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Central and Peripheral Effects of the Non-Neural Substances on Respiration before and after Vagotomy

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ŞAHIN, G., ORUÇ, T., ŞIMŞEK, G. and GÜNER, I. *Central and Peripheral Effects of the Non-Neural Substances on Respiration before and after Vagotomy.* Tohoku J. Exp. Med., 1997, **182** (4), 297-307 —— The central effects of capsaicin, veratrine, histamine and bradykinin were studied by injecting them directly into the cerebrospinal fluid and their peripheral effects were examined by injecting into femoral vein. Our experiments were performed in Na-pentobarbital-anaesthetized dogs. Tidal volume (V_T), respiratory frequency (f/min), systemic arterial pressure (BP) were recorded. A significant increase in f, and an initial apnea or hypoventilation followed by a significant increase in V_T were observed with central and peripheral capsaicin. Vagotomy removed the peripheral V_T response, but not the central one. While central capsaicin administration increased BP, peripheral administration decreased. After vagotomy, a significant increase was observed in BP for both administrations. Respiratory responses to central and peripheral administrations of veratrine were similar to those of capsaicin. Significant increases were observed in f and V_T of the intact group in response to central and peripheral administration of histamine. Response to peripheral administration disappeared after vagotomy. While central and peripheral bradykinin increased V_T significantly, there was no significant change in f. Vagotomy only removed the increase in V_T in response to peripheral administration. In conclusion, respiratory responses to central administration of capsaicin and veratrine are due to direct effects of these substances on respiratory neurons. In peripheral administration, disappearance of the responses after vagotomy indicate that the responses are brought about by stimulation of the lung receptors. ———— capsaicin; veratrine; lung receptors; central respiratory pattern

Capsaicin is known to produce a pulmonary chemoreflex by stimulating pulmonary C-fibers when it is administrated intravenously (Coleridge and Coleridge 1986; Şahin et al. 1987). Veratrine stimulates slowly adaptating lung stretch receptors (SAR) by increasing Na^+ permeability (Coleridge and Coleridge 1986; Şahin et al. 1987). On the other hand, histamine and bradykinine, which are released from bronchial walls of the lungs, initiate the defense reflexes by stimulating unmyelinated C-fibers (Barnes 1986; Coleridge and Coleridge 1986).

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During stimulation of the unmyelinated C-fibers by various substances, sensorial neuropeptides released from these fibers cause neurogenic inflammation by an axonal reflex (Barnes 1986; Widdicombe 1993). These released neuropeptides are claimed to lead facilitation in cholinergic nerves, and therefore to an increase in the cholinergic tone (Widdicombe 1993).

On the other hand, neuron groups responsible for generation of the respiratory rhythm, contain great amount of neurotransmitters and neuroactive substances (Denavit-Saubie et al. 1985). Whether peripherally or centrally, change of concentration of neurotransmitters or neuroactive substances is expected to affect respiration.

The aim of this study was to determine the respiratory responses to specific stimulants of pulmonary C-fibers (capsaicin) and slowly adapting pulmonary stretch receptors (SARs) (veratrine), and further, to investigate the central and peripheral effects of inflammatory mediators (histamin and bradykinin) on respiratory response to these mediators.

Materials and Methods

Our experiments were performed in Na-pentobarbital-anaesthetized (30 mg kg^{-1}, iv) dogs weighed as a mean $\pm$ S.D. of 19 ± 4.3 kg. Respiratory parameters (Tidal volume, V_T; respiratory frequency, f/min) and systemic arterial blood pressure (BP) were recorded by a polygraph, through a tracheal cannula and the right femoral artery, respectively. Respiratory minute volume ($\dot{V}_E$) was calculated. Central effects of the substances were examined by injecting them directly into the cerebrospinal fluid via a catheter settled in cysterna magna by atlantooccipital punction. In addition, peripheral effects of these substances were examined by injecting them into the femoral vein.

