Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 584 (2008) 93–99
www.elsevier.com/locate/ejphar

Electrophysiologic effects of systemic and locally infused epibatidine on locus coeruleus neurons ☆

Arjunan Ganesh [a,b], Alfredo Gonzalez-Sulser [a], Nayla Chaijale [a], Giovanni Cucchiaro [a,b,*]

[a] Department of Anesthesia and Critical Care of Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA, USA
[b] University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Received 10 August 2007; received in revised form 10 January 2008; accepted 24 January 2008
Available online 5 February 2008

Abstract

We evaluated the electrophysiologic response of locus coeruleus neurons to the systemic and local infusion of epibatidine. Rats were anesthetized with 2% halothane and single-unit locus coeruleus discharge was recorded after administration of systemic (2.5, 5 and 10 μg/kg subcutaneously) and intracoerulear (0.03–0.01–0.001 μg) epibatidine. The subcutaneous epibatidine activated locus coeruleus neurons only at the highest dose (10 μg/kg). The 2.5–5 μg/kg doses, previously shown to induce analgesia, did not activate locus coeruleus neurons. The intracoerulear infusion of epibatidine induced excitement of locus coeruleus neurons at every tested dose. Higher doses (0.03 and 0.01 μg) excited 100% of the recorded neurons. A significantly lower number of neurons (50% and 43% respectively) were excited when lower doses (0.005–0.001 μg) were used ($P=0.035$). The intracoerulear infusion of mecamylamine (1 μg) significantly reduced neuronal discharge rate (45%) and blocked the effects of epibatidine. The intra-dorsal raphe infusion of 0.03 μg epibatidine induced significant excitation of locus coeruleus neurons. These data show that the administration of epibatidine induces excitation of locus coeruleus neurons, which is mediated by nicotinic receptors. This activation occurs after systemic and selective local administration of epibatidine. The response of locus coeruleus neurons to systemic and locally administered epibatidine is dose-related.

Keywords: Epibatidine; Locus coeruleus; Electrophysiology; Analgesia; Dorsal raphe

1. Introduction

Recent studies on the analgesic effects of nicotinic agonists have confirmed that serotonergic and noradrenergic midbrain nuclei mediate nicotine induced analgesia. Activation of the nucleus raphe magnus by ABT-594 (Bitner et al., 1998; Boyce et al., 2000), a synthetic derivative of epibatidine has analgesic effects. Similarly, the selective administration of epibatidine into the dorsal raphe and locus coeruleus induces analgesia (Cucchiaro et al., 2005, 2006).

Electrophysiology studies have shown that systemic nicotine excites dorsal raphe noradrenergic neurons in the locus coeruleus (Engberg and Hajos, 1994). These effects of nicotine on locus coeruleus neurons appear to be indirect, and are probably dependent on the local release of excitatory aminoacids (Tung et al., 1989). Dorsal raphe and locus coeruleus have multiple interconnecting projections, which have been shown to play a role in physiologic activities including sleep–wake cycle, arousal and cognitive functions (Aston-Jones et al., 1991; Jacobs and Fornal, 1993; Usher et al., 1999). The serotonergic innervation of the locus coeruleus derives from the dorsal raphe and this probably mediates the autonomic effects of the locus coeruleus (Kaehler et al., 1999; Qiao and Dafny, 1988). There is also evidence of dorsal raphe and locus coeruleus interaction with respect to analgesia. Pain stimuli result in a serotonin sensitive excitation of locus coeruleus neurons (Segal, 1979). Moreover, serotonin antagonists have been shown to attenuate

☆ The present study was funded by FAER, Foundation for Anesthesia Research and Education.
* Corresponding author. Department of Anesthesiology and Critical Care Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA. Tel.: +1 215 590 1884; fax: +1 215 590 141.
E-mail address: cucchiaro@email.chop.edu (G. Cucchiaro).

0014-2999/$ - see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2008.01.034

analgesia produced by locus coeruleus activation (Margalit and Segal, 1979). Nicotine acetylcholine receptors are present in the dorsal raphe (Cucchiaro and Commons, 2003), therefore, nicotine and nicotinic agonists may exert some of their analgesic effects via these neuronal pathways connecting dorsal raphe to locus coeruleus.

In this study we have analyzed locus coeruleus neuronal response after systemic and intracoerulear administration of epibatidine. We have also studied the response of locus coeruleus neurons to the direct administration of epibatidine into the dorsal raphe.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g) were housed three to a cage under a 12:12-h light/dark cycle with water and food available ad libitum. The protocols were in accordance with the animal care guidelines at the University of Pennsylvania and The Children's Hospital of Philadelphia and followed the Guide for the care and use of laboratory animals as adopted and promulgated by the U.S. National Institute of Health.

