Activation of Brain Noradrenergic Neurons during Recovery from Halothane Anesthesia

Perspective of Phasic Activation after Clonidine

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Background: $\alpha_2$-Adrenoceptor agonists, known as antihypertensive agents, may be used during general anesthesia for their anesthetic sparing action and to reduce the occurrence of side effects. Previous studies have shown that the brain’s noradrenergic nucleus, locus coeruleus, is an important target in mediating the hypnotic action of $\alpha_2$ agonists. The authors studied the effects of recovery from halothane anesthesia on the electrical activity of locus coeruleus neurons to examine cellular substrates underlying the clinical effectiveness of $\alpha_2$ agonists.

Methods: Experiments were performed in locally anesthetized rats, whose circulatory and acid-base stabilities were ensured by mechanical ventilation and volume infusion. Locus coeruleus neurons were recorded continuously while the rats were anesthetized with halothane (1%) and/or after the halothane was discontinued.

Results: Under the influence of halothane, locus coeruleus cells exhibited a slow, regular spontaneous discharge (1.95 ± 0.23 Hz), and contralateral foot or tail pinch elicited a prominent, phasic activation in locus coeruleus neurons. Such phasic activation was blocked by local ejection of kynurenic acid, an excitatory amino acid antagonist, close to recorded neurons, but not by clonidine (up to 64 $\mu$g·kg$^{-1}$). Thirty minutes after the halothane was discontinued, the mean firing rate of locus coeruleus neurons was increased by 87 ± 20%. This excitation resulted from a prominent increase in bursting activity (21 ± 5% of spikes in bursts vs. 4 ± 1%) and was reversed by halothane readministration. This activation also was reduced by local ejection of kynurenic acid. Halothane discontinuance revealed the reactivity of locus coeruleus neurons to nonnoxious, sensory stimuli, and considerably reduced the apparent potency of intravenous administration of clonidine to inhibit locus coeruleus activity (effective dose for 50% of maximal effect (ED$_{50}$), 25.48 ± 8.26 $\mu$g·kg$^{-1}$ vs. 4.81 ± 0.80 $\mu$g·kg$^{-1}$ under halothane). This decrease was caused by the persistence of bursting activity after the administration of clonidine, which was completely suppressed by readministration of halothane or local application of kynurenic acid.

Conclusion: The data demonstrate: (1) that halothane withdrawal increases locus coeruleus neuronal activity via excitatory amino acid input, and this withdrawal-induced activity is characterized by a prominent burst (phasic) discharge; (2) that sedative doses of clonidine inhibit the tonic component of locus coeruleus activity but not the phasic activation of locus coeruleus neurons; and (3) that readministration of halothane or local ejection of an excitatory amino acid antagonist fully suppresses the bursting activity unaffected by clonidine. (Keywords: Anesthesia, general. Brain, locus coeruleus. Cells, single: electrophysiology. Neurotransmitter, amino acids: excitatory. Microinjection. Receptors, $\alpha_2$-adrenergic.)

EMERGENCE from anesthesia during the postoperative period may induce cardiovascular instabilities or neurophysiologic changes, e.g., prolonged modifications in rapid eye movement sleep. Many of the physiologic modifications that occur during recovery from general anesthesia seem to result from sympathetic activation. Possible involvement of brain noradrenergic neurons is suggested by the clinical effectiveness of $\alpha_2$-adrenergic agonists known to inhibit these neurons. In particular, noradrenaline-containing neurons in the nucleus locus coeruleus (LC) have been suggested to be an important target in mediating the hypnotic action
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of α2-adrenergic agonists. Such an idea is consistent with the crucial role of the LC in the regulation of the sleep-wake cycle, arousal state, selective attention, and behavior orientation. In addition, various sensory stimuli typically elicit phasic activation (bursts) of LC neurons, whose role may be critical in tuning perceptive or memory processes and in elaborating behavioral strategy in response to environmental changes. Despite the existence of such functional correlates, no study has examined the effects of α2 agonists on the impulse activity of LC neurons during emergence from anesthesia. Such information constitutes a critical step in understanding the cellular processes underlying the clinical effectiveness of α2-adrenergic agonists.