Peripheral doses of the substances we used in the study were selected as specific doses for lung receptors per kg of body weight (Şahin et al. 1987). For central doses, constant quantities on an average of 1/20 of the peripheral doses were used. The central and peripheral doses of the substances were as follows: central; capsaicin 25 μg, veratrine 20 μg, histamine 10 μg, bradykinin 2 μg: periheral; capsaicin 30 μg/kg, veratrine 25 μg/kg, histamine 15 μg/kg, bradykinin 2.5 μg/kg.

For statistical analysis "t-test" in small paired series was used.

Results

Effects of capsaicin. Both central and peripheral administrations of capsaicin have increased f significantly in the control group ($p < 0.05$). In V_T a biphasic response, an initial decrease which was followed by a significant increase was observed ($p < 0.001$) (Table 1, Figs. 1 and 2). $\dot{V}_E$ was found to increase significantly.

After bilateral cervical vagotomy central capsaicin administration produced

TABLE 1. *Mean and standard error (mean ± S.E.) values of f, V_T, $\dot{V}_E$ and BP with central and peripheral capsaicin administrations*

Administration	Experimental Group		f/min	V_T (ml)	$\dot{V}_E$ (liter)	BP (mmHg)
Central	Control ($n=5$)	A	19.0±1.1	169.9±17.2	3.21±0.36	131.5±12.1
		B	22.2±3.7*	55.0±23.8***	1.01±0.47**	185.9±8.1*
				234.5±17.9***	5.76±0.61**	
	Vagotomy ($n=5$)	A	11.6±1.5	286.4±21.2	3.21±0.21	156.4±6.5
		B	12.4±1.7	62.0±26.2***	6.12±0.27**	205.6±12.8*
				326.6±26.7**	3.90±0.28**	
Peripheral	Control ($n=5$)	A	17.4±0.8	162.8±9.5	2.85±0.29	145.9±13.9
		B	21.2±1.1*	64.8±14.5***	1.74±0.33***	88.6±10.5**
				216.2±9.3***	4.61±0.34**	
	Vagotomy ($n=5$)	A	11.0±1.9	346.5±57.3	3.43±0.25	121.5±16.1
		B	10.6±1.8	342.7±59.9	3.33±0.38	179.2±15.3*

*$p<0.05$, **$p<0.01$, ***$p<0.001$
A, Before administration; B, After administration.

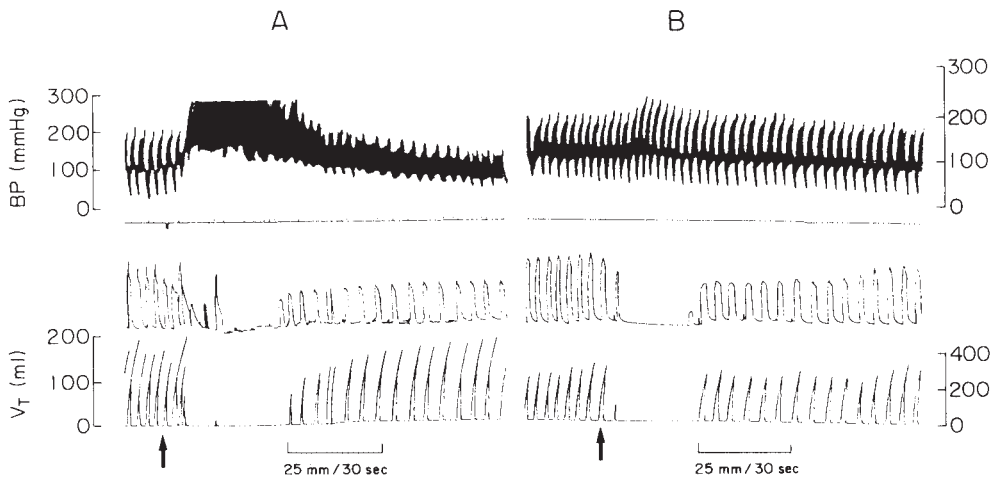

Fig. 1. Responses to central administrations of capsaicin (25 μg) in anaesthetized dog. Traces from top to bottom are systemic arterial blood pressure (BP), tidal airflow, tidal volume (V_T), respectively.
During injections are at the arrows.
A : Control, B : After bilateral cervical vagotomy.