2.2. Electrophysiology experiments

The protocol is similar to the one described in Curtis et al. (2006). Rats were anesthetized with 2% halothane-in-air mixture administered through a nose cone. The anesthetic was maintained at 1.0–1.5% throughout the experiment. Body temperature was maintained at 37.5° by a feedback-controlled heating pad. Rats were positioned in a stereotaxic frame with the head oriented at an 11° angle to the horizontal plane (nose down). The skull was exposed and a hole, centered at 1.2 mm lateral to the midline and 2.8–3.7 mm caudal to lambda, was drilled over the cerebellum in order to reach the locus coeruleus. The dura over the cerebellum was carefully removed with fine iridectomy scissors and tweezers in order to facilitate introduction of the recording micropipette. In the experiments with intra-dorsal raphe infusion of epibatidine a second hole was drilled exposing the brain from 0.5 mm rostral to lambda to 3 mm posterior to bregma. The dura and sagittal sinus were ligated, transected, and reflected to allow for a rostral midline approach to the dorsal raphe with minimal blood loss. The double-barreled micropipette was positioned 2.5 mm rostral to lambda at an angle of 30° from the perpendicular plane. The micropipette was advanced 5.8 mm to the dorsal raphe by hand using the arm attachment of a stereotaxic table (KOPF Tujunga, CA).

2.3. Systemic epibatidine

Single barrel glass micropipettes (2–4 μm diameter tip, 4–7 MΩ) filled with 0.5 M sodium acetate buffer saturated with Pontamine Sky blue dye (PSB) were used to record single-unit locus coeruleus discharge. Four groups of rats received a subcutaneous injection of saline (control group, $n=7$), and epibatidine (2.5 μg/kg, $n=9$; 5 μg/kg, $n=10$; 10 μg/kg, $n=10$). Epibatidine was injected into the back of the rats, in the lumbar area.

2.4. Intra-locus coeruleus epibatidine

Double-barrel glass micropipettes were used to record single-unit locus coeruleus discharge and simultaneously microinfuse epibatidine (Akaoka et al., 1992; Curtis et al., 2006). The microinfusion pipette (Fisher Scientific, Pittsburg, PA; 60–90 μm diameter tip) was angled at 30–45° with its tip adjacent and 130–150 μm dorsal to the tip of the recording pipette and glued in this alignment using a photopolymerizing two-part resin (Silux, 3M Dental Products, St Paul, MN). The electrode was advanced into the locus coeruleus with a micromanipulator. The infusion pipette was connected by tubing to a source of solenoid-activated pneumatic pressure (Picospritzer, General Valve, Fairfield, NJ) and calibrated in such a way that known volumes could be administered. Intracoerulear infusions were made by applying small pulses of pressure (40 psi, 10 ms in duration) to the drug-containing barrel at a frequency of 0.5–1.0 Hz. The movement of the meniscus of the solution through the calibrated injector barrel was viewed with respect to a fixed piece of metric chart paper. Movement of the meniscus by 0.5 mm corresponded to a 30 nl injection. Microinfusions of 30 nl of the epibatidine solution to the microenvironment of the recorded locus coeruleus unit occurred over a period of 1–3 min. This variation in time depended on the difficulty of maintaining the cell in close enough proximity to the electrode in order to keep recording from the neuron. The marking pipette (2–4 m diameter tip, 4–7 MΩ) was filled with 0.5 M sodium acetate buffer saturated with Pontamine Sky blue dye (PSB). The direct infusion of drugs in the locus coeruleus included artificial cerebrospinal fluid (aCSF) (control group; $n=5$), and epibatidine (dissolved in aCSF) (0.001 μg, $n=7$; 0.005 μg, $n=8$; 0.01 μg, $n=7$; 0.03 μg, $n=7$). The volume used to infuse these different doses and aCSF was 30 nl. To confirm that the observed effects of epibatidine were secondary to activation of nicotine acetylcholine receptors, the intracoerulear infusion of epibatidine was preceded by the intracoerulear infusion of mecamylamine ($n=5$), a non-selective nicotine acetylcholine receptors antagonist. At first a double-barrel glass micropipette was used to identify locus coeruleus neurons and to infuse mecamylamine (1 μg). The locus coeruleus neuron activity was recorded for 15 min and this first micropipette was withdrawn. Another pipette was then advanced in the locus coeruleus. After identification of a locus coeruleus neuron, epibatidine (0.03 μg) was infused and the electrical response was recorded. This was possible because of the long lasting effects of locally infused mecamylamine (Nakahara, 2004). In control experiments, artificial cerebrospinal fluid (aCSF) was infused prior to the infusion of epibatidine ($n=4$). The effects of mecamylamine alone (1 μg) on locus coeruleus neurons were also tested in a different group of animals ($n=5$).

2.5. Intra-dorsal raphe epibatidine

In experiments involving intra-dorsal raphe administration of epibatidine, rats were anesthetized as previously described.

