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DIVERGING RESPIRATORY EFFECTS OF SEROTONIN AND NICOTINE IN VAGOTOMISED CATS PRIOR TO AND AFTER SECTION OF CAROTID SINUS NERVES

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Respiratory effects of intravenous serotonin and nicotine were investigated prior to and after bilateral neurotomy of the carotid sinus nerves (CSNs) in eight pentobarbitone/chloralose-anaesthetised, bilaterally vagotomised and superior laryngeal nerves-sectioned cats. Injection of 188 nmol·kg⁻¹ serotonin (hydrogen oxalate salt, 50 µg·kg⁻¹) prior to and after CSNs section induced an expiratory apnoea of, respectively, 7.9±1.25 s and 8.3±1.6 s duration (mean±S.E.M.) in, respectively, five and three of those cats. In all cats, the serotonin challenge produced a period of accelerated breathing ($P < 0.05$) both prior to and after section of CSNs. Injection of a 433 nmol nicotine bolus (hydrogen tartrate salt, 200 µg) increased tidal volume by 25±8% in cats with intact CSNs ($P < 0.01$), but decreased it by 13±10% ($P < 0.05$) after CSNs section. Nicotine, but not serotonin, transiently increased mean arterial blood pressure in our cats, which rise was delayed by CSNs cut. Results of this study indicate that the respiratory response to serotonin occurs beyond carotid body chemoreceptors in vagotomised cats, and suggest that the volume response to intravenous nicotine depends qualitatively on carotid body chemoreceptor input in this experimental model.

Key words: *pulmonary chemoreflex, apnoea, vagotomy, serotonin, nicotine, cat.*

INTRODUCTION

In neurally intact, spontaneously breathing cats, intravenous (i.v.) nicotine and serotonin (5-hydroxytryptamine, 5HT) provoke pulmonary respiratory chemoreflex. Its classical pattern includes apnoea followed by rapid shallow breathing, bradycardia and hypotension, which all are normally abolished by midcervical vagotomy (1). Respiratory sequelae may stray somewhat from this pattern in that the response to nicotine, after variably occurring apnoea, shows hyperpnoea independent of the vagal input (2, 3), whereas the occurrence of 5HT-induced apnoea is only reduced by the removal of the vagal feedback (4-6).

In midcervically vagotomised cats, which are devoid of both pulmonary and aortic body afferentation (7), 5HT and nicotine may elicit ventilatory effects due to excitation of carotid body chemoreceptors. Albeit the presence of a neurotransmitter in a tissue does not imply that such an agent serves as a transmitter at this particular location, 5HT has been immunocytochemically identified in the chemoreceptor cells of the cat carotid body (8). Notably, intracarotid injections of serotonin produce a transient increase followed by a decrease in carotid sinus nerves (CSNs) discharge (9) mediated *via* MDL 72222-sensitive receptors in the cat (10). However, it is not clear whether carotid body chemoreceptors contribute to the pulmonary respiratory chemoreflex induced by *i.v.* 5HT. To the best of our knowledge there is no study quantifying the respiratory response to 5HT in vagotomised cats prior to and after section of the CSNs.

Cat carotid body possesses a predominance of nicotinic receptors (11), and nicotine has been extensively used to explore its function. In the cat, intralingual arterial nicotine injections increase the frequency of discharges in the ipsilateral carotid sinus nerve and produce increases in the tidal volume and breathing frequency; the two latter effects are precluded by CSNs section (12). On the other hand, there is evidence showing that the respiratory reflex response to low intravascular nicotine doses is abolished in peripherally chemodenervated animals only after vagotomy (2, 13).

The question arises as whether the input from carotid bodies modifies the respiratory effects of serotonin-induced pulmonary chemoreflex. In this study we have examined this possibility by comparing the ventilatory effect of serotonin with that of nicotine with the aim of finding out other reflex sources of the respiratory events. A comparison was made of the responses to both drugs in vagotomised cats with those elicited following carotid body deafferentation, by: (i) quantifying the chemoexcitatory effects of 5HT upon ventilation, (ii) testing the possibility that CSNs neurotomy will not affect the respiratory effects of 5HT administered to the pulmonary circulation, but will preclude hyperpnoeic response to nicotine following vagotomy, and (iii) reassessing the role of carotid body chemoreceptors in the respiratory effects of nicotine. To exclude a putative inhibitory influence from the upper airways on ventilatory activity (14), we supplemented the well-established model of bilateral vagotomy with bilateral superior laryngeal nerves (SLNs) section.