no change in f. Changes in V_T, on the other hand, were found to be same as that before vagotomy. An initial decrease was followed by an increase. The responses in f and V_T to peripheral administration of capsaicin in the control group disappeared after vagotomy. No significant change was observed in $\dot{V}_E$ (Table 1, Figs. 1 and 2).

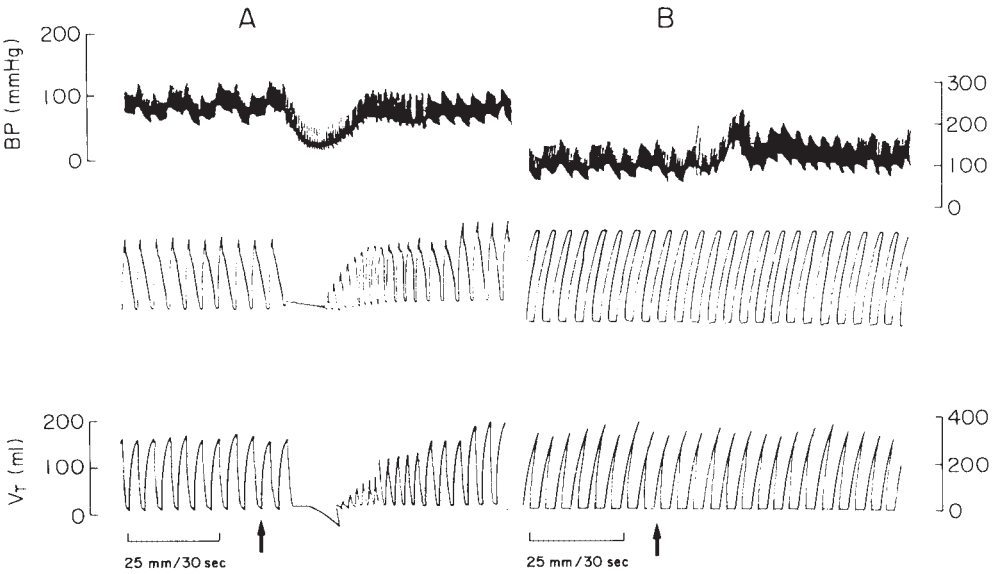

Fig. 2. Responses to peripheral administrations of capsaicin (30 μg/kg) in anaesthetized dog. Traces from top to bottom are systemic arterial blood pressure (BP), tidal airflow, tidal volume (V_T), respectively.
During injections are at the arrows.
A: Control, B: After bilateral cervical vagotomy.

TABLE 2. *Mean and standard error (mean ± S.E.) values of f, V_T, $\dot{V}_E$ and BP with central and peripheral veratrine administrations*

Administration	Experimental Group		f/min	V_T (ml)	V_E (liter)	BP (mmHg)
Central	Control ($n=5$)	A	19.2±0.8	136.7±6.2	2.63±0.14	108.3±4.6
		B	21.9±1.5*	—	—	138.3±15.8*
				205.4±17.1**	4.43±0.66**	
	Vagotomy ($n=5$)	A	13.2±2.6	282.2±34.5	3.63±0.88	138.8±7.2
		B	12.8±2.3	133.7±44.9*	2.69±1.01*	168.3±4.5**
				383.8±56.9*	4.51±0.64*	
Peripheral	Control ($n=5$)	A	16.6±0.6	174.0±17.5	2.88±0.28	131.7±8.5
		B	22.0±1.7*	33.2±18.2**	0.89±038**	94.8±9.9**
				220.0±28.5*	4.75±0.59*	
	Vagotomy ($n=6$)	A	11.8±1.9	313.0±48.0	3.49±0.64	122.2±6.2
		B	12.0±2.2	331.9±49.2	3.69±0.61	149.1±8.2**

*$p<0.05$, **$p<0.01$

A, Before administration; B, After administration.