A double-barreled micropipette, assembled as previously described, was used to infuse epibatidine onto the dorsal raphe and to mark the spot of infusion. The intra-raphe infusion technique was identical to the intra-locus coeruleus infusion. A second double-barrel glass micropipette was used to identify and then record single-unit locus coeruleus discharge. After identification of a locus coeruleus neuron, epibatidine (0.03 μg), a dose shown in previous experiments to induce analgesia (Cucchiaro et al., 2005), was infused into the dorsal raphe. In control experiments, aCSF was infused into the dorsal raphe and locus coeruleus neurons response was recorded.

2.6. Recording

Neuronal signals were amplified and filtered. Impulse activity was monitored with an oscilloscope and loudspeaker to aid in finding the locus coeruleus. Locus coeruleus neurons were tentatively identified during recording by their spontaneous discharge rates (0.5–5 Hz), entirely positive, notched waveforms (2–3 ms duration), and biphasic excitation–inhibition responses to tail pinch. When stable, unitary action potentials were isolated, a window discriminator was used to convert the occurrence of a single action potential into a digital pulse, which was led into a Windows-based computer via a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK) using Spike 2 software for on-line visualization and storage and off-line analysis.

Extracellular single-unit locus coeruleus spontaneous discharge was recorded until it became stable (3–6 min). In the systemic, intra-locus coeruleus and intra-dorsal raphe administrations cells were recorded 4–15 min post-infusion. Only one cell and one single administration were tested per individual rat.

2.7. Histology

The site of recording was labeled by iontophoresis (−15 μA, 25 min) of PSB at the end of each experiment. In the mecamylamine experiments, the site of recording was labeled only after the infusion of epibatidine via the second micropipette. In the dorsal raphe infusion experiments, the site of injection (dorsal raphe) was labeled along with the site of recording (locus coeruleus). Rats were sacrificed after recording with halothane overdose. Brains were removed and frozen. 30-μm-thick coronal sections were cut on a cryostat, mounted on glass slides, and stained with neutral red for localization of the PSB mark. Data presented are from neurons that were histologically identified, under the microscope, as being inside the locus coeruleus.

2.8. Data analysis

Spontaneous locus coeruleus discharge rate was determined by calculating the mean frequency over 1 min intervals. Basal locus coeruleus discharge rate corresponded to the mean of 5 min recording preceding epibatidine administration. The locus coeruleus maximum discharge rate following epibatidine administration was compared to baseline values. Neurons were considered reactive when we observed an increase in the firing rate of at least 10% from baseline values and the firing rate remained constantly higher for at least 3 min. Data were analyzed for normal distribution using the Shapiro–Wilk test. Baseline discharge rates were compared among groups and within groups using two-way ANOVA. Comparison of discharge rates before and after epibatidine was done using one-way ANOVA. The difference in responding neurons between groups was analyzed using the χ^2 test.

3. Results

Baseline discharge rates of locus coeruleus neurons were similar in the different experimental conditions tested in this study.

3.1. Recording after systemic administration of epibatidine

The administration of saline had no effect on neuronal discharge rate. Similarly, the lowest doses tested, 2.5 μg/kg, did not have any effect. The highest dose (10 μg/kg) significantly increased locus coeruleus neurons firing rate in 80% of the recorded neurons (Table 1) (Fig. 1A). The 5 μg/kg dose significantly increased the firing rate only in 30% of the recorded neurons ($P=0.035$, compared to the 10 μg/kg dose) (Table 1). The interval between the subcutaneous administration of epibatidine and the maximum neuronal excitation was 1180±131 s.

3.2. Recording after intra-locus coeruleus administration of epibatidine

The administration of aCSF had no significant effect on neuronal firing rate. The administration of epibatidine directly into the locus coeruleus induced excitement of locus coeruleus neurons (Fig. 1B). As seen in the systemic administration experiments, the neuronal response was dose-related (Table 2). Higher doses of epibatidine excited 100% of the recorded neurons (0.03 and 0.01 μg). A significantly lower number of neurons (50% and 43% respectively) were excited when lower

Table 1
Effects of subcutaneous administration of saline and epibatidine on locus coeruleus neurons discharge rate

Systemic epibatidine	# cells reacted	Hz baseline	Hz max excitation	% change	*P*
10 μg/kg ($n=10$)	8 (80%)[a]	1.4±0.6	5.3±3.9	390%	0.015
5 μg/kg ($n=10$)	3 (30%)[a]	1.3±0.6	2.4±1.3	166%	0.06
2.5 μg/kg ($n=9$)	0 (0%)	1.3±0.6	1.4±0.6	0%	NS
Saline ($n=7$)	0 (0%)	1.4±0.7	1.4±0.5	0%	NS

Rates are in Hz. The administration of 5 and 10 μg/kg epibatidine induced an increased discharged rate in locus coeruleus neurons. 10 μg/kg epibatidine excited a significantly higher number of neurons compared to 5 μg/kg epibatidine ([a]$P=0.035$). The *P* value reflects the differences in firing rates (pre versus post treatment) in each study group. The discharge rate in the excited cells was significantly increased compared to the baseline rate only after 10 μg/kg epibatidine. The statistical analysis (one-way ANOVA) was conducted using data obtained from every sampled neuron. The difference in responding neurons between groups was analyzed using the χ^2 test.