The aim of this study was to examine: (1) the effects of the recovery from halothane anesthesia on single cell electrical activity of LC neurons; and (2) the effects of clonidine on the LC activity during halothane withdrawal. Changes in the average firing rate of single cells were assessed first. We also analyzed the discharge pattern (e.g., presence of bursts) of LC neurons, because previous studies have demonstrated the functional importance of burst discharge in catecholamine release. The present study was performed in rats during mechanical ventilation and appropriate local anesthesia. We have shown previously that such a preparation is necessary to maintain blood pressure and blood acid-base equilibrium within their physiologic ranges.

Materials and Methods

Animal Preparation

Experiments were performed with the approval of the Regional Animal Care Committee and authorization from the French Ministry of Agriculture ("Autorisation de pratiquer des expériences sur les animaux vertébrés vivants"), and followed the guidelines of the American Society for Neuroscience and the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. In particular, no nociceptive stimulation was given during the animals’ emergence from halothane anesthesia.

Fifty-eight male Sprague-Dawley rats (275–375 g) were used in the study. Five animals were in each cage, and they were housed in an animal room for at least 3 days before the experiments were done (12 h cycle, 21 ± 1 °C). Food and water were given ad libitum. Anesthesia was induced initially with halothane (5%) in oxygen via a calibrated vaporizer. Anesthesia was maintained with 1.5–2% halothane during surgery and with 1% halothane throughout the experiment. This latter concentration inhibits corneal reflexes and retraction of hindpaw caused by strong nociceptive stimulation of distal phalanges. This concentration of halothane (1%) is similar to that used in previous reports in rats of similar weight and with a tracheostomy. The incision of skull skin was infiltrated with bupivacaine (0.25%) every 2 h. Swabs soaked with bupivacaine (0.125%) were put at all incision sites. Ear bars were covered with pramocaine, a long-lasting viscous local anesthetic, before they were inserted into ear canals, which also were infiltrated periodically with bupivacaine. We verified that these infiltrations inhibited the responsiveness of LC neurons to strong pressure delivered at any of the locally anesthetized areas. Rectal temperature was maintained at 37 ± 0.5 °C with a heating pad.

When the animal was mounted on the stereotaxic frame, mechanical ventilation of the lungs through tracheostomy was started, with a frequency of 100 breaths per minute, a tidal volume of 12 ml·kg⁻¹, and an inspired oxygen fraction of 0.21. A dose of 3 mg·kg⁻¹ pancuronium dibromide (Pavulon, Organon Teknika, Fresnes, France) in 0.5 ml of physiologic saline was injected intravenously. Subsequently, modified artificial plasma (Plasmion, Roger Bellon, Neuilly-sur-Seine, France), containing pancuronium and sodium bicarbonate, was infused intravenously at a flow rate of 8 ml·kg⁻¹·h⁻¹. Using this protocol, the end-tidal carbon dioxide level was maintained readily at 35 ± 5 mmHg. We verified that 30 min of withdrawal suppresses end-tidal halothane to an undetectable level.

Blood pressure was monitored via a femoral artery catheter. Arterial blood samples (0.125 ml) were assayed on a Corning 178 blood analyzer (Corning, Halstead, Essex, UK). We previously have shown that mechanical ventilation and additional treatments as described above are necessary to maintain a correct arterial pressure and a normal blood gas equilibrium in anesthetized rats. The surgical procedures and subsequent physiological experiment lasted 3 and 9 h, respectively.

Single-cell Recording

Glass micropipettes were pulled, and their tips were broken to an external diameter of 3–5 μm. They were filled with a sodium acetate solution (0.5 M, pH 7.4) containing 2% pontamine sky blue dye. The electrode was lowered into the LC. The extracellular electrical
signal was fed to a high input-impedance preamplifier and treated classically.\textsuperscript{23} LC neurons were identified according to criteria described elsewhere.\textsuperscript{7,18,23} Briefly, under wide band pass filter (0.001–10 kHz), the LC neurons typically exhibited an entirely positive spike of relatively long duration (2–4 ms). Contralateral foot pinch induced a brisk activation followed by a prolonged postactivation inhibition in LC neurons. The impulse activity of a single neuron was detected through a voltage discriminator, whose output was led to a chart recorder and a computer via a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK). Data were stored on floppy disks and analyzed off-line. After recording sessions, dye was iontophoretically deposited from the recording micropipettes, and all recording sites of presumed LC neurons marked by dye were anatomically located within the nucleus LC.