TABLE 3. *Mean and standard error (mean ± S.E.) values of f, V_T, $\dot{V}_E$ and BP with central and peripheral histamine administrations*

Administration	Experimental Group		f/min	V_T (ml)	$\dot{V}_E$ (liter)	BP (mmHg)
Central	Control ($n=5$)	A	17.8±0.5	173.3±6.4	3.07±0.07	129.5±6.2
		B	18.6±0.7*	204.6±10.3*	3.82±0.24*	168.5±11.3**
	Vagotomy ($n=5$)	A	14.8±2.1	250.2±24.0	3.66±0.60	134.0±11.3
		B	16.0±2.2*	299.3±30.8**	4.68±0.73**	168.4±20.3*
Peripheral	Control ($n=5$)	A	19.8±1.7	171.6±8.5	3.36±0.22	154.0±8.3
		B	21.8±2.6*	202.9±6.4**	4.42±0.53*	101.6±10.4*
	Vagotomy ($n=5$)	A	10.4±1.9	290.2±42.7	2.79±0.31	122.1±13.1
		B	10.8±1.8	290.8±46.2	2.94±0.41	88.1±10.1**

*$p<0.05$, **$p<0.01$
A, Before administration; B, After administration.

TABLE 4. *Mean and standard error (mean ± S.E.) vblues of f, V_T, $\dot{V}_E$ and BP with central and peripheral bradykinin administrations*

Administration	Experimental Group		f/min	V_T (ml)	$\dot{V}_E$ (liter)	BP (mmHg)
Central	Control ($n=5$)	A	20.2±2.6	151.4±13.3	3.03±0.40	106.0±7.9
		B	21.2±2.6	207.1±12.5*	4.27±0.31*	134.0±15.6*
	Vagotomy ($n=5$)	A	10.4±1.2	259.8±26.0	2.71±0.45	109.1±6.1
		B	10.4±1.3	335.2±23.8*	3.52±0.61*	143.2±9.0***
Peripheral	Control ($n=5$)	A	15.6±0.6	181.9±4.1	2.84±0.12	153.2±8.6
		B	16.4±0.8	202.1±6.1***	3.31±0.18**	108.2±4.9**
	Vagotomy ($n=5$)	A	10.6±1.3	297.0±21.3	3.16±0.50	126.5±10.1
		B	10.6±11.2	297.1±14.2	3.16±0.44	98.7±8.6*

*$p<0.05$, **$p<0.01$, ***$p<0.001$
A, Before administration; B, After administration.

Central administration of capsaicin increased and peripheral administration decreased BP significantly ($p<0.05$). After bilateral vagotomy both central and peripheral administrations of capsaicin increased BP significantly ($p<0.05$) (Table 1).

Effects of veratrine. Both central and peripheral administrations of veratrine significantly increased f in the control group ($p<0.05$) and biphasic responses, in which a significant decrease followed by a significant increase ($p<0.01$), were observed in V_T (Table 2).

Bilateral cervical vagotomy removed the increase observed with central administration in f and did not affect the V_T response in the control group. For peripheral administration, the responses in f and V_T in the control group were removed after bilateral cervical vagotomy.

Central and peripheral administrations of veratrine produced different effects on blood pressure (Table 2). While central veratrine significantly increased BP ($p<0.05$), peripheral administration significantly decreased it ($p<0.01$). The decrease in BP in response to peripheral administration of veratrine disappear after bilateral vagotomy. The increase in BP in response to central administration of veratrine was not affected by vagotomy (Table 2).