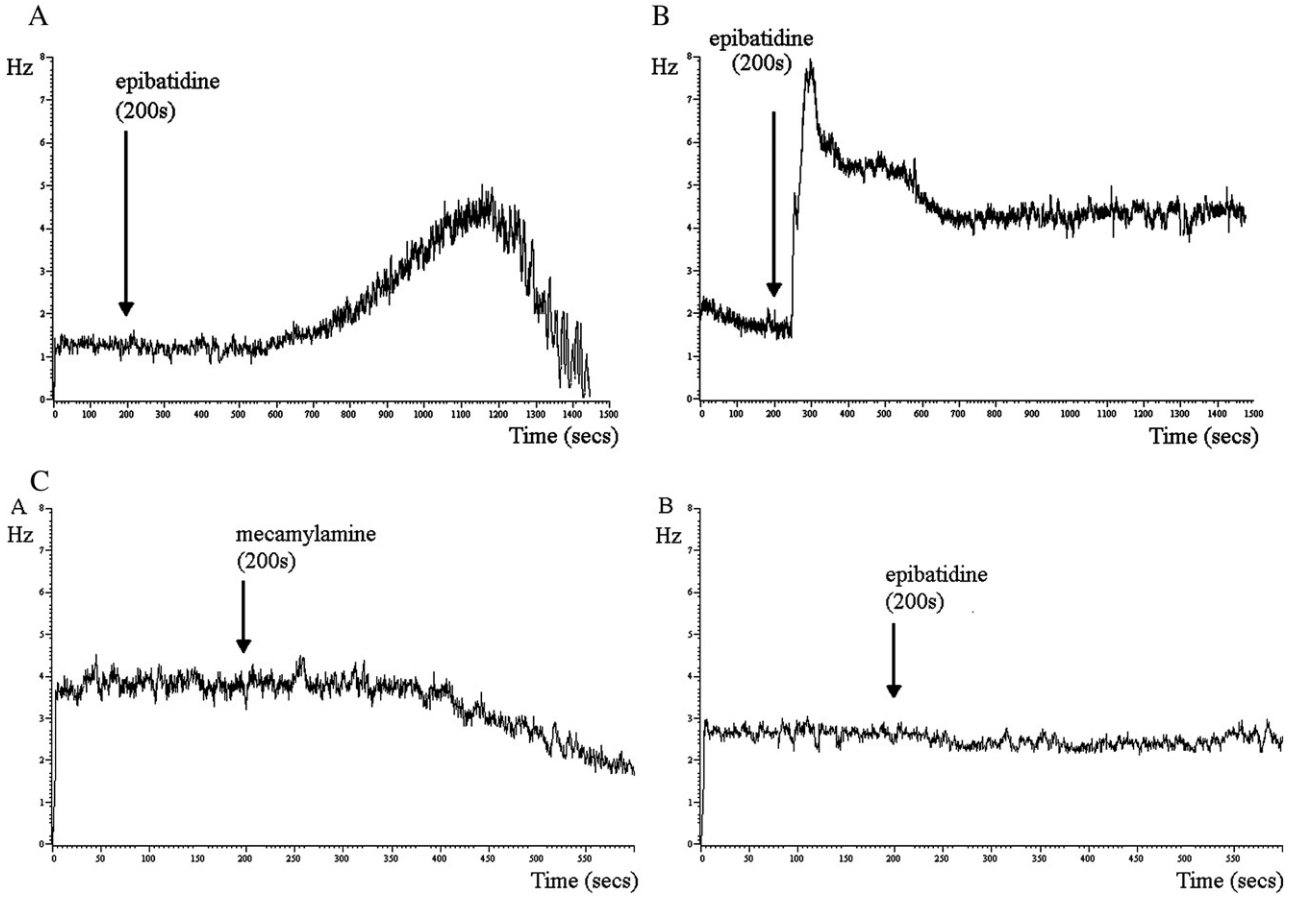

Fig. 1. Electrophysiology studies conducted in different animals. 1A: Effects of high dose systemic epibatidine (10 μg/kg) on the mean frequency (Hz) of action potentials of single locus coeruleus neurons. After a baseline recording of 200 s, epibatidine was injected subcutaneously. An increased firing rate was observed 200 s after epibatidine injection, which peeked 1000 s after the injection. 1B: Effects of 0.03 μg epibatidine infused into the locus coeruleus on the mean frequency (Hz) of action potentials of single locus coeruleus neurons. After a baseline recording of 200 s, epibatidine was infused directly into the locus coeruleus. An increase in firing rate was recorded within 50 s from the infusion of epibatidine. 1C: A) Effect of intracoerulear mecamylamine (1 μg) on the mean frequency (Hz) of action potentials of single locus coeruleus neurons. After a baseline recording of 200 s, mecamylamine was infused directly into the locus coeruleus. A slight decrease in firing rate was observed 200 s after the infusion. B) Effect of intracoerulear epibatidine (0.03 μg) infused 20 min after intracoerulear mecamylamine infusion. After a baseline recording of 200 s, epibatidine was infused directly into the locus coeruleus. No change in the firing rate was recorded.