MATERIALS AND METHODS

A total of eight tomcats weighing 2.8–4.5 kg were anaesthetized with sodium pentobarbitone (30 mg · kg⁻¹, intraperitoneally) and α -chloralose (16 mg · kg⁻¹, *i.v.*). End tidal CO₂ was measured continuously with an Engstrom Eliza Plus capnograph (Gambro) to monitor anaesthesia status, and maintained at 4%. Body temperature was kept at 38°C with a heating pad. Animals were

placed supine, breathing spontaneously through the tracheal cannula. Tidal volume (V_T) was obtained by integrating the flow signal from a model CS6 pneumotachograph (Mercury) attached to the cannula. Arterial blood pressure was measured with a model CK-01 transducer (Mera-Tronik, Poland) and a model 4011S-MCK blood pressure monitor (Mera-Tronik, Poland), and mean arterial blood pressure (MAP) was calculated. The carotid region on both sides was dissected under an operating microscope. The larynx and the oesophagus were reflected to expose both carotid artery bifurcations. The midcervical portions of the vagal and superior laryngeal nerves were isolated and sectioned just prior to the beginning of measurements. CSNs were cut bilaterally at their junctions with the glossopharyngeal nerves later during the experiment. The C_4 - C_5 root of the right phrenic nerve was cleared, cut and desheathed for recording. Phrenic nerve action potentials were amplified with a NL 104 amplifier (Digitimer), and filtered and measured with a model AS 101 (Asbit) leaky integrator (time constant = 100 ms). All recordings were registered on an Omnilight 8M36 apparatus (Honeywell). After control values of respiratory variables were taken, 5HT (hydrogen oxalate salt, Fluka, 188 nmol·kg⁻¹, or 50 µg·kg⁻¹) or nicotine (hydrogen tartrate salt, Sigma, 433 nmol, or 200 µg) boluses were delivered in 0.4 ml aliquots of physiological saline and flushed with 0.4 ml of the saline via the catheter placed in the right femoral vein. The drug doses used in these experiments were selected based on our preliminary dose-response study (not shown), which revealed that this dose of serotonin resulted in most apparent apnoea, and previous reports showing respiratory response to similar doses of nicotine (2, 15). A minimum wash-out period of 10 min was allowed between the nicotine and 5HT challenges. The experimental protocol has been approved by the local animal care committee.

Test i.v. injections of 0.4 ml aliquots of physiological saline showed no volume effect(s). The ventilatory parameters studied were assessed just prior to nicotine or 5HT injection (control values), during the early post-challenge phase (0–5 s after the saline flush or immediately after the first post-apnoeic breath, if apnoea occurred), and at 30 and 60 s after the challenges. Each individual value of V_T , minute ventilation (V_E), inspiratory time (T_I), expiratory time (T_E) and respiratory rate (f) was taken as an average over five consecutive breaths. T_I and T_E were determined, respectively, from the start and the peak of the phrenic neurogram. T_E prolongation was measured as the ratio of the maximum T_E during post-5 HT apnoea or expiration to the respective control T_E value. The duration of apnoeic period in phrenic activity was measured as the time of apnoea (respiratory inhibition). V_T , V_E and f data were analysed by two-way ANOVA with time (pre-challenge, the early post-challenge phase, and 30 s and 60 s post-challenge) and denervation status (vagotomy+SLNs cut, and vagotomy+SLNs cut+CSNs cut) as repeated measures' factors. T_E prolongation data were analysed by one-way ANOVA with denervation status as repeated measures factor. Differences between individual time points and experimental situations were evaluated by contrast analysis. In all cases, $P < 0.05$ was considered significant. All results shown are means \pm 1 standard error.