Effects of histamine. Both central and peripheral administrations of histamine produced significant increases in f in the control group ($p<0.05$). The response in f to central administration and to peripheral administration of histamine disappeared after vagotomy (Table 3). In the control group, the increase observed in V_T with central administration was not affected by vagotomy, the increase observed with peripheral administration disappeared after vagotomy.

In the control group, central histamine administration increased BP significantly ($p<0.01$), but peripheral administration caused a significant decrease in BP ($p<0.05$). Bilateral cervical vagotomy did not affect the responses in BP to central and peripheral histamine administrations (Table 3).

Effects of bradykinin. Central and peripheral administrations of bradykinin produced no significant change in f. V_T on the other hand significantly increased with both administrations (Table 4). Bilateral cervical vagotomy produced no change on the V_T response to central administration of bradykinin. The response observed to peripheral administration, on the other hand, disappeared after vagotomy (Table 4).

Central administration of bradykinin in the control group significantly increased BP ($p<0.05$), while peripheral administration significantly increased it ($p<0.01$). Bilateral cervical vagotomy changed neither the central, nor the peripheral responses (Table 4).

Discussion

Capsaicin is known to stimulate pulmonary C-fibers when it is administrated intravenously at doses of 25-50 μg (Coleridge and Coleridge 1986; Şahin et al.

1987). Action potential recordings from vagal afferents showed capsaicin has no effect on pulmonary stretch receptors (Coleridge and Coleridge 1986). In our findings, disappearance of the response in f and V_T to intravenous injection of capsaicin after vagotomy shows the response is mediated by vagal reflexes and is due to stimulation of the pulmonary C fibers. We observed an increase in f and a decrease in V_T which was followed by an increase, in response to intravenous capsaicin injection. Stimulation of C fibers with capsaicin is known to cause first apnea and then rapid shallow breathing (Coleridge and Coleridge 1986; Widdicombe 1993). On the other hand, Coast and Cassidy (1985) have observed apnea without tachypnea in response to capsaicin. They concluded that the absence of tachypnea was due to the difference in anesthesia. In our findings the increase in V_T following the initial decrease seems to be the result of pulmonary CO_2 reflex, rather than direct effect of capsaicin. It is known that there is an adverse relation between Carbon dioxide concentration in pulmonary artery ($PACO_2$) and SAR activity. When intrapulmonary CO_2 quantity increases, SAR activity decreases and hence, inhibitory effect on inspiratory neurons in the brain stem is delayed and V_T increases (Bradley et al. 1976).

On the other hand, we observed the same responses in V_T and f in the control group to central administration of capsaicin. The increases in frequency and V_T was not affected by vagotomy. Capsaicin, bradykinin, histamine, nicotinic agonists and electrical stimulation lead to release of neuropeptides such as substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) from the sensory fibers in the lungs and the heart (Barnes et al. 1991a). Capsaicin is known to produce acute sensory nerve depolarization by acting on non-selective cation channels (Peter 1991).

Our findings in central administration of capsaicin suggest that capsaicin by acting on afferent axone terminals in respiratory centers causes the release of neuropeptides and neurotransmitters which produce depression in respiratory neurones responsible for tidal volume. On the other hand, neuropeptides and neurotransmitters released from afferent axon terminals in the respiratory centers may be expected to increase cholinergic discharge to airways. In that condition the decrease in tidal volume would be due to increase airway resistance. However, our findings show that respiratory response to central administration of capsaicin persists by vagotomy. Therefore the decrease in V_T cannot be atributed to increase in cholinergic discharge and consequent increase in airway resistance.

Intravenous injection of capsaicin is known to initate pulmonary chemoreflex by stimulating pulmonar C-fibers (Şahin et al. 1987). As a result BP decreases (Şahin et al. 1987). In fact the decrease in BP we observed in peripheral administration of capsaicin disappeared after vagotomy. On the other hand, the increase in blood pressure in response to central administration of capsaicin was not abolished by vagotomy. This finding suggests that centrally administered capsaicin increases pressor activity or inhibits depressor neuron activity by

stimulating axone terminals in the vasomotor center.