doses (0.005–0.001 μg) were used ($P=0.035$) (Table 2). The infusion of mecamylamine (1 μg) resulted in a significant reduction of the neuronal discharge rate ($P=0.03$) in 100% of the recorded neurons (Table 3) (Fig. 1C). The subsequent infusion of epibatidine (0.03 μg) failed to induce neuronal excitation as previously observed (Table 3) (Fig. 1C). In control experiments, the infusion of aCSF prior to the infusion of

Table 2
Effects of selective infusion of aCSF and epibatidine in the locus coeruleus, on locus coeruleus neurons discharge rate

Intra-locus coeruleus epibatidine	# cells reacted	Hz baseline	Hz max excitation	% change	*P*
0.03 μg ($n=7$)	7 (100%)[a]	2.4±1.1	13.2±8.8	450%	0.01
0.01 μg ($n=7$)	7 (100%)[a]	1.9±1.1	7.8±6.5	301%	0.03
0.005 μg ($n=8$)	4 (50%)	1.3±0.9	5.8±5.6	222%	0.04
0.00 1 μg ($n=7$)	3 (43%)	1.9±0.2	7.6±7.5	230%	0.057
aCSF ($n=5$)	0 (0%)	1.5±0.5	1.4±0.5	−7%	0.06

Rates are in Hz. The administration of epibatidine induces activation of locus coeruleus neurons. A significantly higher number of cells is activated by the higher doses of epibatidine (0.03 and 0.01 μg) compared to the lower tested doses, 0.005 and 0.001 μg ([a]$P=0.01$). The *P* value reflects the differences in firing rates (pre versus post treatment) in each study group. The statistical analysis (one-way ANOVA) was conducted using data obtained from every sampled neuron. The difference in responding neurons between groups was analyzed using the χ^2 test.

Table 3
Effects of the intracoerulear infusion of mecamylamine (1 μg) on locus coeruleus neuronal discharge rate

Intra-locus coeruleus antagonist + epibatidine	# cells reacted	Hz baseline	Hz max excitation	% change	*P*
Mecamylamine 1 μg ($n=5$)	5 (100%)	2.7±0.9	1.5±0.7	−45%	0.03
Mecamylamine 1 μg+ epibatidine 0.03 μg ($n=5$)	0 (0%)	1.4±0.8	1.4±0.7	4%	NS
aCSF+Epibatidine (0.03 μg) ($n=4$)	4 (100%)	1.9±0.3	10.7±4.24	468%	0.01

Rates are in Hz. The infusion of mecamylamine resulted in a significant reduction of neuronal discharge rate ($P=0.03$) in 100% of the sampled neurons. The subsequent infusion of epibatidine (0.03 μg) failed to induce neuronal excitation. The infusion of aCSF prior to epibatidine did not alter the baseline firing rate of locus coeruleus neurons. However, neurons responded to the subsequent administration of epibatidine. The neuronal response to epibatidine after administration of aCSF was similar to what seen in previous experiments (Table 2). The statistical analysis (one-way ANOVA) was conducted using data obtained from every sampled neuron.

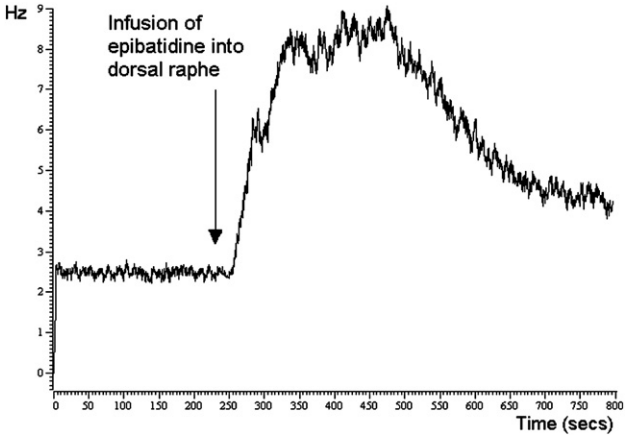

Fig. 2. Effect of intra-dorsal raphe infusion of epibatidine (0.03 μg) on the mean frequency (Hz) of action potentials of single locus coeruleus neurons. After a baseline recording of 200 s, epibatidine was infused into the dorsal raphe. A significant increase in the neuronal firing rate was observed.

epibatidine did not alter neuronal responsiveness to epibatidine (Table 3).

