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A potent excitatory input to the nucleus locus coeruleus from the ventrolateral medulla

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Our recent anatomic experiments reveal major innervation of the nucleus locus coeruleus (LC) from the nucleus paragigantocellularis (PGi), located in the rostral ventrolateral medulla. In the present studies, low intensity, single pulse electrical stimulation of the PGi synaptically activated most LC neurons examined (69%), while a smaller percentage of LC cells (20%) exhibited pure inhibitory responses. Pharmacologic experiments suggest that the excitatory response may be mediated by an amino acid transmitter.

The results of several anatomic studies indicate that the locus coeruleus (LC) in rat is innervated by neurons located in the ventrolateral medulla. The existence of such a projection was first suggested by immunohistochemical studies revealing putative adrenergic cell bodies in the medulla and adrenergic fibers in the LC [19]. In subsequent retrograde tracing experiments [9, 11] injections of horseradish peroxidase into the LC area were reported to label neurons in the lateral reticular nucleus of the medulla. Additional evidence for this projection was provided by anterograde transport of radiolabeled amino acids from the ventrolateral medulla to the LC [22, 26].

Other anterograde tracing experiments indicate that projections to the LC may originate in medullary loci rostral to the lateral reticular nucleus [23]. In agreement with this possibility, our recent anatomic and electrophysiologic studies [5, 6, 13, 15] indicate that the nucleus paragigantocellularis (PGi; ref. 2), rostral to the lateral reticular nucleus, is a major input to the LC, while the lateral reticular nucleus provides no such innervation.

The PGi area contains neurons that react positively for markers of many putative transmitters, including acetylcholine [7, 20], enkephalin [18], substance P [21], serotonin [10, 12] and epinephrine [3, 19], and markers for these neurochemical agents are

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also present within the LC (see also ref. 25). However, the transmitter identity of PGi neurons projecting to the LC has not been established, and there have been no published reports of the influence of this projection on the discharge of LC neurons. We have now investigated the effects of electrical activation of the nucleus PGi on LC impulse activity, and have conducted preliminary pharmacologic experiments to discern the transmitters involved in evoked responses. The results indicate that the nucleus PGi provides a predominantly excitatory input to the LC, possibly mediated by an amino acid transmitter.

Thirteen male albino rats were used in these experiments. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.), and anesthesia was maintained throughout experimental procedures by additional i.v. injections (tail vein) at regular intervals. Body temperature was maintained at 36–37°C with a feedback-controlled heating pad. A tracheal cannula was inserted and the animal was mounted in a stereotaxic instrument with the incisor bar lowered to place the skull at a 20° angle, facilitating electrode implantation.

To prepare for stimulation electrode implantation, the tissue overlying the medulla was excised, and the ventral aspect of the occipital plate was removed. The atlantooccipital membrane was removed to expose the obex. A stimulation electrode, consisting of twisted stainless-steel wires (250 μm diameter), insulated except for bluntly cut tips, was implanted in the PGi (2.1 mm rostral to the obex, 1.9 mm lateral to midline, and 6.5–7.0 mm ventral to the cerebellar surface). In addition, a 23-gauge stainless-steel guide cannula was implanted in a lateral ventricle (2.0 mm posterior to bregma, 1.5 mm lateral to midline, 4.0 mm ventral to skull surface) in two animals for intraventricular drug administration. In these animals the dura overlying the medulla was left intact to prevent loss of cerebrospinal fluid, and stimulation electrodes were implanted through the cerebellum. The cannula was secured to adjacent skull screws with dental acrylic.

A hole was drilled in the skull above the LC (3.7 mm posterior to lambda, 1.2 mm lateral to midline), and the dura and pia mater were reflected. A glass micropipette filled with a Pontamine sky blue solution was lowered into the LC (approximately 5.7–6.5 mm ventral to the skull surface) with a hydraulic microdrive. Extracellular recordings from individual neurons were amplified and displayed as filtered (500 Hz–10 kHz bandpass) and unfiltered electrode signals, and monitored with a loudspeaker. LC neurons were tentatively identified at the time of recording on the basis of their characteristic impulse waveforms, spontaneous discharge patterns, and response to foot or tail pinch, as previously reported [1, 8, 27].