We observed the similar respiratory responses with central and peripheral administration of veratrine, to those of capsaicin. Vagal afferent endings carrying the impulses from stimulated SARs to the brain stem, caused a decrease followed by an increase in V_T. After vagotomy, disappearance of the responses in f and V_T shows the response is mediated by vagal impulses generated in lung receptors. The biphasic responses we observed in V_T to central administration of veratrine seem to be due to stimulation of axone terminals in respiratory centers. As is well known veratrine stimulates axone terminals by increasing Na^+ permeability (Coleridge and Coleridge 1986). In fact the responses obtained by central administration of veratrine were not affected by vagotomy. Peripheral administration of veratrine caused a significant decrease in blood pressure due to stimulation SARs. This response was abolished by vagotomy. The increase in blood pressure in response to central administration of veratrine seems to be brought about by the same mechanism as that of capsaicin.

Histamine in low doses causes bronchoconstriction by acting through H_1 receptors in airway smooth muscle (Coleridge and Coleridge 1986). High doses of histamine produces bronchoconstriction by cholinergic reflex activation (Widdicombe 1993). This response is reduced by atropine or vagotomy (Widdicombe 1993). In addition, it modulates neuropeptide release from sensory nerves via H_3 receptors in the airways. It may also increase acetylcholine release from the postganglionic nerves in the airways (Barnes 1986).

Histamine is known to stimulate rapidly adaptating lung stretch receptors (RAR) secondary to its bronchoconstrictor effect (Barnes 1986; Coleridge and Coleridge 1986). The impulses from RARs are known to increase the threshold of inspiratory off-switch (IOS) mechanism. Thus, central inspiratory activity (CIA) and V_T are increased (Coleridge and Coleridge 1986). In fact the increase we observed in f and V_T in response to peripheral administration of histamine was abolished after vagotomy. This finding confirms that peripherally administered histamine increases V_T by its action on RAR and that the increase in f occures by vagal reflexes secondary to increase in V_T. On the other hand, histamine has various neuromodulatory effects on the central nervous system. It provides neurotransmission by H_3 receptors (Barnes et al. 1991b). Our results show that respiratory response to central administration of histamine is not affected by vagotomy. This finding indicates the direct action of histamine on respiratory neurones. Our finding of no change in blood pressure response to peripheral administration of histamine after vagotomy suggests the direct action of histamine on vascular smooth muscle via histamine receptors.

Bradykinin, the classical mediator of inflammatory pain, is a selective stimulator for bronchial C-fibers (Barnes et al. 1991b). It is known to produce bronchoconstriction by cholinergic reflex activation and by stimulating the afferent nerve endings (Barnes et al. 1991b). Further more bradykinin causes the release

of prostaglandines (Barnes et al. 1991b). The increase we observed in V_T with peripheral bradykinin administration is probably brought about by stimulation of RARs secondary to bronchoconstrictor effect of bradykinin. If the respiratory response we obtained were due to stimulation of bronchial C-fibers, we would have observed a decrease in V_T, instead of an increase. Our finding of increase in V_T with central administration of bradykinin may be explained by direct action of bradykinin on the respiratory neurons responsible for generation tidal volume. Our results show that blood pressure responses to both administrations of bradykinin were not affected by vagotomy. The changes in blood pressure may be receptor mediated or due to neuropeptide released from nerve terminals by the effect of bradykinin.

Responses to central administration of all substances could be considered to occur by passage of these substances into the peripheral circulation. However, immediate appearance of the responses after central administration, and most importantly, persistence of central responses after vagotomy nullifys this possibility.

It is concluded that respiratory responses to peripheral administration of capsaicin, veratrine, histamine and bradykinin are vagaly mediated by impulses generated in lung receptors. The central effects of these substances seem to be due to direct action of these substances on respiratory centers. It is possible that these substances act on axon terminals in respiratory centers and produce their effect by neuropeptides released.

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