**Drug Administration**

Local pressure applications of kynurenic acid, an excitatory amino acid (EAA) antagonist, were performed as described in detail elsewhere.\textsuperscript{24,25} Microinjections were made with a double pipette assembly, consisting of an ejection micropipette of narrow inner diameter glued next to the recording pipette, whose tip was positioned 60 μm beyond the ejection micropipette’s tip. Kynurenic acid (0.6 μl in 6 nl) was ejected locally using a pneumatic pressure device. We have shown previously that local application of a similar amount of kynurenic acid produces robust blockade of EAA receptor in vivo.\textsuperscript{26} The ejected volume was quantified on-line under microscopic control by measuring the movement of liquid column inside the pipette. Kynurenic acid was dissolved in Dulbecco’s saline buffer, containing calcium and magnesium ions (pH 7).\textsuperscript{24,25} The spontaneous activity of LC neurons was not affected by local application of the vehicle.

**Chemicals**

The following substances were used: clonidine hydrochloride (Boehringer-Ingelheim, Ingelheim, Germany), idazoxan hydrochloride (RBI, Natick, MA), kynurenic acid (Sigma, St. Louis, MO), and naloxone hydrochloride (Sigma).

**Data Analysis**

All multunit recordings were discarded. Only cells yielding stable, high quality (signal/noise at least 3) recording throughout testing were used for quantitative analysis. Baseline spontaneous impulse activity was recorded for each cell for 3–5 min before halothane withdrawal. Regularity of discharge was assessed by the coefficient of variation of intervals between two consecutive spikes, which was computed as the ratio between the SD and mean values of the intervals.\textsuperscript{27} The firing pattern of LC neurons was analyzed off-line according to criteria of burst, defined previously.\textsuperscript{27} The onset of a burst was defined by an interspike interval shorter than 80 ms and the termination of a burst by the next interval longer than 160 ms. The number of bursts, percentage of spikes in bursts, and the coefficient of variation as defined above were calculated over a set of 100 consecutive spikes.

As previously described,\textsuperscript{28} third-order polynomial regression was applied for each neuron to fit the dose–response curve from data obtained by cumulative injection of clonidine. The average ED\textsubscript{50} during halothane administration versus withdrawal were compared by Student’s \textit{t}-test for independent samples. Firing rates and coefficients of variation of the same neurons under different experimental conditions were compared with the Student’s \textit{t}-test for paired observations. The percentage of spikes in bursts and the number of bursts for pairs were compared using the nonparametric Wilcoxon signed ranks test for pairs. All other comparisons were performed by the Student’s \textit{t}-test for independent samples or the Mann–Whitney U test for nonpaired observations. Results are given as the mean ± SEM.

**Results**

**Spontaneous Activity of Locus Coeruleus Neurons Under Halothane Anesthesia**

Under halothane (1%), 37 LC neurons were recorded before any pharmacologic manipulation was performed and halothane was discontinued. Their basal discharge was slow (mean firing rate of 1.99 ± 0.17 Hz) and regular (4 ± 1% of spikes in bursts; coefficient of variation, 0.47 ± 0.01). A brisk activation followed by a prolonged inhibitory period was induced in all LC neurons in response to contralateral foot pinch but not to nonnoxious stimulation, such as air puff on the back. Local application of kynurenic acid, the broad spectrum EAA antagonist, into the LC did not affect the
Fig. 1. Effects of halothane withdrawal on single cell activity of a noradrenergic neuron of the rat locus coerules (LC). (Top) Integrated rate histogram of LC discharge. Under halothane (1%, hatched bar above), the spontaneous discharge of the LC neuron was slow and regular. Halothane withdrawal increased the average firing rate and made the pattern of LC discharge irregular. Reintroduction of halothane returned the LC discharge to the prewithdrawal rate and pattern. (Bottom) Direct oscilloscopic trace of the same LC neuron for the same recording period as shown in the upper traces. (A) Regular discharge under halothane. Note the relative constancy of interspike intervals. (B) Activity during withdrawal. Note the irregular discharge.

spontaneous activity of noradrenaline neurons (n = 8), but suppressed the activation of the same LC neurons evoked by contralateral footpinc (n = 4).

