Title
Lung C-fiber CNS reflex: role in the respiratory consequences of extended environmental tobacco smoke exposure in young guinea pigs.

Permalink
https://escholarship.org/uc/item/53r4m254

Journal
Environmental health perspectives, 109 Suppl 4(SUPPL. 4)

ISSN
0091-6765

Authors
Bonham, AC
Chen, CY
Mutoh, T
et al.

Publication Date
2001-08-01

DOI
10.1289/ehp.01109s4573

Peer reviewed
Environmental Tobacco Smoke and Neural Control of the Lung

Extended exposure to environmental tobacco smoke (ETS) adversely affects the respiratory health of children. Those exposed to ETS have more coughs, wheeze, airway obstruction, and increased airway reactivity, and increased sputum production. ETS exposure is associated with an increased risk of lower respiratory tract illnesses, an increased rate and earlier onset of asthma, and an increased risk of sudden infant death syndrome (SIDS). Although the association between extended ETS exposure and harmfult effects on children's respiratory health has been established by epidemiologic studies, much less is known about the mechanisms by which ETS causes these effects. Identifying the mechanisms may help to discourage exposing children to ETS, perhaps preventing these adverse effects, and may also help in the development of novel therapeutic strategies for those exposed.

The purpose of this research is 2-fold: a) to highlight evidence obtained in an animal model of postnatal ETS exposure supporting the overall hypothesis that an upregulation of the lung C-fiber CNS reflex system may contribute to the asthmalike symptoms associated with extended postnatal ETS exposure in children, and b) to develop the proposal that the neuropeptide substance P (SP) may contribute to the upregulation.

Sensory nonmyelinated C fibers innervating the lung are vigorously stimulated by components of ETS including nicotine, acrolein, acrylonitrile, and oxidants as well as by mainstream tobacco smoke. When stimulated, these lung C fibers can trigger profound respiratory responses through a local axon and central nervous system (CNS) reflex. Some of the reflex responses resemble the symptoms associated with extended ETS exposure: bronchoconstriction, mucous secretion, and increased microvascular leak. The responses evoked through the local axon reflex include bronchoconstriction, mucous secretion, and increased microvascular leak and are thought to be mediated by the local release of substance P and neurokinin A contained in the C fibers. The CNS reflex respiratory responses also include bronchoconstriction, mucous secretion, and increased microvascular leak but are not limited to the airways and include breathing changes composed of an expiratory apnea sometimes followed by rapid shallow breathing and cardiovascular changes such as decreases in blood pressure and heart rate. Figure 1 gives a simplified overview of the lung C-fiber CNS reflex pathway illustrating the reflex responses evoked by stimulation of the lung C fibers by an injection of capsaicin into the left atrium of a young guinea pig. As shown in Figure 1A, the first-order afferent lung C fibers course in the vagus nerve with their cell bodies located in the nodose or jugular ganglia. Upon entering the medulla, the central axons become part of the tractus solitarius (TS), then exit the tractus to make excitatory synapses onto second-order neurons in the caudomedial region of the nucleus of the tractus solitarius (NTS) as shown in the coronal section in the inset (19, 20). The signals are conditioned in the NTS and then transmitted to distal synapses in the medulla to ultimately elicit the coordinated reflex responses illustrated in Figure 1B.

Our specific hypothesis is that extended exposure to ETS increases the excitability of the primary lung afferent C fibers and that this increase in afferent traffic to the CNS triggers a further increase in lung C-fiber signal transmission, ultimately augmenting the CNS reflex responses, which could contribute to the respiratory symptoms and SIDS associated with ETS exposure in children. We tested the hypothesis in young guinea pigs randomly assigned to a group exposed to either sidestream smoke, the surrogate for ETS, or filtered air for 6 hr/day, 5 days/week, from 1 to 6 weeks of life (a 5-week exposure period). Like the human, the guinea pig shows advanced development of lung function and morphology at birth. The age of puberty in guinea pigs is 7-10 weeks, and the maximum life span is approximately 7 years. Thus, these guinea pigs were...
exposed during a period equivalent to human childhood and were tested during the period equivalent to human adolescence. Sidestream smoke was generated by an automated cigarette-smoking machine that smoked conditioned 1R4F cigarettes (1.2 mg nicotine/cigarette) from the University of Kentucky Tobacco and Health Research Institute in Lexington, Kentucky. The smoke was collected from the smoldering end of the cigarette, then aged and diluted to a particulate concentration of 1.00 ± 0.07 mg/m³ (23). Sixteen hours after the last exposure, we anesthetized each guinea pig and recorded the spiking activity of individual primary lung C fibers in the vagus nerve. We compared the baseline activity and the increase in the intrinsic postsynaptic excitability of the primary sensory fibers that occurs at these synapses that the primary sensory information is first subject to neuronal modulation before it is ultimately transformed into a complex output to the lungs, airways, respiratory muscles, heart, and blood vessels (24). The studies were performed using the same exposure protocol as the study described previously. We simultaneously recorded extracellular action potentials of second-order NTS neurons in the lung C-fiber reflex pathway and phrenic nerve activity as an index of respiratory output. As shown in the two upper traces in Figure 2B, NTS neurons recorded from sidestream smoke-exposed guinea pigs were more excited by lung C-fiber activation than those neurons recorded from the filtered air-exposed control animals. The lower panel of Figure 2B shows the grouped data from the highest dose of capsaicin and confirms that sidestream smoke exposure increased both the magnitude and the duration of the synaptic responses, the excitability of NTS neurons (23). The peak increase in NTS activity in the sidestream smoke-exposed animals was significantly greater than that in the filtered air-exposed animals, as was the duration of the response. In addition, as shown in Figure 2C, the apnea (prolongation of the expiratory time, Tₑ) was significantly greater in the sidestream smoke-exposed animals compared to that in the filtered air-exposed control group (25).