3.3. Recording after intra-dorsal raphe administration of epibatidine

The administration of epibatidine (0.03 μg) into the dorsal raphe resulted in excitation of locus coeruleus neurons (Fig. 2). Most of the locus coeruleus neurons (86%) responded with an increase in the firing rate from 2.5±0.5 to 8.6±4.4 Hz (n=7; P=0.004). The infusion of epibatidine in areas adjacent to the v had no significant effect on locus coeruleus neuronal discharge rate, (from 2.0±0.7 to 1.3±0.7 Hz) (n=8; P=0.2). Similarly the infusion of aCSF into the dorsal raphe had no effect on locus coeruleus neurons (2.2±0.6 versus 2.2±0.5) (n=4; P=0.8) Fig. 3 shows the location of the micropipettes infusing epibatidine and aCSF in relationship with the dorsal raphe.

4. Discussion

The results from this study show that the administration of epibatidine induces excitation of locus coeruleus neurons. This activation occurs after systemic and selective local administration of epibatidine and is mediated by nicotine acetylcholine receptors. The number of neurons responding to systemic and locally administered epibatidine as well as the magnitude of the response seems to be dose-related. We also observed that it possible to excite locus coeruleus neurons by infusing epibatidine directly into the dorsal raphe.

We have previously shown that systemic epibatidine, at doses used in the present study, activates both locus coeruleus using c-Fos expression as an indirect marker of neuronal activation (Cucchiaro and Commons, 2003). However, c-Fos expression is an imprecise predictor of neuronal excitation and, opposite to electrophysiologic studies, does not allow for qualitative and quantitative analysis of single neuron response to drugs. In this study we observed that locus coeruleus neurons response to epibatidine is not uniform and is dose-related.

The locus coeruleus and other midbrain noradrenergic (A5–A7) and serotonergic nuclei (dorsal raphe nucleus raphe magnus) have been shown to directly or indirectly control pain transmission in the dorsal horn of the spinal cord via descending projections (Proudfit and Clark, 1991; Wang and Nakai, 1994). Henceforth, activation of the locus coeruleus by epibatidine could indicate a role of locus coeruleus in mediating the analgesic effects of epibatidine and possibly other nicotinic agonists. However, it is unknown whether nicotinic agonists induce analgesia by acting directly on locus coeruleus neurons or their effects are mediated by other midbrain nuclei.

We found in the current study that low systemic doses of epibatidine, previously shown to induce analgesia (Cucchiaro and Commons, 2003) did not induce uniform locus coeruleus neurons excitation. The systemic administration of 2.5, 5 and 10 μg/kg epibatidine induces analgesia in every animal

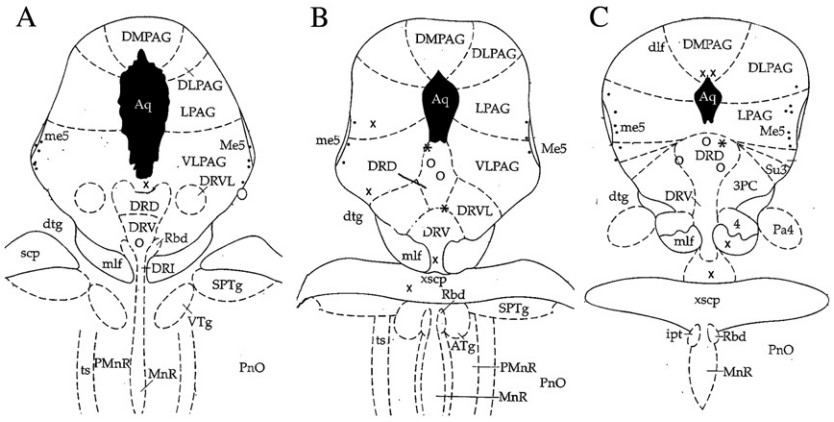

Fig. 3. Coronal sections of the midbrain showing the localization of the dorsal raphe micropipettes infusing epibatidine. Sections are arranged in caudal to rostral order. A, dorsal raphe nucleus; B, dorsal raphe and ventrolateral section of the dorsal raphe; C, caudal section of the dorsal raphe. * indicates intra-dorsal raphe cannulas in aCSF experiments. O indicates intra-dorsal raphe cannulas in epibatidine experiments. X indicates cannulas placed outside the dorsal raphe. 4, trochlear nucleus; Aq, aqueduct; DLPAG, dorsolateral periaqueductal grey; DMPAG, dorsomedial periaqueductal grey; DRD, dorsal raphe nucleus; DRI, interfascicular dorsal raphe; DRV, ventral dorsal raphe; DRVL, ventrolateral dorsal raphe; LPAG, lateral periaqueductal grey; Rbd, rhabdoid nucleus; VLPAG ventrolateral periaqueductal grey; xscp, decussation superior peduncle.

