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A 5-HYDROXYTRYPTAMINE$_2$ AGONIST AUGMENTS γ-AMINOBUTYRIC ACID AND EXCITATORY AMINO ACID INPUTS TO NORADRENERGIC LOCUS COERULEUS NEURONS

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Abstract—We examined the effects of the 5-hydroxytryptamine$_2$ receptor agonist, (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, on spontaneous and evoked discharge of locus coeruleus neurons in the rat. Extracellular recordings were obtained from single locus coeruleus neurons while (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane was injected systemically or locally into the locus coeruleus. Systemic, but not local, administration of (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane decreased spontaneous discharge of locus coeruleus neurons in a dose-dependent manner while simultaneously increasing responses evoked by somatosensory stimulation, consistent with previous studies using 5-hydroxytryptamine$_2$ agonists. Increased responsiveness was observed after both low- and high-intensity stimulation and, in the latter, resulted from the addition of a second, longer latency response after (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane administration, when cells typically responded to each stimulation with two driven spikes instead of one. Both of these effects could be completely reversed by systemic administration of the 5-hydroxytryptamine$_2$ receptor antagonist, ketanserin.

Furthermore, we report that: (i) the (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-induced decrease in spontaneous firing was blocked by local infusion of the GABA antagonists bicuculline or picrotoxin into the locus coeruleus, but not by local infusion of the alpha-2 adrenoceptor antagonist, idazoxan; and (ii) the enhancement of locus coeruleus sensory responses after high-intensity stimulation was blocked by local application of the selective antagonist of *N*-methyl-D-aspartate receptors, 2-amino-5-phosphonopentanoic acid, but not by local infusion of the preferential antagonist of non-*N*-methyl-D-aspartate receptors, 6-cyano-7-nitroquinoxaline-2,3-dione. Together, these results lead us to propose that systemic 5-hydroxytryptamine$_2$ agonists influence locus coeruleus indirectly, causing tonic activation of a GABAergic input to the locus coeruleus, and facilitating sensory inputs that act via excitatory amino acid receptors within locus coeruleus.

The noradrenergic nucleus locus coeruleus (LC) contains a dense plexus of serotonin (5-hydroxytryptamine, 5-HT)-immunoreactive nerve fibers and terminals.[41,55,74] Studies *in vivo* in rat have demonstrated that although iontophoretically applied 5-HT does not consistently affect LC spontaneous discharge, 5-HT markedly attenuates LC responses to iontophoretic glutamate;[7,22] this was shown to be a 5-HT$_{1a}$-mediated effect.[8,19] This is consistent with *in vitro* work showing that 5-HT and 5-HT agonists inhibit excitatory amino acid-mediated synaptic potentials in LC.[15]

The modulatory effects of 5-HT on LC discharge are not, however, limited to receptor activation within the LC itself. Several studies have reported that hallucinogens and a variety of 5-HT$_2$ receptor agonists decrease LC spontaneous firing by acting outside the LC proper, because this effect is seen with systemic but not local drug administration.[33,61,64] These agents also increase the responsiveness of LC cells to somatosensory stimulation,[2,61,64] an effect that seems specific to 5-HT$_2$ agonists, as other drugs that also decrease LC discharge (amphetamine, desipramine, clonidine) do not enhance LC sensory responses.[2]

A widely used 5-HT$_2$ agonist is (±)1-(2,5-dimethoxy - 4 - iodophenyl) - 2 - aminopropane (DOI[32,37,65]). Behaviorally, it has been reported that rats can discriminate between DOI and non-5-HT$_2$ agonists while stimulus generalization occurs between DOI and the 5-HT$_2$ agonist, 2,5-dimethoxy-4-methylamphetamine (DOM).[31] In addition, DOI downregulates rat brain 5-HT$_2$ receptors after chronic administration.[16,45] In the present study, we used DOI to examine the mechanism by which 5-HT$_2$ receptor activation modulates LC spontaneous and sensory-evoked discharge.

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Abbreviations: AP-5, 2-amino-5-phosphonopentanoic acid; BIC, bicuculline methiodide; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CRF, corticotropin releasing factor; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; DOM, 2,5-dimethoxy-4-methylamphetamine; EAA, excitatory amino acid; 5-HT, 5-hydroxytryptamine, serotonin; FS, footpad stimulation; ISH, interspike interval histogram; LC, locus coeruleus; NMDA, *N*-methyl-D-aspartate; PSTH, peri-stimulus time histogram.

EXPERIMENTAL PROCEDURES

Extracellular recording technique

Male Sprague–Dawley rats (Taconic; 300–400 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments). Body temperature was maintained at 36–38°C using a thermistor-controlled heating pad. The incisor bar was lowered to tilt the head at a 15° angle to spare the transverse sinus overlying LC. Extracellular recordings of single LC neurons were made through glass micropipettes (3.0–4.0 μm tip) filled with a 2% solution of Pontamine Sky Blue dye in 0.5 M sodium acetate buffer. Identification of LC cells during recordings was based on properties described elsewhere[12,27,39] and included a positive, notched waveform in unfiltered records, slow (0.5–2.0 Hz) tonic discharge, and biphasic (excitatory–inhibitory) response to noxious stimuli such as foot or tail pinch. Action potentials were amplified (Grass P16), displayed continuously on a storage oscilloscope as unfiltered and filtered (500 Hz–10 KHz bandpass) signals, and also monitored with a loudspeaker. Footpad stimulation (FS) consisted of pulses (4 or 9 mA, 0.5 ms duration) from a square-wave stimulator (Grass S48) and a constant-current stimulus isolation unit (Grass PSIU6) administered through two 26-gauge needles inserted subcutaneously in the medial aspect of the contralateral footpad to activate the sciatic nerve. Impulses from single neurons were used to generate digital pulses via a waveform discriminator, which led to a computer for on-line generation of activity histograms. Interspike interval histograms (ISHs) were used to analyse the rate and pattern of basal discharge, and peristimulus time histograms (PSTHs) were constructed to determine response to FS.