These data, linking changes in NTS neuronal behavior to an augmentation of at least one respiratory motor output, suggest the possibility that one mechanism by which extended ETS exposure may worsen respiratory symptoms in children is by an upregulation of the lung C-fiber CNS reflex at synapses in the NTS. The question is what is the underlying mechanism(s). We propose substance P as one possibility, based on several lines of evidence. First, substance P is synthesized in the cell bodies of the vagal afferent C fibers located in the nodose and jugular ganglia, making the neuropeptide available for neuronal release (26–29). Most studies have focused on the local release of substance P in the axon reflex, documenting that neuropeptide can be transported peripherally (26) and released locally in the airways (30) where it has been implicated in causing allergen-induced asthma (30,31). What may have been under-appreciated is the considerable morphologic and physiologic evidence suggesting that substance P may also be released at central synapses in the NTS. Second, this NTS region contains a high density of substance P-containing nerve terminals (26,27,32–37), some of which have been shown to emanate from vagal afferent C fibers (10,28) as well as from axons and somata throughout the CNS (38). Third, there is a parallel distribution of substance P (neurokinin-1 (NK1) receptors with respect to the nerve terminals, providing targets for substance P release in the nucleus (29,39–41). Fourth, there is a striking parallel between neural changes that can occur with extended ETS exposure and those that occur during extended neuropathic or inflammatory pain. Prolonged peripheral inflammation increases the sensitivity of pain sensory fiber endings to noxious stimuli (42) (analogous to extended ETS exposure increasing the sensitivity of the lung afferent C fibers) and also increases the synaptic excitation of spinal cord neurons (analogous to increases in synaptic excitation of NTS neurons in the lung C-fiber reflex pathway). The augmentation of synaptic excitation in the spinal cord has been characterized by Woolf and colleagues as an increase in the intrinsic postsynaptic excitability of spinal neurons (42,43) that may be triggered in part from an induced novel (substance P) input from peripheral Aβ afferent fibers and an increased substance P input from the peripheral nociceptive C fibers.
The grouped data from 12 guinea pigs are shown in Figure 3B. Modest increases in TP (upper panel) and TE (lower panel) evoked by left atrial capsaicin injections (LA CAP) were statistically significantly augmented following discrete injections of substance P in the caudomedial NTS (SP + LA CAP). The augmentative effects of substance P were abolished by prior injection of the NK1 receptor antagonist, but not by the inactive enantiomer. The reflex-evoked decreases in ABP and HR were also statistically significantly augmented (data not shown).

The findings suggested that activation of substance P receptors in the NTS can have a physiologically relevant effect by augmenting the lung C-fiber reflex output; however, the data did not address the question of whether substance P can directly increase the baseline activity of lung C-fiber neurons (action potential discharge rate) or their response to synaptic activation by lung sensory input.

Does Substance P Enhance Synaptic Transmission at NTS Synapses?

