of parturition in the human. Oxytocin and prostaglandins, potent stimulators of uterine contractions, are secreted during human parturition (*2*), but whether their concentrations in the maternal circulation increase as a cause or as a consequence of uterine contractions is not known; nor has a stimulus been detected for the increased production of prostaglandins during labor.

The absence in pregnant women of any of the clear and consistent changes in the concentrations of humoral factors that are associated with parturition in many animal species prompted us to search for changes at the tissue level. Soloff *et al.* (*3*) demonstrated that myometrial oxytocin receptor concentrations in pregnant rats increased shortly before parturition and reached maximum levels at delivery. Our purpose in the present study was to measure the concentration of oxytocin receptors in the uterus of pregnant women and to determine whether this concentration increases at the time of parturition. The discovery, in the course of this investigation, of high levels of oxytocin receptors in uterine decidua prompted a further study of the role of these receptors in uterine physiology.

Samples of myometrium and decidua parietalis were obtained from women delivering by cesarean sections before or at term. Samples of myometrium and endometrium were also obtained from the uteri of nonpregnant women undergoing hysterectomy. All tissues were placed on ice and transported to the laboratory for storage at −85°C until assayed. The oxytocin receptor concentrations were measured in a crude membrane fraction of myometrial and decidual homogenates (pellet sedimenting between 10,000*g* and 100,000*g*) as described (*4*), with [^{3}H]tyrosine-oxytocin being used as the radioactive ligand. The buffer used for homogenization contained 1 m*M* EDTA to dissociate endogenous oxytocin from its binding sites. This dissociation permitted us to determine the total number of receptor sites when the samples were exposed to endogenous oxytocin in vivo (*4*). Scatchard analyses were performed with increasing concentrations of unlabeled oxytocin. Nonspecific binding was measured by the addition of 0.2 μ*M* unlabeled oxytocin. In many instances, a single point assay was performed in duplicate with a subsaturating concentration of [^{3}H]oxytocin (0.6 n*M*). This concentration of [^{3}H]oxytocin was used to minimize nonspecific binding, which was about 20