Drug solutions and administration

(±)DOI–HCl (Research Biochemicals, Inc.), ketanserin tartrate (Research Biochemicals, Inc.), bicuculline methiodide (BIC; Sigma), picrotoxin (Sigma), idazoxan (Reckitt and Coleman, Ltd), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Ferrosan and Research Biochemicals, Inc.) and 2-amino-5-phosphonopentanoic acid (AP-5; Research Biochemicals, Inc.) were used in these experiments. All systemically administered solutions were intravenously injected in 0.9% saline through a polyethylene catheter (PE 10) into the right femoral vein. Solutions infused locally into LC were prepared in an artificial cerebrospinal fluid solution containing (in mM): NaCl, 122; KCl, 3.1; NaH_2PO_4, 0.4; $MgSO_4$, 1.2; $CaCl_2$, 1.3; $NaHCO_3$, 25; glucose, 10, and were adjusted to a pH of approximately 7.4.

Local infusions of drugs into the LC were made using composite micropipettes, as described elsewhere.[4,68] These were constructed from two glass micropipettes glued immediately adjacent to each other with a light-curing dental fixative (3M Silux); one was the recording pipette which was filled with the Pontamine Sky Blue solution described above, while the injection pipette (30–50 μm tip) was filled with a drug solution and connected to a pneumatic pressure source (Picospritzer; General Valve, Inc.) for controlled microvolume infusion. The recording pipette tip extended approximately 125 μm beyond the injection pipette tip. Briefly, while an LC cell was recorded, an infusion was made by engaging the Picospritzer and gradually increasing the pressure until the meniscus in the injection pipette moved slowly (usually not greater than 30 nl/min) and steadily. Injection volumes (usually between 60 and 300 nl total, over 2–10 min) were monitored by observing the movement of the meniscus (accuracy = approximately 20 nl). This type of continuous-flow pressure injection allowed administration of drug solutions directly into the area of the cell being recorded without disrupting the recording.[4,67,68]

Systemic and local injection protocols

Three experimental protocols were used in this study: one for examining DOI effects on LC cells, one for examining the mechanism by which DOI decreases LC discharge, and one for examining the mechanism by which DOI enhances LC sensory responses.

In the first protocol, when a stable recording of a single LC cell was obtained, a pre-drug PSTH and ISH were taken to establish the control FS response and spontaneous discharge of the cell, respectively. DOI was then injected (intravenously or locally); post-drug ISHs (2 min duration) were taken every 10 min starting immediately after injection until the cell could no longer be recorded or until 60 min post-drug, while post-drug PSTHs were also taken every 10 min, starting at 3 and 5 min post-drug (for 4 and 9 mA stimuli, respectively). In similar experiments, ketanserin was injected between 8 and 9 min after DOI (50 μg/kg). In other experiments examining possible adrenergic involvement in DOI effects on LC-evoked responses, idazoxan (0.5 mg/kg i.v.) was substituted for DOI. Only 9 mA FS data were taken in these idazoxan experiments.

In the experiments investigating the mechanism of the DOI-induced decrease in tonic LC activity, after an LC cell was isolated and a baseline ISH and PSTH were generated, idazoxan, BIC, or picrotoxin was slowly infused into the LC. During this infusion, DOI (50 μg/kg) was injected systemically. ISHs were constructed both immediately before and immediately after the DOI injection, and a PSTH was generated at 5 min post-DOI.

In the experiments investigating the mechanism of DOI-induced enhancement of LC sensory responses, after an LC cell was isolated and a baseline ISH and PSTH were collected, DOI (50 μg/kg) was injected intravenously. PSTHs were generated at 5 and 10 min post-DOI. After the latter PSTH, either CNQX or AP-5 was infused locally into the LC. PSTHs were taken at 5-min intervals starting immediately after the local infusion.

Histology

Recording electrode placements were marked by iontophoretically depositing dye with negative current pulses (7.0 μA, 50% duty cycle for 12 min) through the recording pipette tip. Rats were deeply anesthetized and killed by Nembutal overdose. The brain was removed, snap-frozen in an isopentane solution, and placed in a −70°C freezer. Brains were cut on a cryostat into 50-μm-thick sections, stained with Neutral Red, and coverslipped with Permount to histologically verify recording sites (Fig. 1).

Data analysis

Discharge rates during pre-drug ISHs and FS response magnitudes (R_{mag}s) during pre-drug PSTHs were compared to those for post-drug ISHs and PSTHs using two-tailed paired t-tests. Dose effects on spontaneous and evoked discharge were examined by performing a one-factor ANOVA on the differences between the pre- and post-drug rates and R_{mag}s for each dose. Reversal of DOI effects by ketanserin was analysed by paired t-test to compare post-ketanserin values to control. A one-factor ANOVA was also performed on the raw data for idazoxan, BIC, and picrotoxin for the three conditions: baseline, infusion, and post-drug.

In each PSTH, a baseline period was defined as the 500-ms epoch preceding stimulation. R_{mag}s were calculated by the following formula: (number of counts in predefined response epoch) − (mean counts per baseline bin × number of bins in predefined response epoch). FS responses were quantified separately for early (20–60 ms post-stimulation) and late (60–100 ms post-stimulation) response epochs, denoted herein as the 1st and 2nd response components, respectively. Total FS response magnitude was calculated as the sum of the 1st plus 2nd response components.