(Cucchiaro and Commons, 2003) using a model of chronic inflammatory pain. However, only the highest dose (10 μg/kg) induced a significant increase in the number of excited neurons and their firing rate in the current study and the lower doses (2.5 and 5μg/kg), failed to induce significant excitation of locus coeruleus neurons.

There are a few possible explanations to these findings. An effect of halothane on neuronal responsiveness to epibatidine cannot be excluded. Halothane, like many other volatile anesthetic agents, has been shown to decreased cellular excitability in multiple areas of the cortex (Hentschke et al., 2005; Nishikawa and MacIver, 2001) and locus coeruleus (Sirois et al., 2000). Inhibition of locus coeruleus neurons by halothane in our experimental conditions cannot be excluded and higher doses of epibatidine may have been necessary to overcome the baseline inhibition caused by halothane.

Another possible explanation is that other midbrain nuclei may mediate the analgesic effects of low dose epibatidine. We have previously shown that the dorsal raphe also mediates epibatidine induced analgesia, at the same doses used in this study (Cucchiaro et al., 2005). Dorsal raphe neurons may have a lower excitatory potential for epibatidine compared to locus coeruleus neurons. Henceforth, low dose epibatidine (2.5–5 μg/kg) may be ineffective on locus coeruleus and induce analgesia by activating the dorsal raphe.

The infusion of epibatidine directly into the locus coeruleus caused neuronal excitation. Direct activation of locus coeruleus neurons by a non-selective nicotinic agonist is not surprising given previous studies, which have shown expression of α4 nicotinic receptors in noradrenergic cells of the locus coeruleus (Cucchiaro and Commons, 2003). The low doses of epibatidine infused directly into the locus coeruleus (0.05–0.001 μg), although previously shown to induce analgesia (Cucchiaro et al., 2006), excited a significant lower number of neurons compared to the higher doses, with only 43–50% of the recorded neurons responding, and the amplitude of the response of the excited neurons was also lower. The reasons for this dose-related response of locus coeruleus neurons to epibatidine are unclear.

The effects of epibatidine on locus coeruleus neurons seem to be dependent on nicotine acetylcholine receptors activation because the local administration of mecamylamine, a non-selective nicotine acetylcholine receptors blocker, prior to the infusion of epibatidine, prevents locus coeruleus activation. We observed a significant reduction in the baseline neuronal firing rate after the local administration of mecamylamine. This finding may indicate the presence of a baseline cholinergic tone in the locus coeruleus.

Several studies have shown the presence of neuronal interconnections between the dorsal raphe and locus coeruleus, which seem to link multiple physiologic activities between these two nuclei (Kim et al., 2004). In particular, it has been suggested that the antinociceptive effects of the dorsal raphe is exerted, among other possible sites, on the locus coeruleus (Segal, 1979). This relationship seems to be confirmed by our findings because the infusion of epibatidine into the dorsal raphe induced excitation of locus coeruleus neurons. Locus coeruleus excitation is strictly dependent of the infusion of epibatidine into the dorsal raphe because the infusion of epibatidine in areas adjacent to these nuclei had no effect on locus coeruleus neuronal firing rate.

We cannot rule out the possibility that the local administration of epibatidine into the locus coeruleus remained confined into the locus coeruleus and did not spill out into the IV ventricle and then to other brain regions, such as the dorsal raphe. In previous histological studies where c-Fos expression was used as an indicator of neuronal activation and significant higher volumes of epibatidine were infused into the locus coeruleus, we failed to detect activation of the dorsal raphe after local administration of epibatidine into the locus coeruleus (data not shown). Moreover, the neuronal activation was also strictly limited to the locus coeruleus and did not spread to the surrounding areas (Cucchiaro and Commons, 2003). Although more cumbersome, the use of double-barrel micropipettes in the locus coeruleus seemed more appropriate than the use of triple-barrel micropipettes. Preliminary histological studies had shown significant structural damage in the locus coeruleus when we used triple-barrel micropipettes, due to their larger size.

In conclusion, this study shows that locus coeruleus neurons are activated by systemic and local infusion of epibatidine into selective nuclei. Systemic epibatidine may induce neuronal excitation by direct activation of locus coeruleus and/or indirectly via excitation of the dorsal raphe.

References

Akaoka, H., Saunier, C.F., Chergui, K., Charlety, P., Buda, M., Chouvet, G., 1992. Combining in vivo volume-controlled pressure microejection with extracellular unit recording. J. Neurosci. Methods 42, 119–128.

Aston-Jones, G., Akaoka, H., Charlety, P., Chouvet, G., 1991. Serotonin selectively attenuates glutamate-evoked activation of noradrenergic locus coeruleus neurons. J. Neurosci. 11, 760–769.