RESULTS

Two-way ANOVA yielded a significant effect of CSNs section ($F_{1,7} = 12.6$, $P = 0.01$), no effect of serotonin ($F_{3,21} = 1.04$, $P = 0.40$), and no interaction between the effects of the two main factors on tidal volume ($F_{3,21} = 1.06$, $P = 0.39$). There were significant effects of serotonin ($F_{3,21} = 4.22$, $P = 0.02$) and CSNs neurotomy ($F_{1,7} = 7.87$, $P = 0.03$), but no significant interaction between the effects of serotonin and CSNs neurotomy on minute ventilation ($F_{3,21} = 2.33$, $P = 0.10$). Two-way ANOVA of respiratory rate data showed a significant effect of serotonin ($F_{3,21} = 8.50$, $P = 0.001$), no effect of CSNs

section ($F_{1,7} = 0.12$, $P = 0.73$) and no interaction between the effects of serotonin and CSNs section ($F_{3,21} = 0.26$, $P = 0.86$). 5HT bolus evoked expiratory apnoea in five out of eight vagotomised cats (mean duration 7.9 ± 1.25 s, $n = 5$). After the CSNs section, the expiratory arrest appeared in only three cats (all of which showed the apnoeic response prior to the neurotomy) and lasted for 8.3 ± 1.6 s. Mean T_E prolongation after 5HT injection was 2.4 ± 0.84 — fold and 1.98 ± 0.7 — fold prior to and after CSNs neurotomy, respectively, indicating similar levels of expiratory inhibition ($P = 0.70$). The carotid deafferentation resulted in significant decreases in tidal volume both during the early phase and at 60 s after 5HT challenge; a similar tendency ($P < 0.06$) was apparent also prior to the serotonin challenge and at 30 s post-challenge. CSNs cut resulted also in serotonin challenge-induced increase in minute ventilation not reaching significance and being significantly reduced compared with that prior to the carotid deafferentation during the early post-serotonin phase. Breathing that followed the 5HT challenge was of increased rate both prior to and after CSNs section (*Fig. 1*). The changes were mainly due to decrease in T_I ($P < 0.001$), with no statistically significant shortening of T_E . Statistical analysis of MAP data (not shown) revealed no effect of 5HT ($F_{3,21} = 0.47$, $P = 0.70$) and CSNs section ($F_{1,7} = 0.34$, $P = 0.60$), and no interaction between the two main factors ($F_{3,21} = 1.3$, $P = 0.30$).

Two-way ANOVA yielded significant effects of nicotine and CSNs neurotomy, and a significant interaction between the effects of the two main factors on V_T ($F_{3,21} = 9.95$, $P = 0.0003$; $F_{1,7} = 17.8$, $P = 0.004$; and $F_{3,21} = 14.9$, $P = 0.00002$, respectively). Effects of these factors on V_E paralleled those on V_T ($F_{3,21} = 4.86$, $P = 0.010$ for nicotine, $F_{1,7} = 5.46$, $P = 0.052$ for CSNs cut, and $F_{3,21} = 10.02$, $P = 0.0003$ for nicotine \times CSNs cut interaction). There was no significant effect of the two main factors and no interaction between nicotine and CSNs section on respiratory rate ($F_{3,21} = 2.88$, $P = 0.060$; $F_{1,7} = 2.50$, $P = 0.15$; and $F_{3,21} = 0.67$, $P = 0.58$, respectively). In contrast to the 5HT challenge, the nicotine bolus did not produce apnoea, but caused a moderate change in V_T , the direction of which was reversed by the CSNs section. Before the neurotomy, V_T rose immediately after the nicotine challenge, returned to control value within 30 s and decreased further at 60 s. After CSNs section, V_T presented significantly lower pre-challenge values, and the nicotine challenge caused an immediate, slight but significant decrease in V_T that persisted during the 1-min post-challenge period. Changes in V_E roughly paralleled those in V_T (*Fig. 2*). Two-way ANOVA of MAP data yielded significant effect of nicotine ($F_{3,21} = 4.26$, $P = 0.02$), no effect of CSNs section ($F_{1,7} = 1.83$, $P = 0.20$), and a significant interaction between the two main factors ($F_{3,21} = 5.30$, $P = 0.007$). CSNs cut resulted in a clear-cut delay in nicotine challenge-induced rise in MAP (*Table 1*).