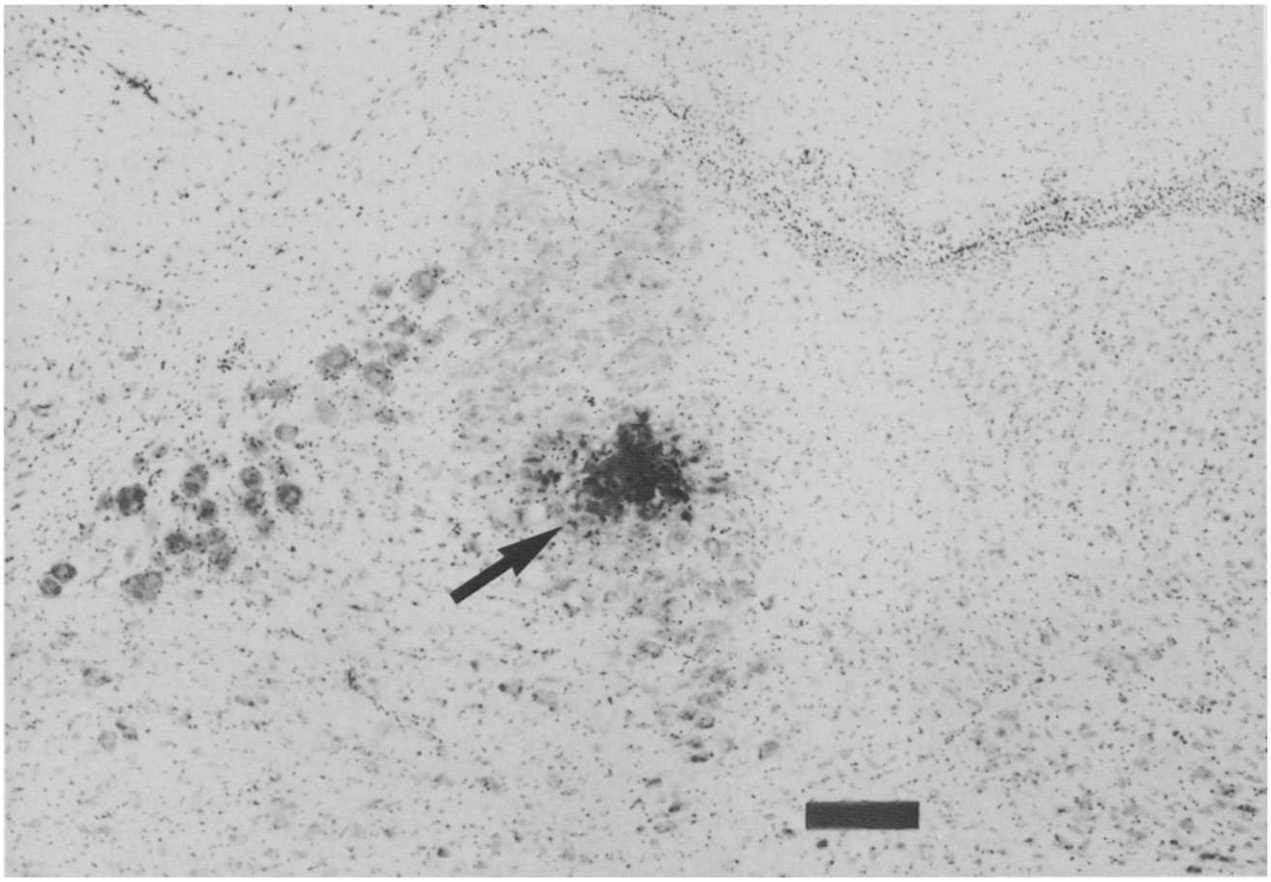

Fig. 1. Photomicrograph of a coronal section through the LC, stained with Neutral Red. Blue spot in LC (at arrow) was created by iontophoresis of dye at the end of the recording penetration. Scale bar = 150 μm. Dorsal is at top, medial is at right. All recording sites were histologically verified from such sections.

RESULTS

Effects of 1-(2,5-*dimethoxy*-4-*iodophenyl*)-2-*amino*-*propane on spontaneous locus coeruleus activity*

Systemic DOI (5, 16, and 50 μg/kg, i.v.) significantly decreased LC spontaneous firing, as follows. At the 5 μg/kg dose, DOI reduced LC discharge from a mean (±S.E.M.) of 1.3 ± 0.4 to 1.1 ± 0.3 spikes/s ($n = 4$; $P < 0.05$); at 16 μg/kg DOI reduced firing rates from a mean of 1.1 ± 0.0 to 0.4 ± 0.1 spikes/s ($n = 5$; $P < 0.001$); and at 50 μg/kg LC discharge decreased from a mean of 1.8 ± 0.1 to 0.5 ± 0.1 spikes/s ($n = 21$, $P < 0.001$). The decrease in activity with DOI was immediate, and was typically apparent by the end of the injection. Comparison of the absolute change in firing rate elicited by each DOI dose (see Experimental Procedures) revealed a dose-dependent effect on LC spontaneous discharge ($F_{2,29} = 13.9$, $P < 0.001$; Fig. 2).

Effects of 1-(2,5-*dimethoxy*-4-*iodophenyl*)-2-*amino*-*propane on evoked locus coeruleus activity*

In addition to decreasing discharge rates, systemic DOI enhanced LC responses to FS. This enhancement reflected a significant increase in the absolute number of evoked spikes, not just a relative increase resulting from decreased spontaneous discharge (total spikes in the 20–100 ms epoch post-stimulation over 50 sweeps, pre-drug = 45.2 ± 1.8, post-drug = 57.6 ± 3.7, $P < 0.001$). Furthermore, with 9 mA FS intensity, the larger responses appeared to result from the addition of a second, longer latency response peak (Fig. 3A). This new response was apparent during recordings after DOI administration, when cells often responded to each FS stimulation with two driven spikes instead of just one, which was typical pre-drug.

To quantitatively test whether increased responsiveness following DOI was due to the addition of a second response epoch, we divided the FS response into early and late response components, 20–60 ms and 60–100 ms post-stimulation, respectively. As summarized in Fig. 3B, this analysis for the 9 mA FS intensity revealed that the 50 μg/kg DOI dose had no effect on the early response component but substantially increased the second response component (24/24 cells, $P < 0.001$). In addition, 5 μg/kg DOI did not affect the first component of LC responses and

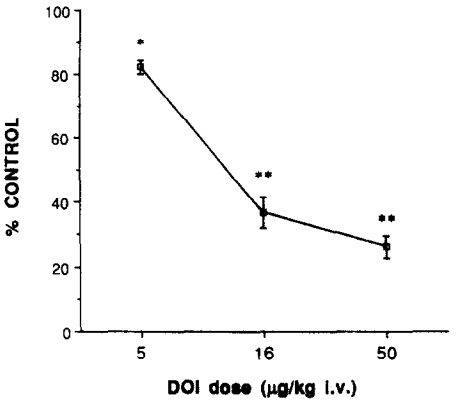

Fig. 2. Graph showing the effect of systemically administered DOI on LC spontaneous discharge rate. Systemic DOI produced a dose-dependent ($F_{2,29} = 13.9$, $P < 0.001$) decrease in LC firing (*$P < 0.05$, **$P < 0.0001$; paired t-tests on pre- and post-DOI rates).

did not elicit a significant second component ($P = 0.2$ for both, $n = 4$), while the 16 μg/kg DOI dose ($n = 5$) decreased the first response component ($P < 0.05$) and increased the second component (four out of five cells, $P < 0.05$) of LC responses to FS. Comparison of the absolute change in second component R_{mag}s elicited by each DOI dose (see Experimental Procedures) revealed that the ability of DOI to produce a second component in LC responses to FS was a dose-dependent effect ($F_{2,32} = 4.9$, $P = 0.01$). Regardless of dose, all augmentations of FS responses were observed by 5 min after DOI injection, consistent with previous work using other 5-HT_2 agents.[61]