Putative LC neurons encountered during micropipette penetrations were examined for responsiveness to stimulation of the PGi. Peri-stimulus time histograms (PSTHs) were generated on-line by computer for a minimum of 50 consecutive stimulus trials, presented at a frequency of 0.5 Hz. Stimuli were monophasic square wave pulses, 0.5 ms in duration and 0.1–1.0 mA in intensity. To quantitate evoked responses, individual PSTHs were analyzed by computer to determine excitatory and inhibitory epochs, as previously described [4]. In brief, a baseline period was defined as the 500-ms epoch preceding stimulation and the onset of significant excitation was defined

as the first of 5 consecutive bins whose mean value exceeded mean baseline activity by two standard deviations. Inhibition was defined as a 50% reduction in baseline activity for at least 250 ms. For statistical comparisons of excitatory responses in pre- and post-drug PSTHs, response magnitudes were defined as the number of spikes in the response epoch normalized by baseline activity.

The muscarinic antagonist scopolamine hydrochloride (Sigma) or the amino acid antagonist kynurenic acid (Sigma) was administered in some animals in an attempt to antagonize evoked responses in LC neurons. Scopolamine was injected into the lateral tail vein as a 5 mg/ml aqueous solution. Kynurenic acid was dissolved in a 0.1 M phosphate buffer to form a 0.1 M solution (pH 7.4) of this drug, and was injected into the lateral ventricle through a 30-gauge needle inserted into the previously implanted guide cannula.

Recording sites were marked by iontophoretic ejection of dye (7 μA of cathodal current for 5 min). At the end of experimental sessions, 30 μA of anodal current was passed through the stimulation electrode for 50 s. Five microliters of dye (Neutral red) was injected through the guide cannula to confirm ventricular placement. Animals were then deeply anesthetized, perfused with a 10% formalin solution, and brains were removed for histologic analysis of recording, stimulation and cannula sites. All data reported here are from animals in which these sites were histologically verified (see Fig. 1).

Single pulse stimulation of the nucleus PGi at low intensities activated most LC neurons (mean intensity for activation, 375 μA), as illustrated in Fig. 2A. Significant excitation occurred for 24/35 LC neurons examined. The mean ($\pm$S.E.M.) onset latency for this excitation was 11.7 ± 4.9 ms, and the mean duration was 35.4 ± 4.3 ms. Such excitation was typically followed by inhibition lasting 200–500 ms, similar to post-activation inhibition previously reported for LC neurons [1, 8, 14].

A purely inhibitory response to PGi stimulation was also observed for some LC neurons (7/35; see Fig. 2B). The mean onset latency for this inhibition was 22.8 ± 4.1

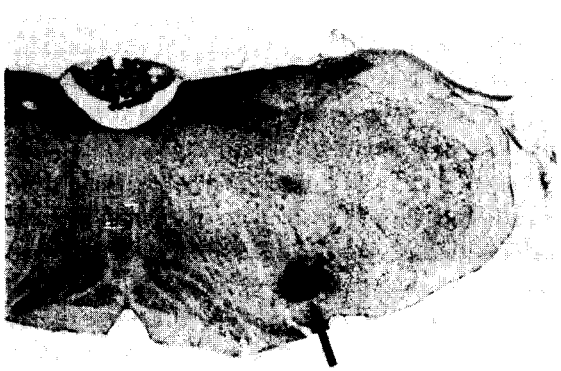

Fig. 1. Photomicrograph of a Nissl-stained, coronal section taken through the medulla of an experimental rat brain showing the stimulation site in the PGi. Prussian blue spot (at arrow) was created at site of iron deposited from stimulation electrode tip. All stimulation sites were histologically verified from such tissue sections. For calibration, length of arrow = 630 μm.

ms, and the mean duration was 404.6±47.1 ms. Another 4 LC neurons exhibited no response to PGi stimulation. Cells that exhibited inhibition only or no response