Bitner, R.S., Nikkel, A.L., Curzon, P., Arneric, S.P., Bannon, A.W., Decker, M.W., 1998. Role of the nucleus raphe magnus in antinociception produced by ABT-594: immediate early gene responses possibly linked to neuronal nicotinic acetylcholine receptors on serotonergic neurons. J. Neurosci. 18, 5426–5432.

Boyce, S., Webb, J.K., Shepheard, S.L., Russell, M.G., Hill, R.G., Rupniak, N.M., 2000. Analgesic and toxic effects of ABT-594 resemble epibatidine and nicotine in rats. Pain 85, 443–450.

Cucchiaro, G., Commons, K.G., 2003. Alpha 4 nicotinic acetylcholine receptor subunit links cholinergic to brainstem monoaminergic neurotransmission. Synapse 49, 195–205.

Cucchiaro, G., Chaijale, N., Commons, K.G., 2005. The dorsal raphe nucleus as a site of action of the antinociceptive and behavioral effects of the alpha4 nicotinic receptor agonist epibatidine. J. Pharmacol. Exp. Ther. 313, 389–394.

Cucchiaro, G., Chaijale, N., Commons, K.G., 2006. The locus coeruleus nucleus as a site of action of the antinociceptive and behavioral effects of the nicotinic receptor agonist, epibatidine. Neuropharmacology 50, 769–776.

Curtis, A.L., Bethea, T., Valentino, R.J., 2006. Sexually dimorphic responses of the brain norepinephrine system to stress and corticotropin-releasing factor. Neuropsychopharmacology 31, 544–554.

Engberg, G., Hajos, M., 1994. Nicotine-induced activation of locus coeruleus neurons—an analysis of peripheral versus central induction. Naunyn Schmiedebergs Arch. Pharmacol. 349, 443–446.

Hentschke, H., Schwarz, C., Antkowiak, B., 2005. Neocortex is the major target of sedative concentrations of volatile anaesthetics: strong depression of firing rates and increase of GABAA receptor-mediated inhibition. Eur. J. Neurosci. 21, 93–102.

Jacobs, B.L., Fornal, C.A., 1993. 5-HT and motor control: a hypothesis. Trends Neurosci. 16, 346–352.

Kaehler, S.T., Singewald, N., Philippu, A., 1999. Dependence of serotonin release in the locus coeruleus on dorsal raphe neuronal activity. Naunyn Schmiedebergs Arch. Pharmacol. 359, 386–393.

Kim, M.A., Lee, H.S., Lee, B.Y., Waterhouse, B.D., 2004. Reciprocal connections between subdivisions of the dorsal raphe and the nuclear core of the locus coeruleus in the rat. Brain Res. 1026, 56–67.

Margalit, D., Segal, M., 1979. A pharmacologic study of analgesia produced by stimulation of the nucleus locus coeruleus. Psychopharmacology (Berl) 62, 169–173.

Nakahara, D., 2004. Influence of nicotine on brain reward systems: study of intracranial self-stimulation. Ann. N.Y. Acad. Sci. 1025, 489–490.

Nishikawa, K., MacIver, M.B., 2001. Agent-selective effects of volatile anesthetics on GABAA receptor-mediated synaptic inhibition in hippocampal interneurons. Anesthesiology 94, 340–347.

Proudfit, H.K., Clark, F.M., 1991. The projections of locus coeruleus neurons to the spinal cord. Prog. Brain Res. 88, 123–141.

Qiao, J.T., Dafny, N., 1988. Dorsal raphe stimulation modulates nociceptive responses in thalamic parafascicular neurons via an ascending pathway: further studies on ascending pain modulation pathways. Pain 34, 65–74.

Segal, M., 1979. Serotonergic innervation of the locus coeruleus from the dorsal raphe and its action on responses to noxious stimuli. J. Physiol. 286, 401–415.

Sirois, J.E., Lei, Q., Talley, E.M., Lynch III, C., Bayliss, D.A., 2000. The TASK-1 two-pore domain K+ channel is a molecular substrate for neuronal effects of inhalation anesthetics. J. Neurosci. 20, 6347–6354.

Tung, C.S., Ugedo, L., Grenhoff, J., Engberg, G., Svensson, T.H., 1989. Peripheral induction of burst firing in locus coeruleus neurons by nicotine mediated via excitatory amino acids. Synapse 4, 313–318.

Usher, M., Cohen, J.D., Servan-Schreiber, D., Rajkowski, J., Aston-Jones, G., 1999. The role of locus coeruleus in the regulation of cognitive performance. Science 283, 549–554.

Wang, Q.P., Nakai, Y., 1994. The dorsal raphe: an important nucleus in pain modulation. Brain Res. Bull. 34, 575–585.