This effect of DOI on LC evoked activity was not mimicked by the alpha-2 adrenoceptor antagonist, idazoxan (0.5 mg/kg, i.v.), a drug which has previously been reported to increase LC responsiveness to sensory stimulation.[70] In three cells tested before and 1–5 min after idazoxan, R_{mag}s for 9 mA FS were unaffected ($P = 0.97$). In particular, there was no second response component for FS pre- or post-idazoxan. In the same three rats, a total of 15 cells were tested for FS responses within one hour of idazoxan injection, and their R_{mag}s were compared (unpaired t-test) to those of the pre-DOI control group ($n = 24$). This analysis showed a decrease in the first response component R_{mag} (control = 38.0 ± 1.5, post-idazoxan = 32.0 ± 2.0, $P = 0.02$) and no second response component (control $R_{mag} = -1.1 \pm 0.7$, post-idazoxan $R_{mag} = -1.7 \pm 1.7$, $P = 0.7$). The decrease in the first component R_{mag} may be a reflection of increased LC spontaneous discharge immediately after idazoxan injection in each of the three rats tested (from 1.3 ± 0.1 to 2.6 ± 0.5 spikes/s; $P = 0.06$).

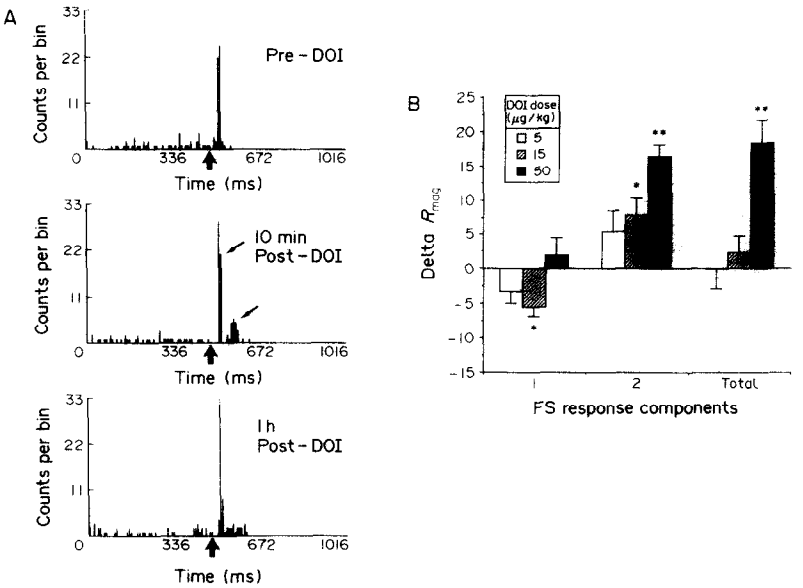

Fig. 3. (A) PSTHs generated during FS (0.5 Hz, 9 mA, stimulation onset indicated by large arrows, 50 sweeps). In the upper panel, such stimulation yielded potent, short-latency excitation of this LC neuron (bins containing driven spikes are seen shortly after arrow; bin width = 8 ms). Excitation is followed by a longer-lasting inhibition of impulse activity, similar to the characteristic postactivation inhibition of LC neurons.[3,26] The middle panel shows a similar PSTH for FS stimulation, but 5 min after DOI (50 μg/kg, i.v.) injection. Note that baseline activity is decreased while the FS response is increased by the addition of a second, longer-latency response component to the original response (response components indicated by small arrows). In the lower panel, a PSTH for the same cell shows partial recovery of the FS response 1 h after DOI injection. (B) Graph showing the effect of different doses of systemic DOI on LC responses to electrical stimulation of the contralateral footpad (FS; 9 mA). Data are represented as the mean change in R_{mag} (delta R_{mag}) induced by DOI. Note that DOI produces a dose-dependent increase in the second response component (one-way ANOVA, $F_{2,32} = 4.9$, $P = 0.01$; for 5 μg/kg dose, $n = 4$; for 16 μg/kg dose, $n = 5$; for 50 μg/kg dose, $n = 24$). Statistical significance of delta R_{mag} values was assessed using paired t-tests on pre- and post-DOI R_{mag}s. *$P < 0.05$, **$P < 0.001$.

We also tested the effects of DOI (50 μg/kg) on LC responses to FS at a lower stimulation current (4 mA), which produced responses in approximately 50% of trials in naive animals. In these tests ($n = 5$), DOI enhanced the early response component ($P < 0.01$) but did not produce a late response component ($P = 0.71$). This enhancement also reflected a significant increase in the absolute number of evoked spikes, not just a relative increase resulting from decreased spontaneous discharge (total spikes in the 20–60-ms epoch post-stimulation over 50 sweeps, pre-drug = 23.8 ± 3.3, post-drug = 31.6 ± 4.5). For both the 4 mA and 9 mA FS intensities, the patterns of enhanced FS response were apparent at the first time point tested (3 and 5 min after injection, respectively).

Spontaneous and evoked LC activity at least partially recovered to baseline values by 30–40 min post-DOI for four of five cells examined.

Reversal of 1-(2,5-*dimethoxy*-4-*iodophenyl*)-2-*aminopropane effects by ketanserin*

Injection of the 5-HT_2 antagonist, ketanserin (1.0 mg/kg), 8 min after DOI injection (50 μg/kg) completely restored LC spontaneous activity to control levels ($P = 0.12$, $n = 5$). In four of the same cells, examination of LC responses to FS (9 mA) showed that ketanserin also completely reversed the DOI-induced late component ($P = 0.58$) without affecting the early component ($P = 0.28$; Fig. 4).

Local infusions of 1-(2,5-*dimethoxy*-4-*iodophenyl*)-2-*aminopropane into locus coeruleus*

In contrast to these effects of systemic DOI, we found that neither spontaneous nor evoked LC discharge was significantly affected by local infusion of either 0.5 μM ($n = 5$ and 9 for spontaneous and evoked activity, respectively; mean of approximately 70 nl per infusion) nor 20 μM DOI ($n = 2$ and 5 for spontaneous and evoked activity, respectively; mean of approximately 80 nl per infusion). In contrast, similar microinfusions of other agents were markedly effective on LC discharge (see below).