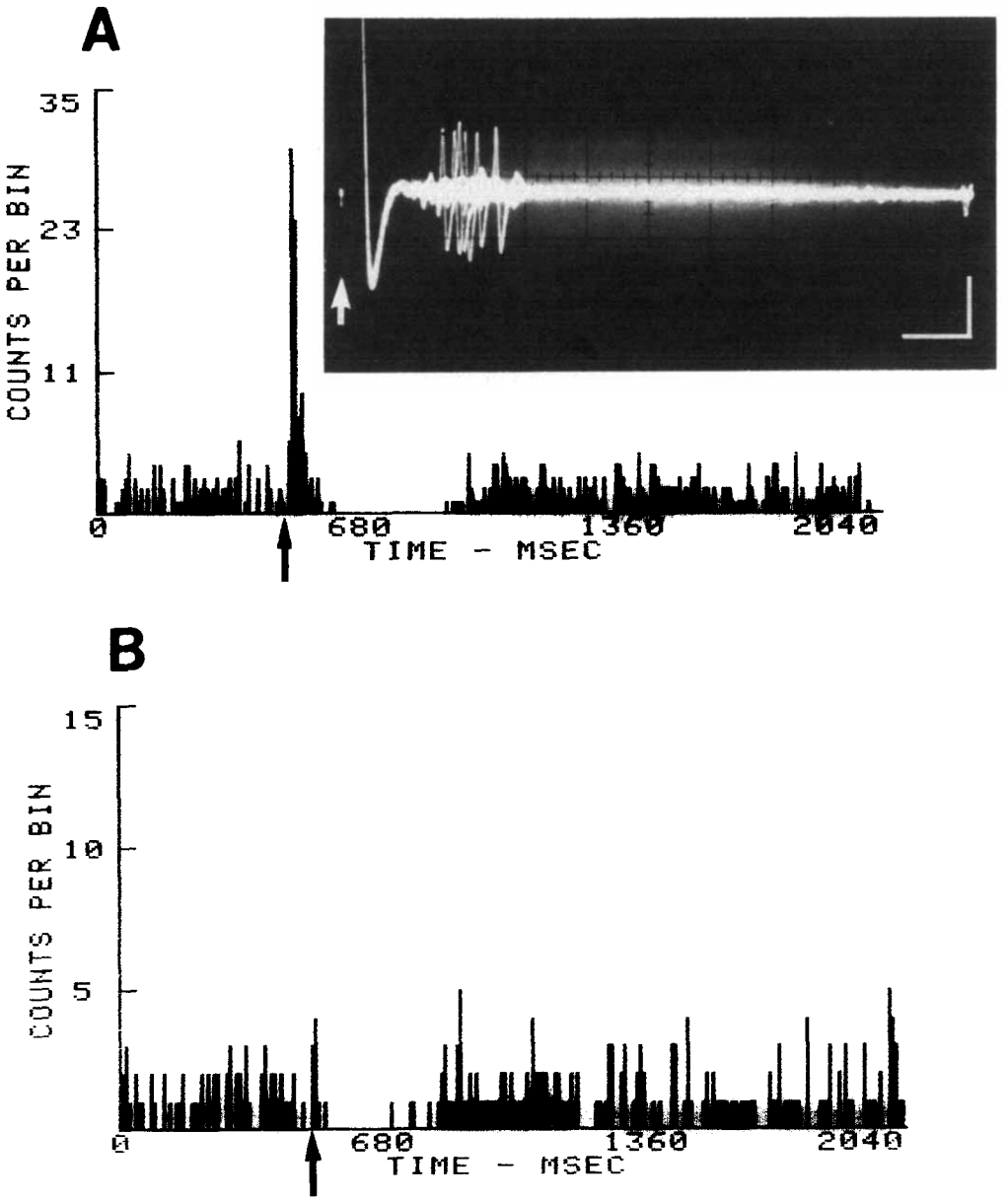

Fig. 2. A: PSTH generated for an LC neuron during PGi stimulation (at arrow, 400 μA intensity; 50 sweeps). Note short latency, potent excitation and subsequent inhibition. Inset: photograph of 10 superimposed oscilloscope sweeps (filtered records) showing activation of another LC neuron. Single pulse stimulation (at arrow, 950 μA, 1.5 × threshold) of the PGi activated this cell at latencies ranging from 9 to 16 ms. Calibrations: 5.0 ms, 0.5 mV. B: PSTH showing pure inhibitory response of an LC neuron to PGi stimulation (at arrow, 300 μA intensity; 50 sweeps).

were found in animals in which other LC neurons were synaptically activated from the PGi.