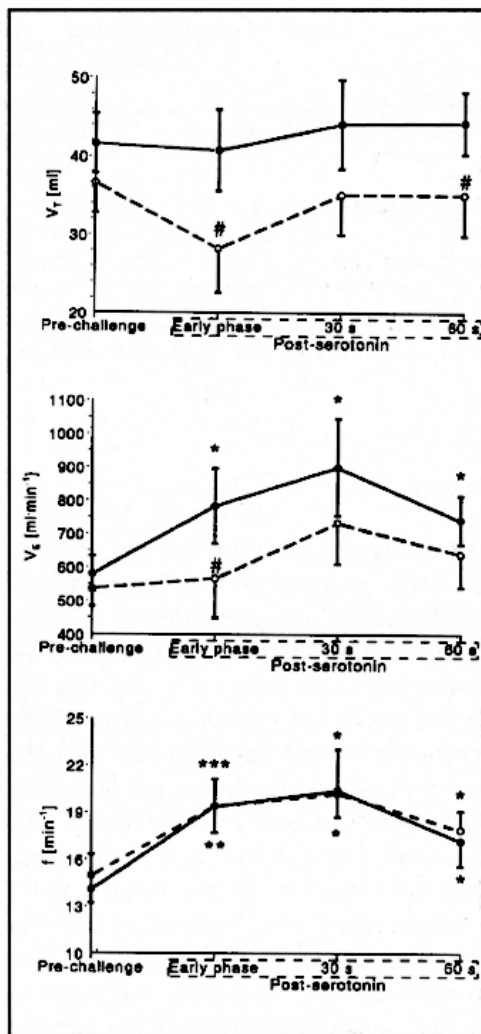


Fig. 1. Effect of i.v. serotonin (hydrogen oxalate salt, $50 \mu\text{g} \cdot \text{kg}^{-1}$) challenge on tidal volume (V_T , top panel), minute ventilation (V_E , middle panel), and respiratory rate (f , bottom panel) in bilaterally vagotomised and superior laryngeal nerves-sectioned cats prior to (solid lines, closed circles) and after (dashed lines, open circles) bilateral carotid sinus nerves' section. All values are means ± 1 S.E.M. of $N = 8$; * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$ vs. the respective pre-serotonin value, * $P < 0.05$ vs. the corresponding pre-carotid neurotomy value (two-way ANOVA followed by planned contrasts' analysis).

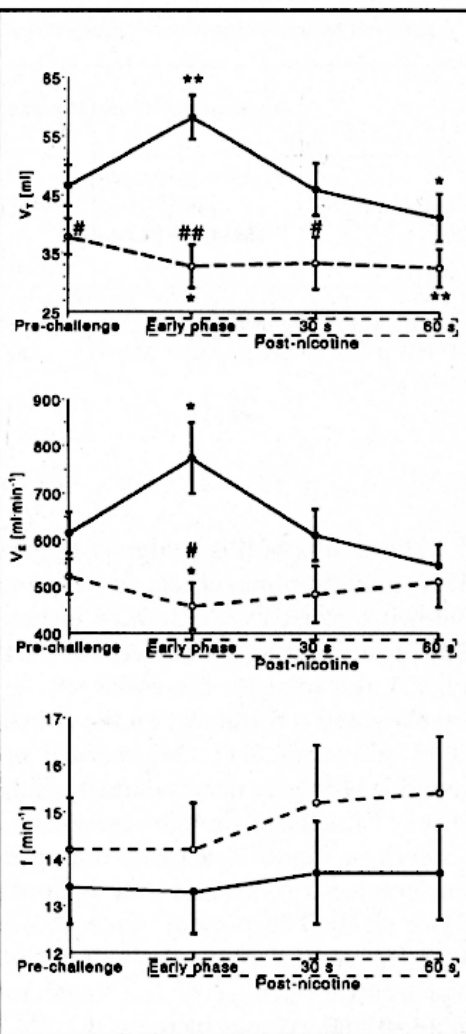


Fig. 2. Effects of i.v. nicotine (hydrogen tartrate salt, $200 \mu\text{g}$) challenge on tidal volume (V_T , top panel), minute ventilation (V_E , middle panel) and respiratory rate (f , bottom panel) in bilaterally vagotomised and superior laryngeal nerves-sectioned cats prior to (solid lines, closed circles) and after (dashed lines, open circles) bilateral carotid sinus nerves' section. All values are means ± 1 S.E.M. of $N = 8$; * $P < 0.05$, ** $P < 0.01$ vs. the respective pre-nicotine value, * $P < 0.05$ vs. the corresponding pre-carotid neurotomy value (two-way ANOVA followed by planned contrasts' analysis).

Table 1. Mean arterial blood pressure (MAP) changes induced by nicotine challenge*.