GABA mediates 1-(2,5-*dimethoxy*-4-*iodophenyl*)-2-*aminopropane suppression of locus coeruleus tonic activity*

Previous studies have shown that the LC receives potently inhibitory adrenergic/noradrenergic[6,13,57] and GABAergic[13,28,29,59,69] inputs. To investigate whether such inhibitory afferents play a role in the decreased LC spontaneous firing seen after systemic DOI, we locally infused idazoxan (0.1 mM), or one of the GABA antagonists, BIC (0.01, 0.1, and 0.5 mM) or picrotoxin (0.5 mM), into the LC. During these infusions, DOI (50 μg/kg) was injected intravenously.

Local infusion of BIC (0.5 mM; Fig. 5A, B) increased LC discharge (to 145% of baseline, $P < 0.005$), as expected.[66] Such BIC infusion also blocked the DOI-induced decrease in LC discharge (rate post-DOI = 111% of the post-BIC rate, $P = 0.5$, $n = 7$). This action of BIC was reproduced by infusion of picrotoxin (rate post-DOI = 94% of the rate post-picrotoxin, $P = 0.4$, $n = 5$; Fig. 5A, C). Infusions of picrotoxin alone also increased LC firing (to 134% of baseline, $P < 0.05$, $n = 5$). Local infusion of idazoxan increased LC spontaneous discharge, as expected (to 135% of baseline, $P = 0.05$, $n = 5$). However, in contrast to results with BIC and picrotoxin, a subsequent injection of DOI decreased LC discharge substantially (to 40% of the post-idazoxan rate, $P < 0.02$), indicating that idazoxan was not effective in preventing the decrease in LC firing caused by DOI (Fig. 5A, D). One-way ANOVA comparisons of the three locally infused drugs showed that the baseline firing rates ($F_{2,16} = 1.65$, $P = 0.23$) and post-infusion firing rates ($F_{2,16} = 1.57$, $P = 0.24$) were not significantly different, and they confirmed the ineffectiveness of idazoxan in blocking the DOI effect in a comparison of post-DOI firing rates ($F_{2,16} = 9.0$, $P = 0.003$).

It is noteworthy that BIC's antagonism of the DOI-induced decrease in LC discharge was dose-

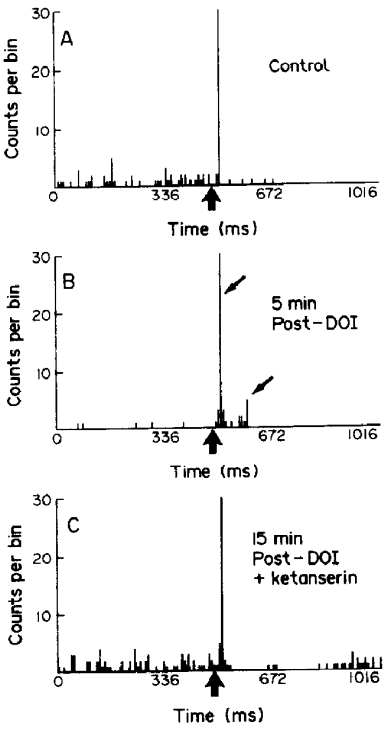

Fig. 4. (A–C) PSTHs generated during FS (0.5 Hz, 9 mA, stimulation onset indicated by large arrows, 50 sweeps) showing reversal of the DOI effect on LC spontaneous and evoked discharge by the 5-HT_2 antagonist ketanserin. (A) Response of a single LC neuron to FS before drug injection. (B) Response of the cell 5 min after DOI (50 μg/kg) injection. Note that baseline activity is decreased while the FS response is increased by the addition of a second, longer-latency response component to the original response (response components indicated by small arrows). (C) The baseline activity and evoked discharge of the cell have returned to pre-drug levels 15 min after injection of DOI and the 5-HT_2 antagonist, ketanserin (1.0 mg/kg). Ketanserin was injected 8 min after DOI injection.

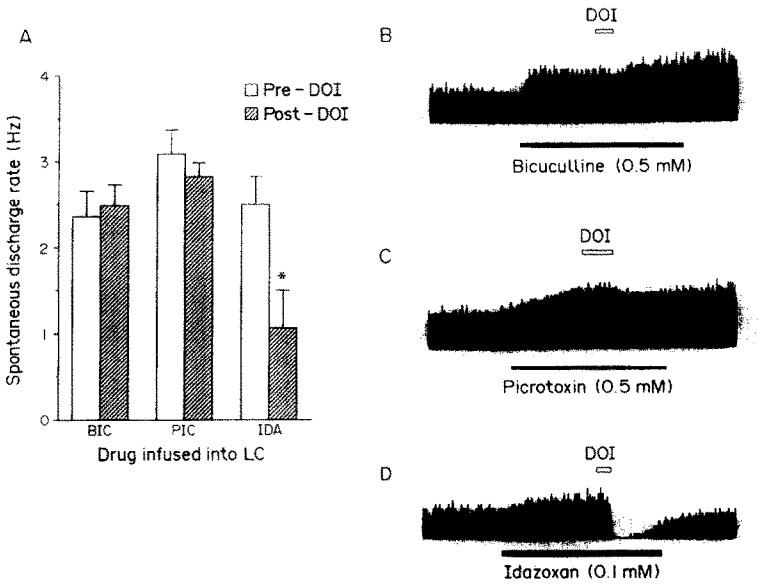

Fig. 5. Blockade of the DOI-induced decrease in LC spontaneous firing by local infusion of GABA antagonists but not an adrenergic antagonist. (A) Locally infused BIC (0.5 mM, $n = 7$) or picrotoxin (PIC: 0.5 mM, $n = 5$) blocked the DOI effect ($P = 0.5$ and $P = 0.4$, respectively), while locally infused idazoxan did not (IDA: 0.1 mM, $n = 5$, pre- vs post-DOI, $^*P < 0.02$). Open columns represent pre-DOI rates, while striped columns represent post-DOI rates. (B–D) Ratemeter records of three different LC cells treated with local infusions (solid bars) of BIC, picrotoxin, or idazoxan and with systemic DOI (50 μg/kg, i.v., open bars). Note that DOI is ineffective after local BIC or PIC, but that it still potently inhibits LC discharge after local IDA.

dependent ($F_{2,11} = 14.4$, $P < 0.001$). The lowest concentration of BIC tested (0.01 mM) was ineffective in blocking the DOI-induced decrease in LC discharge (rate post-DOI = 27.4 ± 9.3% of the post-BIC rate, $P < 0.01$, $n = 4$), 0.1 mM BIC attenuated the effect of DOI (rate post-DOI = 74.8 ± 8.9% of the post-BIC rate, $P = 0.15$, $n = 3$), and the 0.5 mM dose of BIC was the most effective (results given above). All concentrations of BIC increased LC spontaneous discharge (175.8 ± 18.6% and 191.6 ± 4.9% of baseline for the 0.01 and 0.1 mM doses, respectively; $P < 0.05$ for each).