Intraventricular administration of 5 μl of 0.1 M kynurenic acid, an excitatory amino acid antagonist [16, 24], completely blocked excitation in all 5 LC neurons tested with this agent ($P < 0.01$, Mann–Whitney test). Intraventricular administration of vehicle (0.1 M phosphate buffer, pH 7.4) had no effect in another 4 LC neurons. In contrast, i.v. or i.p. administration of the muscarinic antagonist scopolamine, at doses of up to 3.0 mg/kg, had no consistent effect on PGi-induced excitation in the 5 LC neurons tested with this agent ($P > 0.10$, Mann–Whitney test).

The present results demonstrate a strong excitatory influence of the nucleus PGi activation on LC neuronal discharge. Approximately 69% of LC neurons examined were synaptically activated by focal stimulation of the nucleus PGi at relatively low intensities. Interestingly, a smaller population, corresponding to 20% of the cells examined, exhibited a purely inhibitory response to stimulation.

Two results indicate that the excitatory influence of the nucleus PGi on LC discharge is monosynaptically mediated: (1) the narrow range of latencies observed for spikes driven with low stimulation intensities, and (2) the latency for synaptic activation of LC neurons is similar to latencies obtained for PGi neurons antidromically activated from the LC (range of latencies, 2.0–22.5 ms [13]).

The finding that stimulation of the nucleus PGi excites some LC neurons while inhibiting others may indicate that two populations of afferents, one excitatory and the other inhibitory, project to the LC from this nucleus. Our results suggest that, at least in anesthetized animals, the set of excitatory afferents are predominant. It is also possible, however, that the inhibitory responses were mediated by a collateral feedback mechanism among LC neurons [1, 8, 14].

Previous studies have demonstrated potent excitation of LC neurons by cholinergic agonists [17], and cells exhibiting cholinergic markers have been localized to the PGi area [7, 20]. However, the inconsistent effect of the muscarinic antagonist scopalamine on PGi-induced excitation of LC neurons observed here indicates that cholinergic mechanisms may not be substantially involved in this response. Additional studies (e.g. with nicotinic receptor antagonists) are needed to confirm this initial conclusion. The ability of kynurenic acid, on the other hand, to antagonize PGi-induced excitation of LC neurons suggests an amino acid transmitter may be a strong component of this pathway. Kynurenic acid has been demonstrated to potently antagonize excitation induced by quinolinic acid, N-methyl-D-aspartate, quisqualic acid, and to a lesser extent, that induced by acetylcholine [16, 24]. Additional testing on a greater number of cells and use of more specific amino acid receptor antagonists are now in progress to identify the transmitter(s) and receptor(s) responsible for this potent excitatory regulation of LC discharge.