These data were obtained from extracellular recordings of action-potential responses obtained from NTS neurons in the whole animal and from whole-cell patch-clamp recordings obtained from NTS neurons in a coronal brainstem slice preparation. The slice contained the caudomedial NTS, the same region studied in vivo, and the peripheral (including vagal) afferent fibers in the TS. To determine whether exogenous substance P can enhance the transmission of input from peripheral vagal afferent C fibers, we compared the action-potential responses of NTS neurons evoked by electrical stimulation of the vagal afferent C fibers before and during iontophoretic application of substance P in vivo. The findings are illustrated in the example in Figure 4A. Under control conditions, 30 sequential stimuli applied to the ipsilateral vagus nerve at intensities to activate C fibers evoked 16 action potentials for a 53% action-potential response rate. During the iontophoresis of substance P in amounts that did not change the baseline activity, the same stimulation protocol evoked 30 responses for a 100% response rate. The grouped data are shown in the right panel. Seven of 8 NTS neurons tested exhibited a statistically significant increased response rate (number of action potentials evoked divided by the number of stimuli delivered × 100) to vagal afferent C-fiber stimulation.

The in vitro studies performed in the NTS slice (Figure 4B) confirmed the in vivo findings. As shown in the example (left
Bonham et al.

**Figure 3.** (A) Model illustrating the hypothesis that substance P injections in the NTS would increase neuronal responsiveness to lung C-fiber input and result in an augmented reflex output. (B) Substance P (SP) injections in the NTS augmented the C-fiber evoked increase in TP and T_e (n = 12). (B, upper panel) Left atrial capsaicin (LA CAP, 0.5 µg/kg) evoked small increases in TP that were not statistically significant. After bilateral NTS injections of SP, the LA-CAP-evoked increase in TP was increased (LA CAP vs SP + LA CAP; p < 0.02, Fisher’s test). The augmented effects of SP were prevented by prior injection of the NK1 receptor antagonist, CP-96345 (SP + LA CAP vs CP-96345 + SP + LA CAP; p > 0.49, Fisher’s test). (B, lower panel) LA CAP produced a slight increase in T_e that was significantly augmented by bilateral NTS injections of SP (LA CAP vs SP + LA CAP; p < 0.0001, Fisher’s test). The augmented effects of SP were prevented by prior injection of CP-96345 (SP + LA CAP vs CP-96345 + SP + LA CAP; p < 0.0001, Fisher’s test) but not of the inactive enantiomer, CP-96344 (SP + LA CAP vs CP-96344 + SP + LA CAP; p > 0.49, Fisher’s test). The grouped data are expressed as means ± SE. Modified from Mutoh et al. (45).

**Figure 4.** (A) Substance P applied at NTS synapses enhanced the postsynaptic responses to vagal afferent C-fiber input in vivo. (A, left panel) Peristimulus time histogram shows the number of action potentials evoked in NTS neurons by 30 sequential electrical stimuli applied to the vagus nerve under control conditions and during the iontophoretic application of substance P onto the neuron. (A, right panel) The grouped data confirmed that application of substance P in the NTS augmented synaptic transmission between the vagal afferent C fibers and NTS neurons (p = 0.02, paired t-test). (B) Stimulation of sensory afferent fibers in the TS also evoked action potential responses in NTS neurons in an in vitro slice preparation. As shown in the example (left panel) and grouped data (right panel), perfusion of the slice with substance P statistically significantly augmented the action potential response rate to TS stimulation, confirming the results obtained in vivo. The grouped data are expressed as means ± SE.

To determine whether substance P has excitatory effects on NTS neurons that receive vagal afferent C-fiber input, we performed extracellular recordings of the baseline spiking activity of NTS neurons before and during the local iontophoretic application of substance P in the in vitro preparations. The in vivo data are shown in Figure 5A. In the example (left panel), substance P modestly increased the baseline action potential firing rate. As shown in the right panel, a statistically significant increase was confirmed in nine of ten neurons tested; the spiking activity increased from a mean resting activity of 11 ± 2 Hz in the preceding 30 sec to a peak 18 ± 3 Hz averaged over 60 sec.

Because all neurons may not display spiking responses to substance P, we further determined whether substance P could depolarize the membrane potential with or without concomitant spiking in the NTS in the brainstem slice in vitro. The in vitro data are shown in Figure 5B. An example of substance P-induced depolarization and spiking in one NTS neuron is shown in the left panel. The grouped data are shown in the middle panel; in 11 or 14 neurons, perfusion of the NTS with substance P (0.5–10 µM) modestly but statistically significantly depolarized the resting membrane potential from a holding potential of ~60 mV. In a separate group of 14 neurons, we counted the number of action potentials evoked by substance P. The grouped data are shown in the right panel. In 7 of the 14 neurons, substance P modestly but statistically significantly increased the action-potential firing rate (p = 0.04). The data suggest that substance P has the capacity to modestly excite some but not all postsynaptic neurons in the NTS; thus, substance P acting in the NTS can facilitate synaptic transmission between vagal afferent C fibers and postsynaptic NTS neurons by augmenting the postsynaptic neuronal responsiveness to the sensory input.
In summary, substance P can act in the NTS to augment synaptic transmission between sensory afferent fibers and NTS neurons and can also increase the baseline excitability of NTS neurons. Either or both effects could contribute to the ability of substance P acting in the NTS to augment lung C-fiber reflex output.