N-*Methyl*-D-*aspartate receptor activation mediates second response component following* 1-(2,5-*dimethoxy*-4-*iodophenyl*)-2-*aminopropane*

As previous work from our laboratory had indicated that the FS response in LC is mediated by an excitatory amino acid (EAA) pathway,[9,21,27] it seemed possible that the enhanced FS response following systemic DOI injection may result from augmented EAA neurotransmission within LC. To test this hypothesis, we made local microinjections of the preferential non-*N*-methyl-D-aspartate (NMDA) antagonist, CNQX, or of the selective NMDA antagonist, AP-5, into the LC following DOI (50 μg/kg) and examined FS response amplitudes (9 mA FS). In each cell tested, local infusion of CNQX (50 μM; Fig. 6A–C) attenuated the first component of FS responses ($P < 0.001$) as expected,[9] but was relatively ineffective on the new, DOI-elicited, second component ($P = 0.4$, four out of four cells). One cell each for two lower doses of CNQX was also tested on the FS response after DOI; at 25 μM, CNQX reduced the first component (from an R_{mag} of 43.0 to 17.5) while not affecting the second response component, and at 12 μM, CNQX had no effect on either the first or second response component. In contrast, local AP-5 (50 μM; Fig. 6D–F) selectively attenuated the second response component ($P < 0.05$) but not the first in each cell tested ($P = 0.1$, four out of four cells). These results are summarized in Fig. 7.

In contrast to the attenuation of FS response components by EAA antagonists, local infusion of GABA antagonists did not alter LC-evoked responses despite their blockade of DOI's inhibitory effects on LC tonic discharge. Local infusion of 0.5 mM picrotoxin by itself had no effect on the LC response to FS, as shown in Fig. 8; it also did not affect the DOI enhancement of LC-evoked discharge despite its blockade of the DOI-evoked decrease in LC spontaneous discharge. Similarly, locally infused idazoxan had no effect on DOI's ability to augment LC-evoked discharge. One-way ANOVA analysis of these enhanced responses showed that the second component elicited by DOI in the presence of the above antagonists was not different from that elicited by DOI alone ($F_{3,41} = 0.86$, $P = 0.47$).

DISCUSSION

The present results show that DOI affects LC neuronal discharge in much the same way as other 5-HT$_2$ agonists.[33,61] Specifically, DOI decreased basal

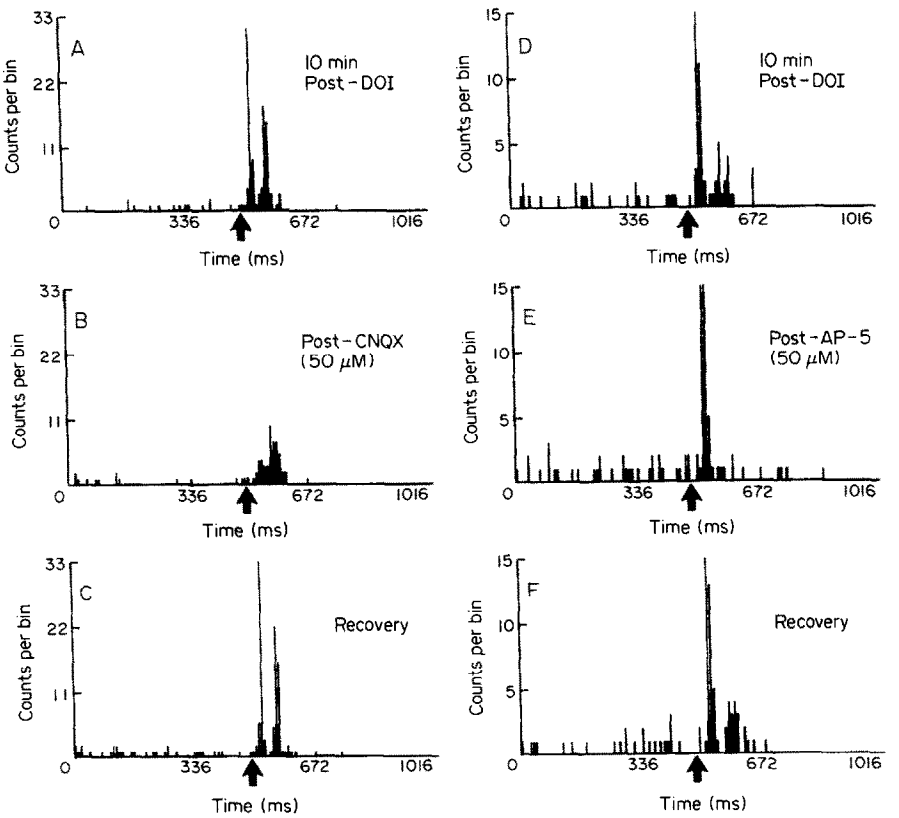

Fig. 6. PSTHs illustrating the contrasting effects of locally infused CNQX and AP-5 on the DOI-enhanced FS responses of two different LC cells. Fifty stimuli (at arrows) in each PSTH. (A) FS response of an LC neuron 10 min after DOI (50 μg/kg). Note the slow basal discharge (0.5 spikes/s) and the presence of two response components (number of spikes in first component = 48, second component = 41). (B) Selective attenuation of the first FS component after local CNQX (50 μM): number of spikes in first component = 14 (a 71% decrease), second component = 34 (a 17% decrease). CNQX infusion does not affect the basal discharge of the cell (0.5 spikes/s). (C) Recovery of the pre-infusion FS response approximately 20 min after CNQX infusion. (D) FS response of a different LC neuron 10 min after DOI (50 μg/kg). Number of spikes in first component = 48, second component = 12. (E) Selective blockade of the second FS component after local AP-5 (50 μM): number of spikes in first component = 47 (a 2% decrease), second component = 3 (a 75% decrease). AP-5 also has little effect on basal discharge of this cell (1.25 spikes/s compared with 1.0 spikes/s in D). (F) Recovery of the pre-infusion FS response approximately 15 min after AP-5 infusion.

discharge and increased the evoked activity of these cells when administered systemically, but had no effect when administered locally. Furthermore, our results indicate that systemic DOI exerts its influence on LC neurons via two separate sites outside of the LC: an action at one site tonically activates an inhibitory GABA input to LC, while DOI acting at a second site facilitates EAA inputs to LC which mediate sensory responses of these cells. Our ability to block the decrease in spontaneous discharge without affecting the increase in the sensory response (Fig. 8), and vice versa (Fig. 6), clearly illustrates that the two effects of DOI can be manipulated separately.