**Effects of Halothane Withdrawal on the Activity of Locus Coerules Neurons**

Because the recorded unit could be lost or the single cell recording could become multunit, a continuous and unambiguous recording of single cell activity before and during halothane withdrawal was possible in only 15 of 37 cells. Their discharge characteristics before withdrawal were very similar to those observed for the whole sample: mean firing rate, 1.95 ± 0.23 Hz; 4 ± 1% of spikes in bursts; and coefficient of variation, 0.47 ± 0.02.

Thirty minutes after withdrawal, there were significant increases in the average firing rate, coefficient of variation, and proportion of spikes in bursts of the 15 LC neurons continuously recorded (3.66 ± 0.41 Hz, 0.71 ± 0.04, 21 ± 5%, respectively; P < 0.01; fig. 1). Although variable from cell to cell, episodes of bursts generally were superimposed on a tonic discharge activity augmented by halothane withdrawal. At 30 min after withdrawal, LC neurons were highly sensitive to nonnoxious sensory

Fig. 2. Effects of local pressure application of kynurenic acid (KYN; 0.6 nm in 6 nl), a broad spectrum excitatory amino acid antagonist, on the single-cell activity of a noradrenergic neuron of the rat locus coerules (LC). (Left) Integrated rate of LC discharge. Under halothane (H on), the spontaneous discharge is not affected by the ejection of kynurenic acid. (Right) The same neuron after halothane withdrawal. Kynurenic acid locally applied reduces the mean firing rate to the previous value.

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stimuli, such as air puff on the back, which elicited a prolonged phasic activation of these neurons.

Microinjection of kynurenic acid into the LC potently attenuated the activation of noradrenaline neurons induced by halothane withdrawal, significantly reducing the mean firing rate after withdrawal by 46%, from 2.8 ± 0.31 Hz to 1.59 ± 0.33 Hz (n = 5, P < 0.05, fig. 2). Local kynurenic acid also completely blocked the activation of LC neurons evoked by air puff (n = 4).

During halothane withdrawal, the single cell activity of LC neurons was not affected by intravenous injection of physiologic saline (0.5 ml, n = 10), pancuronium (n = 7), or supplementary infiltration of bupivacaine for local analgesia (n = 6).

Effects of readministration of halothane could be examined in 12 LC neurons among the 15 cells recorded at least 30 min after withdrawal. Upon halothane readministration, the activity of the LC neurons rapidly returned to their prewithdrawal firing rate (from 4.29 ± 0.7 Hz to 1.97 ± 0.3 Hz; P < 0.01). The latter value, obtained after halothane readministration, was not statistically different from the basal firing rate of the same 12 cells before any withdrawal and pharmacologic challenge (2.26 ± 0.3 Hz, P > 0.4).

**Effects of Intravenous Injection of Clonidine**

In preliminary experiments, intravenous injection of clonidine (16 μg·kg⁻¹, single bolus) inhibited the activity of LC neurons (n = 6) recorded during halothane withdrawal. A short period of marked inhibition (less than 30 s) was followed by a slow but clear return to previous basal firing (fig. 3). At the beginning, there were only short bursts of 2-6 spikes (frequency within bursts, generally 10–20 Hz), occurring every 1–5 s (fig. 3B). This repetitive bursting pattern returned slowly (30–60 min) to the previous pattern (i.e., irregular and augmented activity).

Effects of cumulative intravenous injections of clonidine (8, 16, 32, 64, and 128 μg·kg⁻¹, every 2 min) were examined in further experiments after 30 min of halothane withdrawal. The ED₅₀ of clonidine was 25.48 ± 8.2 μg·kg⁻¹ (n = 10). The proportion of spikes in bursts and the coefficient of variation of the interspike interval increased with cumulative dose of clonidine (fig. 4).