Conclusions and Speculation

The increased responsiveness of NTS neurons in the lung C-fiber reflex pathway and prolonged inspiratory times suggest that extended ETS exposure can change respiratory function via the CNS. The results may provide a mechanism to explain some of the respiratory symptoms in children exposed to ETS. If extended exposure to ETS increases some aspect of the substance P system—either production or release in the lung C-fiber CNS reflex pathway, the neuropopetic may act in the NTS to augment lung C-fiber reflex output to increase the apnea and also the bronchoconstriction, mucous secretion, and microvascular leak—responses that resemble asthmatic symptoms associated with ETS exposure in children. Future studies will be required to determine if ETS increases the expression of substance P and if such increases affect the lung C-fiber CNS reflex. Understanding the mechanisms will underscore efforts to discourage exposing children to ETS and direct attention to new therapeutic strategies.

REFERENCES AND NOTES

1. Dodge R. The effects of indoor pollution on Arizona children. Arch Environ Health 37:151–155 (1982).
2. Eksow RR, Weinberger MM, Lachenbruch PA, Huntley WH. Relationship of parental smoking and gas cooking to respiratory disease in children. Chest 84:682–689 (1983).
3. Wang X, Wyjo D, Gold DR, Speizer FE, Ware JH, Ferris BF Jr, Dockery DW. A longitudinal study of the effects of parental smoking on pulmonary function in children 6-18 years. Am J Respir Crit Care Med 149:1420–1425 (1994).
4. Frischer T, Kuehl J, Meinen R, Karmans W, Barth R, Hermann-Kutz E, Ubbekane R. Maternal smoking in early childhood: a risk factor for bronchial responsiveness to exercise in preschool children. J Pediatr 121:71–72 (1992).
5. Strachan DP, Cook DG. Health effects of passive smoking. 1: Parental smoking and lower respiratory illness in infancy and early childhood. Thorax 52:907–914 (1997).
6. Weitzman M, Gortmaker S, Walker DK, Sobol A. Maternal smoking and childhood asthma. Pediatrics 85:505–511 (1990).
7. Klonoff-Coher HS, Edelstein SL, Lefkowitz ES, Srinivasan IP, Kunz E, Urbanek R. Maternal smoking in early childhood: a risk factor for bronchial reactivity. Am J Respir Crit Care Med 155:1727–1731 (1997).
8. Gold DR. Environmental tobacco smoke, indoor allergens, and childhood asthma (Review). Environ Health Perspect 108(suppl 1):643–651 (2000).
9. Joad J. P. Smoking and respiratory pediatric health. Clin Med 21:37–46, vi–vii (2000).
10. Sarnia A, Mantling CR, Yan ZQ, Theodorsson-Norheim E, Gamse 8. Gold DR. Environmental tobacco smoke, indoor allergens, and childhood asthma (Review). Environ Health Perspect 108(suppl 1):643–651 (2000).
39. Davis BJ, Smith H. Neurokinin-1 receptor immunoreactivity in the nucleus of the solitary tract in the hamster. Neuroreport 10:1003–1006 (1999).

40. Mazzone SB, Hinrichsen CF, Geraghty DP. Substance P receptors in brain stem respiratory centers of the rat: regulation of NK1 receptors by hypoxia. J Pharmacol Exp Ther 282:1547–1556 (1997).

41. Baude A, Shigemoto R. Cellular and subcellular distribution of substance P receptor immunoreactivity in the dorsal vagal complex of the rat and cat: a light and electron microscope study. J Comp Neurol 402:181–196 (2000).

42. Neumann S, Doubell TP, Leslie T, Woolf CJ. Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. Nature 384:360–364 (1996).

43. Woolf CJ, Doubell TP. The pathophysiology of chronic pain—increased sensitivity to low threshold Aβ-fibre inputs. Curr Opin Neurobiol 4:525–534 (1994).

44. Undem BJ, Hunter DD, Liu M, Haak-Frendscho M, Oakragly A, Fischer A. Allergen-induced sensory neuroplasticity in airways. Internl Arch Allergy Immunol 118:130–153 (1999).

45. Mutoh T, Bonham AC, Joad JP. Substance P in the nucleus tractus solitarius (NTS) augments bronchopulmonary C-fiber reflex output. Am J Physiol (Reg) 279:R1215–R1223 (2000).