Recently, there has been debate about the specificity of DOI for the 5-HT_2 receptor. DOI is a phenalkylamine compound which has been shown to bind selectively to the 5-HT_2 receptor.[32,37,65] However, the 5-HT_2 receptor is structurally homologous to the 5-HT_{1c} receptor,[36,38] and there is evidence that DOI has equal affinity for both receptor subtypes.[36,42,46,47] The predominant site in the brain where 5-HT_{1c} receptors are localized is the choroid plexus,[54] but they are also found to a lesser degree in the cerebral cortex (particularly the hippocampus) and, unlike 5-HT_2 receptors, are widely distributed throughout subcortical regions including the subthalamic nucleus, the substantia nigra pars compacta, inferior olive, vestibular nucleus, and the LC itself.[50,60] As there is currently no specific agonist or antagonist for 5-HT_{1c} receptors, it is impossible to directly assess the role these receptors may play in the effects of DOI on LC cells. However, our results demonstrate that ketanserin, a potent 5-HT_2 antagonist which has relatively low affinity for 5-HT_{1c} receptors,[49] completely reverses DOI's effects on both spontaneous and evoked LC discharge. This finding is consistent with previous results showing that spiperone reverses

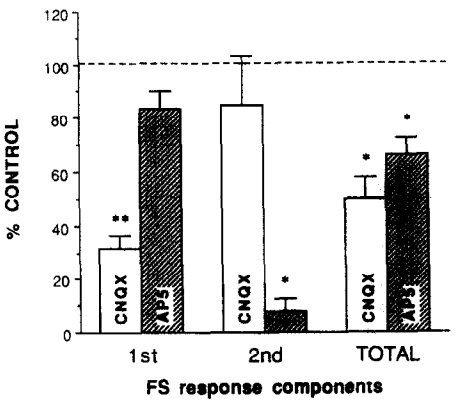

Fig. 7. Graph illustrating the contrasting effects of locally infused CNQX or AP-5 on DOI-enhanced responses of LC neurons to FS (9 mA). One hundred per cent represents the FS response of LC neurons 10 min after systemic DOI (50 μg/kg iv). While both drugs reduced the total FS response in LC, the first response component was preferentially attenuated by CNQX (four out of four cells), and the second response component (seen following DOI) was selectively attenuated by AP-5 (four out of four cells). $^{*}P < 0.05$, $^{**}P < 0.001$ (paired t-tests on absolute R_{mag} values).

the effects of 5-HT_2 agonists on LC neurons.[62] Therefore, we propose that our data reflect 5-HT_2 receptor activation.

Agonists at 5-HT_2 receptors increase the release of corticotropin releasing factor (CRF) in brain: the bromine-substituted analog of DOI, 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane, increases activity in the hypothalamic-pituitary axis to stimulate CRF release in the median eminence,[52] while DOI can stimulate release of CRF from explanted rat hypothalami.[17] Although the LC contains CRF-immunoreactive fibers,[48,72] the DOI effect in LC is not likely due to intracoerulear CRF release because CRF increases spontaneous firing and decreases FS responsiveness of LC neurons,[71] opposite to the results obtained here with DOI.

The present finding of NMDA-mediated LC activation is consistent with recent studies showing that LC neurons can be activated by NMDA both *in vitro*[20,40,51] and *in vivo*.[30,67] Recent studies in our laboratory have also demonstrated AP-5-sensitive, NMDA receptor-mediated FS responses in LC neurons following local infusion of Mg^{2+}-free artificial cerebrospinal fluid or a bicuculline solution into the LC *in vivo*.[67] NMDA receptor-mediated synaptic potentials have also been observed in LC neurons *in vitro* bathed in Mg^{2+}-free solution.[14] These results suggest that excitatory synaptic mechanisms may exist within the LC similar to those reported in hippocampal pyramidal cells where two types of EAA-mediated postsynaptic currents have been recorded: a fast, CNQX-sensitive response com-