The cumulative doses cited above could not be used with a dose of 1% halothane, because 8 or 16 μg·kg⁻¹ of clonidine was sufficient to inhibit LC neurons completely (n = 6, data not shown). Incremental doses of 0.5, 1, 2, 4, 8, 16, and 32 μg·kg⁻¹ were used instead to obtain the ED₅₀ under halothane anesthesia. In conjunction with halothane, clonidine was substantially more effective in depressing LC neurons (ED₅₀ of 4.81 ± 0.8 μg·kg⁻¹, n = 8, P < 0.01) and failed to increase significantly bursting activity and variability of discharge (P > 0.1, fig. 4). As a result, less clonidine was

![Fig. 3. Effects of intravenous administration of clonidine on the hyperactivity of a locus coeruleus (LC) neuron during halothane withdrawal. (Top) Integrated rate histogram showing recording of an LC cell 30 min after halothane withdrawal. Clonidine (16 μg/kg, administered intravenously) inhibited LC neurons. A short period of marked inhibition (less than 30 s) was followed by a slow but clear recovery. This effect is mediated by α₂ receptors, because it is reversed by the specific antagonist, idazoxan (100 μg/kg, administered intravenously). (Bottom) Direct oscilloscopic traces recorded for the same period, illustrating the pattern of discharge. (A) Irregular pattern during withdrawal. (B) After clonidine, the LC neuron discharged almost only in bursts. (C) Idazoxan, administered intravenously, antagonized the effects of clonidine.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931316/ on 06/22/2017)
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Fig. 4. (A) Dose-response curves illustrating the inhibitory effects of cumulative intravenous administration of clonidine on the firing rate of single locus coeruleus neurons under halothane (dashed line) and during withdrawal (continuous line). The effective dose values for 50% of maximal inhibition computed for each curve were as follows (mean ± SEM): under halothane anesthesia: 4.81 ± 0.8 μg/kg, n = 8; 30 min after halothane withdrawal: 25.48 ± 8.2 μg/kg, n = 10. The values found for 30 min after halothane withdrawal were significantly different from those found for halothane-anesthetized rats (P < 0.01). (B and C) The relationship between the inhibitory effect of clonidine (expressed as percentage of basal firing rate) and variables characterizing the regularity of discharge pattern. During halothane withdrawal (continuous lines), but not during its administration (dashed lines), the coefficient of variation (B) and the percentage of spikes in burst (C) increased as the firing rate decreased with cumulative dose of clonidine. All mean values are significantly different from control values in anesthetized rats (P < 0.05).

necessary to reduce the absolute firing rate of LC neurons; for example, a decrease from 1.5 Hz to 1 Hz required 10.39 μg·kg⁻¹ during halothane withdrawal but only 1.26 μg·kg⁻¹ during halothane administration.

Fig. 5. Effect of the local application of kynurenic acid on burst activity of locus coeruleus (LC) neurons after clonidine. Integrated rate histogram of an LC neuron recorded during emergence. Local pressure application of kynurenic acid (KYN; 0.6 μM in 5 nl), a broad spectrum excitatory amino acid antagonist, suppressed the bursting activity persisting after clonidine treatment (15 μg/kg, administered intravenously). The inhibition lasted for 2 min, and LC activity progressively recovered.

The bursting activity that persisted after halothane withdrawal and the addition of clonidine (cumulative doses up to 64 μg·kg⁻¹, intravenously) was suppressed completely by local ejection of kynurenic acid into the LC (n = 7, fig. 5) or by re-administration of halothane (1%, n = 5, fig. 6). Even after the administration of a high dose of clonidine (32 or 64 μg·kg⁻¹), such readministration did not inhibit the responsiveness of LC neurons to contralateral foot pinch (n = 4, fig. 6). Naloxone (10–40 μg·kg⁻¹), administered intravenously, had no effect on the LC activity during withdrawal alone or withdrawal plus clonidine (n = 5, in both cases).