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1 Aghajanian, G.K., Cederbaum, J.M. and Wang, R.Y., Evidence or norepinephrine mediated collateral inhibition of locus coeruleus neurons, Brain Res., 136 (1977) 570–577.
2 Andrezik, J.A., Chan-Paley, V and Palay, S.L., The nucleus paragigantocellularis lateralis in the rat: conformation and cytology, Anat. Embryol., 161 (1981) 355–371.
3 Armstrong, D.M., Ross, C.A., Pickel, V.M., Joh, T.H. and Reis, D.J., Distribution of dopamine-, noradrenaline-, and adrenaline-containing cell bodies in the rat medulla oblongata: demonstrated by the immunocytochemical localization of catecholamine biosynthetic enzymes, J. Comp. Neurol., 212 (1982) 173–187.
4 Aston-Jones, G. and Bloom, F.E., Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli, J. Neurosci., 1 (1981) 887–900.
5 Aston-Jones, G., Ennis, M., Pieribone, V.A., Nickell, W.T. and Shipley, M.T., The brain nucleus locus coeruleus: restricted afferent control of a broad network, Science, in press.
6 Aston-Jones, G., Shipley, M.T., Nickell, W.T., Ennis, M. and Pieribone, V., Afferents to locus coeruleus are largely restricted to two medullar nuclei: anatomic and physiologic studies, Soc. Neurosci. Abstr., in press.
7 Butcher, L.L. and Woolf, N.J., Histochemical distribution of acetylcholinesterase in the central nervous system: clues to the localization of cholinergic neurons. In A. Björklund, T. Hökfelt and M.J. Kuhar (Eds.), Handbook of Chemical Neuroanatomy, Vol. 3, Classical Transmitters and Transmitter Receptor in the CNS, Part II, Elsevier, Amsterdam, pp. 1–45.
8 Cederbaum, J.M. and Aghajanian, G.K., Activation of the locus coeruleus by peripheral stimuli: modulation by a collateral inhibitory mechanism, Life Sci., 23 (1978) 1383–1392.
9 Cederbaum, J.M. and Aghajanian, G.K., Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique, J. Comp. Neurol., 178 (1978) 1–16.
10 Chan-Palay, V., Indolamine neurons and their processes in the normal brain and in chronic diet-induced thiamine deficiency demonstrated by uptake of ^{3}H serotonin, J. Comp. Neurol., 176 (1977) 467–494.
11 Clavier, R.M., Afferent projections to the self-stimulation regions of the dorsal pons, including the locus coeruleus, in the rat as demonstrated by the horseradish peroxidase technique, Brain Res. Bull., 4 (1979) 497–504.
12 Dahlstrom, A. and Fuxe, K., Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons, Acta Physiol. Scand., 62, Suppl. 232 (1964) 1–55.
13 Ennis, M. and Aston-Jones, G., Electrophysiologic studies of neurons projecting to nucleus locus coeruleus, Soc. Neurosci. Abstr., 11 (1985) 830.
14 Ennis, M. and Aston-Jones, G., Evidence for neighbor and self-mediated postactivation inhibition of locus coeruleus neurons, Brain Res., 374 (1986) 299–305.
15 Ennis, M. and Aston-Jones, G., Potent excitation of the locus coeruleus by stimulation of the ventrolateral medulla, Soc. Neurosci. Abstr., 12 (1986) 138.
16 Ganong, A.H., Lanthorn, T.H. and Cotman, C.W., Kynurenic acid inhibits synaptic and acidic acid-induced responses in the rat hippocampus and spinal cord, Brain Res., 273 (1983) 170–174.
17 Guyenet, P.G. and Aghajanian, G.K., Excitation of neurons in the locus coeruleus by substance P and related peptides, Brain Res., 136 (1977) 178–184.
18 Hökfelt, T., Elde, R., Johansson, O., Terenius, L. and Stein, L., The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system, Neurosci. Lett., 5 (1977) 25–31.
19 Hökfelt, T., Fuxe, K., Goldstein, M. and Johansson, O., Immunohistochemical evidence for the existence of adrenaline neurons in the brain, Brain Res., 66 (1974) 235–251.
20 Kimura, H., McGeer, P.L. and Peng, J.-H., Choline acetyltransferase-containing neurons in the rat brain. In: A. Bjorklund, T. Hökfelt and M.J. Kuhar (Eds.), Handbook of Chemical Neuroanatomy, Vol. 3, Classical Transmitter and Neurotransmitter Receptors in the CNS, Part II, Elsevier, Amsterdam, 1984, pp. 51–65.

21 Ljungdahl, A., Hökfelt, T. and Nilsson, G., Distribution of substance P-like immunoreactivity in the central nervous system of the rat. I. Cell bodies and nerve terminals, Neuroscience, 3 (1978) 861–943.
22 Loewy, A.D., Wallach, J.H. and McKellar, S., Efferent connections of the ventral medulla oblongata in the rat, Brain Res. Rev., 3 (1981) 63–80.
23 McKeller, S. and Loewy, A.D., Efferent projections of the A1 catecholamine cell group in the rat: an autoradiographic study, Brain Res., 241 (1982) 11–29.
24 Perkins, M.N. and Stone, T.W., An iontophoretic investigation of the actions of convulsant kynurines and their interaction with the endogenous excitant quinolinic acid, Brain Res., 247 (1982) 184–187.
25 Pickel, V.M., Joh, T.H. and Reis, D.J., A serotonergic innervation of noradrenergic neurons in the nucleus locus coeruleus: demonstration by immunocytochemical localization of the transmitter specific enzymes tyrosine and tryptophan hydroxylase, Brain Res., 131 (1977) 197–214.
26 Sawchenko, P.E. and Swanson, L.W., The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat, Brain Res. Rev., 4 (1982) 275–325.
27 Valentino, R.J. and Aston-Jones, G., Activation of locus coeruleus neurons in the rat by a benzazocine derivative (UM 1046) that mimics opiate withdrawal, Neuropharmacology, 22 (1983) 1363-1368.