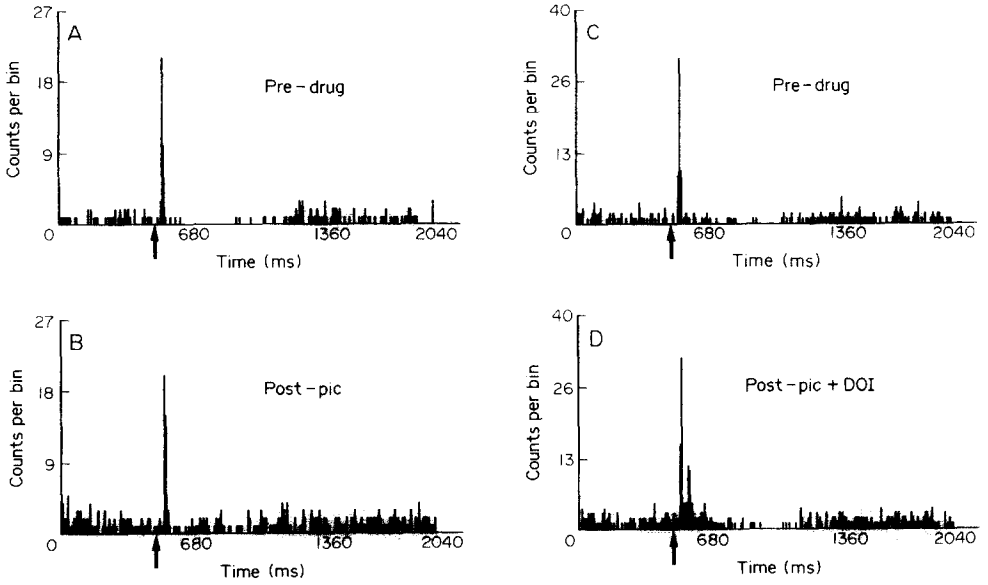

Fig. 8. PSTHs illustrating the ability of systemic DOI to enhance the LC FS response in the presence of locally infused picrotoxin (pic). Stimuli (50 sweeps per PSTH) were presented at arrows. (A) Pre-drug response of an LC neuron to FS. (B) FS response of the same LC cell after locally applied picrotoxin (0.5 mM). Note that the evoked response is not substantially changed (number of spikes 20–100 ms post-stimulation, pre-picrotoxin = 37, post-picrotoxin = 43) while the baseline activity has increased (rate pre-picrotoxin = 1.75 spikes/s, rate post-picrotoxin = 3.0 spikes/s). (C) Pre-drug response of a different LC neuron to FS. (D) FS response of this cell after locally infused picrotoxin (0.5 mM) and systemic DOI (50 μg/kg) injection. Note that while the increase in the cell's spontaneous activity in the presence of picrotoxin is not substantially changed by DOI (rate pre-drug, from C, = 2.75 spikes/s, rate post-picrotoxin + DOI = 3.5 spikes/s), the FS response is still enhanced (number of spikes 20–100 ms post-stimulation, pre-drug = 57, post-picrotoxin + DOI = 92), as shown by the prominent second response component.

ponent and a slower, AP-5-sensitive response component.[35] These previous results also indicate that the EAA input from the nucleus paragigantocellularis that may mediate the FS response of LC neurons through non-NMDA receptors[10,21,27] may also activate NMDA receptors upon decrease of the Mg^{++} blockade of the NMDA receptor.[14,67]

As there is no evidence for DOI- or other 5-HT_2 agonist-induced alteration of Mg^{2+} levels, and as DOI acts outside LC to increase FS responses, we propose that the potentiated NMDA-receptor-mediated response in LC to high-intensity FS is due to augmented FS-evoked release of an EAA transmitter within the LC, sufficient to activate NMDA receptors despite physiological levels of Mg^{2+}. This may involve, e.g., disinhibition of EAA inputs to LC, so that their influence on LC with FS is increased. This hypothesis is consistent with the present finding that, when given with low-intensity FS stimulation (4 mA), DOI increased the early component of LC responses but did not elicit a late FS response component. This suggests that DOI increases FS-evoked release of an EAA transmitter within the LC such that with low stimulation intensity the increased EAA release activates a greater number of non-NMDA receptors on LC cells, while at high stimulation intensity the greater EAA release is sufficient to activate NMDA receptors as well, yielding the second response component. It is unclear why the first response component of LC cells after 9 mA FS decreases after the 16 μg/kg dose of DOI. This may indicate that DOI has a sensitive but low-potency influence on LC neurons (depression of FS responses) which is overridden at higher DOI doses to enhance these same responses. Further experiments are necessary to test this and other possibilities.

It has been previously reported that alpha-2 adrenoceptor blockade may enhance LC responses to sensory stimulation.[70] Our laboratory has reported in other studies[5,27,58] that an inhibitory adrenergic input to the LC arises from the nucleus paragigantocellularis, and it is conceivable that DOI may increase LC responsiveness, in part, by inhibiting this pathway. However, our present results suggest that this is unlikely. Our finding that local infusion of idazoxan, as well as BIC and picrotoxin, into the LC did not significantly alter the second FS response component elicited by DOI indicated that alpha-2 adrenoceptor or $GABA_A$ receptor blockade does not play a role in producing this DOI effect. This idea is supported by our data showing that systemically administered idazoxan does not mimic DOI, as would be expected if DOI acted by inhibiting an adrenergic inhibitory input to the LC. Rather, idazoxan increased LC spontaneous discharge and did not elicit a second FS response component.

While this study does not specify at what extracoerulear site(s) DOI acts to influence LC cells, the results, in view of previous findings, indicate possible pathways which may be tested in future experiments. The present results indicate that the sources of GABA and EAA afferents to LC may be sites through which 5-HT_2 agonists act directly or indirectly to influence LC. Anatomical[11,13,59,69] and physiological studies[28,29] indicate that the LC receives a potent GABA input from the nucleus prepositus hypoglossi in the dorsomedial medulla; additional GABAergic afferents to LC may originate in the PGi and other areas (Aston-Jones, Zhu and Pieribone unpublished observations; see also Ref. 14). A major EAA input to the LC originates from the nucleus paragigantocellularis in the ventrolateral rostral medulla.[11,30] Our results also indicate that the FS response is mediated through this input[21] (however, see also Ref. 63). Thus, the two major afferents to LC, the nucleus prepositus hyperglossi and the nucleus paragigantocellularis, appear to be good candidates for mediating the effects of DOI on LC, and our results are consistent with 5-HT_2 depolarization or disinhibition of these afferents. However, as autoradiographic studies have demonstrated that these areas are relatively sparsely populated with 5-HT_2 receptors,[44,53] it also seems possible that the nucleus paragigantocellularis and nucleus prepositus hypoglossi may indirectly convey the effect of 5-HT_2 agonists on LC neurons, reflecting activation of 5-HT_2 mechanisms in brain structures afferent to nucleus paragigantocellularis and nucleus prepositus hyperglossi that are more densely populated with such receptors.[44,53,56] This hypothesis is supported by the recent work of Gorea *et al.*,[34] who have shown that lesions of the nucleus prepositus hypoglossi attenuate the inhibitory effects of systemic quipazine on LC discharge, but that local injection of quipazine into the nucleus prepositus hypoglossi does not affect the LC.

CONCLUSION

The functional significance of the present results remains to be elucidated, but the influence of 5-HT_2 receptor activation on central noradrenergic cells may be important because 5-HT_2 mechanisms play a role in several of the same processes that are also associated with LC function. For example, mescaline, acting at 5-HT_2 receptors, can potentiate acoustic startle reflexes[24] while LC lesions attenuate such startle responses.[1] In addition, 5-HT_2 receptor changes are seen in affective disorders[25,75,76] and Alzheimer-type dementia,[23] dysfunctions associated with norepinephrine or LC anomalies.[18,43,73] It is therefore possible that indirect 5-HT_2 influences on the LC noradrenergic system may play a vital, yet currently undefined, role in such normal and abnormal behavioral states.

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