The inhibitions of LC neurons produced by clonidine during emergence were reversed by an α₂ antagonist, idazoxan (100 μg·kg⁻¹, administered intravenously, n = 9, fig. 3). Idazoxan alone had no effect on LC neurons during halothane withdrawal (n = 5).

Blood Pressure

During halothane (1%) administration, blood pressure was stable (97 ± 3 mmHg, 29 animals) for time periods exceeding 12 h. Thirty min after halothane withdrawal, the mean blood pressure significantly
increased from 97 ± 3 mmHg to 146 ± 5 mmHg (n = 29, P < 0.05). Clonidine (16 µg·kg⁻¹, administered intravenously) lowered the mean blood pressure from 151 ± 5 mmHg to 128 ± 5 mmHg (n = 9, P < 0.05). Local ejection of kynurenic acid into the LC did not affect these circulatory changes. This outcome indicates that the increase in LC activity is not due to the change in blood pressure, because LC neuronal activity is correlated inversely with arterial blood pressure.³⁹

Discussion

Although many studies have been dedicated to the mechanisms of the onset and maintenance of general anesthesia, less attention has been paid to the neurophysiologic effects of emergence from anesthesia. However, clinical disturbances that occur during emergence may be severe and even lethal.³⁰ More consistently, arousal from anesthesia induces psychologic discomfort and produces many sensory and physiologic symptoms, which complicate the care of the patient during the recovery period. Both clinical and experimental evidence have suggested that the activation of brain noradrenergic or adrenergic neurons is part of the neuronal mechanism involved in anesthesia withdrawal. For example, α₂-receptor agonists substantially alleviate clinical symptoms of emergence from anesthesia.⁴¹,²¹

In this study, we have shown that noradrenaline neurons of the brain nucleus LC are activated strongly during halothane withdrawal. The activation of LC neurons during withdrawal is mediated primarily by EAA input within the LC, as this excitation was attenuated significantly by microinjection of kynurenic acid into the LC. In agreement with the clinical effectiveness of α₂-adrenergic agonists, intravenous clonidine also potently reduced the degree of LC activation induced by halothane withdrawal, and its effect was reversed by an α₂-adrenergic antagonist. However, clonidine did not block the phasic activation of LC neurons during recovery from anesthesia. This activity, which persisted after clonidine administration, was blocked by local kynurenic acid into the LC.

Clinical Relevance of the Animal Model of Recovery from Anesthesia

Our animal experiments satisfy all critical conditions to reproduce the principal signs observed during recovery from general anesthesia in humans. Animals were carefully anesthetized locally at all wounds and pressure points. The ear bars were covered abundantly with pramocaine, and bupivacaine also was periodically injected around the ear canals. Because the tissue near and underneath the ears is very thin in the rat, the local anesthetics probably reached and deadened the pain in deeper structures, including the skull surface. This view is supported strongly by our and others' physiologic data: (1) It has been established that various stressors, including painful stimuli, potently activate LC neurons.³²-³⁵ Therefore, LC neuronal activity is a good index for examining whether rats are in pain. During halothane withdrawal, we verified that local anesthesia was fully effective, because mechanical pressure on any wound did not excite LC neurons. The effect of the local anesthetic, bupivacaine, was long-lasting, because its single infiltration into the contralateral paw was sufficient to inhibit an excitatory response of LC neurons to foot pinch for 4 h. (2) Halothane is a poor analgesic agent, as ascertained by phasic activations of LC neurons evoked by foot or tail pinches in rats. If the augmented and irregular electrical activity of LC neurons during withdrawal resulted from pain (e.g., the pressure applied by ear bars), such augmented and irregular activity also would be observed under halothane anesthesia. This was not the case. (3) It has been established that responses of LC neurons to
sensory and noxious stimulation are mediated by EAA pathway terminating within the LC.\textsuperscript{15,36} It is probable that our animals did not experience pain, because LC activity under halothane anesthesia was not affected by local application of the EAA antagonist. (4) Korf \textit{et al.},\textsuperscript{37} in a study on paralyzed, locally anesthetized, and mechanically ventilated rats, showed that irregular and bursting LC activity, similar to our results, persisted and was even enhanced by systemic administration of morphine. If the augmented and irregular activity resulted from defective pain management, morphine would suppress rather than enhance such activity.

In the present study, a steady symptomatology of withdrawal could be observed because of the fast and reproducible elimination of halothane from the circulatory and nervous systems, and the use of paralyzed, mechanically ventilated rats. It was essential to use this preparation to avoid any physiologic changes, such as hypoxia and hypercapnia, which would occur in spontaneously respiring animals during halothane withdrawal and its readministration. Indeed, LC neuronal discharge is modified profoundly by such physiologic challenges.\textsuperscript{38} All arterial blood parameters, including pH, blood gases, oxygen saturation, and bicarbonate content, were maintained within their respective physiologic ranges, by combining mechanical ventilation and continuous perfusion with an appropriate physiologic solution.\textsuperscript{20,22} Stress induced by halothane withdrawal was minimized. A previous study in awake cats\textsuperscript{39} showed that during restraint stress, systemic naloxone increases LC activity, because it relieves the depressant effect of endogenous opiates released during immobilization. In the present study, the intraventricular administration of naloxone during halothane withdrawal was ineffective, suggesting that the stress induced by halothane was not sufficient to release endogenous opiates.

Finally, we used the pharmacologic agents most commonly employed in humans and animals: halothane as the anesthetic and clonidine as the $\alpha_2$ agonist. However, further studies should be performed with more specific $\alpha_2$ agonists or other anesthetics to confirm our results.

\textbf{Increase in Locus Coeruleus Activity During Halothane Withdrawal: Possible Mechanisms}

Halothane withdrawal markedly increased and made the impulse activity of LC neurons irregular. Similar activity has been reported during recovery from chloral hydrate anesthesia\textsuperscript{40} and in immobilized, unanesthe-
tized rats.\textsuperscript{37} Readministration of halothane reversed the withdrawal-induced activation of LC neurons, indicating a close relationship between circulating halothane and LC neuronal activity. In addition, there was a significant, fivefold decrease in the $ED_{50}$ of intravenously administered clonidine during halothane administration \textit{versus} emergence (fig. 4). A similar change in apparent inhibitory potency has been described for morphine, which is much less effective\textsuperscript{41} or even excitatory\textsuperscript{42} in awake animals.

There are several possible mechanisms induced by halothane withdrawal that may contribute to an increase of LC activity and a decrease in the effectiveness of clonidine. These are: (1) relief from halothane-induced membrane hyperpolarization;\textsuperscript{43} (2) a synergistic effect of halothane and $\alpha_2$-adrenoceptors on the LC neuronal firing; (3) a decrease in the potency of $\alpha_2$ receptors; and (4) the presence of excitatory afferents (e.g., EAA) in the LC, which was revealed by the unavailability of halothane.\textsuperscript{44} The possible implications of these and other excitatory or disinhibitory mechanisms need to be investigated fully. The lack of an effect of naloxone on withdrawal-induced changes in the LC discharge pattern indicates that endogenous opiates cannot be involved.

Our results suggest that an endogenous EAA pathway(s) to the LC constitutes a major neurotransmitter in stimulating LC activity during halothane withdrawal, because this is attenuated significantly by the local administration of an EAA antagonist. However, the possible contribution of other excitatory transmitters or peptides, such as acetylcholine, substance P, and vasoactive intestinal peptide, need to be evaluated carefully. In the present study, no attempt was made to determine the neuroanatomic pathway(s) responsible for the increase in LC activity during recovery from halothane anesthesia because of the relative complexity of the afferent network terminating within and around the LC.\textsuperscript{16,45} Nevertheless, an excitatory pathway from the nucleus paragigantocellularis in the rostroventral medulla probably is involved in the LC activity during halothane withdrawal, as has been demonstrated for a number of stimuli that elicit LC activation, such as opiate withdrawal\textsuperscript{16} and electrical stimulation of hindpaw.\textsuperscript{47} Furthermore, activation of LC neurons by these stimuli or by urinary bladder distension is mediated by EAA input within the LC.\textsuperscript{15,46,48,49}

The dose response curve of clonidine administered intravenously during halothane withdrawal was shifted approximately five times with respect to the curve ob-
tained for clonidine administered in conjunction with halothane. The value we found for the ED50 of clonidine under halothane is in agreement with that cited in previous reports.9,50 The lesser effect of clonidine on the LC firing during halothane withdrawal seems to result from a prominent burst discharge of LC neurons, as clonidine considerably increased the proportion of bursts. In contrast, under halothane anesthesia, the firing of LC neurons after the administration of clonidine was virtually devoid of bursts (fig. 4). The LC activity that followed the administration of clonidine during halothane withdrawal probably was caused by the activation of an extrinsic EAA pathway to the LC, because this activation was suppressed by a local EAA antagonist.

**Clinical Implications**

Our results suggest that the discharge pattern of LC neurons, which changes from a regular pattern to an increased, irregular bursting pattern, depends on the level of halothane. These observations are consistent with the proposed role of the LC in the regulation of the arousal state: excitation of LC neurons elicits cortical and hippocampal electroencephalographic desynchronization, indicating an increased arousal state.51 Conversely, inhibition of the same neurons induces an electroencephalographic synchronization.52 Thus, it is likely that the increase in LC activity plays a critical role in patients' emergence from anesthesia.

**In vitro** (i.e., without any functional afferents), the spontaneous discharge of LC neurons is very regular.53,54 Our results in *vivo* suggest that during halothane withdrawal, a phasic bursting discharge is superimposed on the tonic, regular activity of the LC neurons, as observed in *vivo*. This phasic activity of LC neurons probably results from an activation of afferents containing EAs, which convey sensory or other internal environmental stimuli to the LC, and is not suppressed by clonidine, even at high doses (up to 64 μg·kg⁻¹). Such a pharmacologic profile is in striking contrast with the one for the tonic component of LC activity, which is readily suppressed by low doses of clonidine but not by EAA antagonists. The depressant effect of clonidine on the tonic component of LC discharge may be the primary mechanism in the sedative properties of α2 agonists, which decrease the dose requirement of the coadministered main anesthetic agent. However, the doses of clonidine required to produce sedative effects55 cannot suppress the readiness of the LC to respond to unexpected stimuli. In particular, clonidine, at doses that induce sedative effects, does not suppress responses to auditory or sensory stimuli.56 This pharmacologic property may allow clonidine to be used safely during the postoperative period. However, our results concerning the responses to foot pinch (fig. 6) are also in agreement with previous reports,57 which is consistent with the clinical findings that clonidine, though it reduces the requirement for opioids during recovery,58 is not sufficient on its own to abolish perioperative acute suffering and pain.59

Alternatively, as clonidine did not suppress the EAA-mediated activation of LC neurons during recovery from anesthesia (fig. 3), drugs that directly or indirectly antagonize such LC activation also may be clinically effective. Such a strategy may be especially useful, as phasic (burst) neuronal discharge is more effective than regular discharge in increasing the metabolic activity of LC system60 and in releasing catecholamines.19 Therefore, although they induce certain psychotic symptoms, derivatives of the dissociative anesthetic ketamine or other direct EAA receptor antagonists should be investigated to assess their effectiveness for such clinical use. Rather, our previous studies on the selective blockade of EAA-evoked activation of the LC by serotonin25,64 suggest that medication with serotonergic agonists specifically depresses LC activity during patient recovery from general anesthesia. Such blockade of LC activation by serotonergic agonists have been demonstrated recently for opioid withdrawal.62 Finally, possible implications of other brainstem noradrenergic or adrenergic systems cannot be eliminated completely, as these groups are implicated more critically in various autonomic functions, such as cardiovascular and respiratory regulation. Such an idea is consistent with the clinical findings that clonidine eases the circulatory course of recovery from anesthesia.63,64

The present findings suggest: (1) that halothane withdrawal increases LC neuronal activity *via* EAA input, and this withdrawal-induced activity is characterized by a prominent burst (phasic) discharge; (2) that sedative doses of clonidine inhibit the tonic components of LC activity, but not the phasic activation of LC neurons, acting as a high-pass filter; and (3) that the EAA-driven activity of LC neurons may be a critical pharmacologic target for the future development of effective medications for administration during general anesthesia or the recovery period.

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