	Denervation status	Pre-nicotine	Pre-nicotine		
			Early phase	30 s	60 s
MAP (mm Hbg)	Vagotomy + SLNs cut	130.4 ± 12.0	142.0 ± 11.0*	140.4 ± 11.1*	134.4 ± 12.1
	Vagotomy + SLNs cut + CSNs cut	138.0 ± 13.0	137.0 ± 13.0	152.5 ± 9.8	150.4 ± 10.6**

* All values are means ± 1 S.E.M. of N = 8; * P < 0.05 vs. the respective pre-challenge value, ** P < 0.05 the respective pre-CSNs cut value (2-way ANOVA followed by planned contrasts' analysis).

DISCUSSION

The results of this study are in general agreement with the reports showing 5HT and nicotine effects on the respiratory pattern in vagotomised cats following either i.v. (2, 3, 6) or intracarotid arterial administration (4, 12, 16). Both nicotine and 5HT affect the excitability of peripheral chemoreceptors (4, 12, 17) and increase the traffic of chemosensory discharges (2, 9, 10, 18). We have focused our interest on the contribution of CSNs to the ventilatory effects of nicotine and 5HT. Midcervical neurotomies of the vagal nerves and the superior laryngeal nerves eliminate afferent inputs from the lungs and laryngeal airway, which are thought essential for the occurrence of apnoeic spells. The absence in vagotomised cats of apnoeic response to nicotine bolus given into the pulmonary vascular bed is consistent with earlier studies using similar doses (2, 3). The present study showed an immediate short-lived increase in V_T after nicotine challenge, without noticeable changes in the respiratory rate. Apparently, opening of the vagal loop excludes the frequency response to nicotine and exposes increase(s) in V_T uniquely due to activation of the afferent input from the carotid body. Similar observations have been made by Haxhiu *et al.* (15). For unknown reasons, the relative magnitude of V_T response to nicotine in our cats with intact CSNs was small compared with that reported with similar doses in cats with intact SLNs (2); however, the present results are consistent with our earlier study in vagotomised cats with SLNs cut (3).

It is well established that carotid body denervation (19) and lidocaine blockade of the CSNs (20) reduce V_T in cats, which is obviously due to the absence of afferent drive from the carotid body. In our study, CSNs section resulted in both lowering of V_T (see Fig. 2) and a qualitative change in the pattern of V_T response to nicotine challenge. This latter effect, which has not been reported in the earlier study employing no SLN section (2), suggests the existence of opposing pathways mediating chemosensory effects of nicotine on

respiration. Whereas the precise mechanism of the nicotine-induced V_T depression in vagotomised and peripherally chemodenervated cats remains to be elucidated, it likely involves nicotine action at the CNS level. Nicotine readily crosses the blood-brain barrier, and it was shown that respiratory depression can be produced by nicotine application to the floor of the 4th ventricle (21); no recent studies are available that would shed more light on the exact determinants of the CSNs cut-associated inversion of V_T response to nicotine.

Our finding of the CSNs-associated delay in nicotine-induced rise of MAP in this study confirms results obtained by others in cats treated by both vagal and CSNs sections (2, 15, 22). The action of nicotine on blood pressure is obviously a complex one and may involve direct stimulation of the vasomotor center and/or activation of the sympathetic nervous system that promotes catecholamine release from the adrenal medulla (23).

This is the first report comparing the respiratory response to serotonin in vagotomised and CSNs-sectioned cats. Whereas the 5HT dose used in this study was rather large, it failed to affect V_T , suggesting no effective stimulation of carotid body chemoreceptors. It is yet possible that the i.v. 5HT bolus after traversing the pulmonary circulation with its high 5HT-binding capacity did not result in carotid arterial blood 5HT level high enough to cause chemoexcitation. We believe higher 5HT doses given into the femoral vein might excite carotid body, because intracarotid artery administration of 5HT doses in a low microgram range has been found to produce a transient CSN excitation in the cat (9, 10). However, those reports showed no data on V_T , and transient excitation of the sinus nerves activity may not be readily translated into increased ventilation.

Nucleus tractus solitarii, which takes part in respiratory drive generation by integrating, among others, peripheral inputs carried by the CSNs (24), receives also projections from the serotonergic system in vagal fibres and cell bodies in the nodose ganglia (25). In our experimental design (see also ref. 6), 5HT challenge resulted in virtually identical patterns of increased respiratory rate prior to and after CSNs section in the cat. This finding together with the occurrence of apnoea after bilateral carotid body deafferentation imply that the serotonin-induced timing component of the breathing pattern as well as the inhibition of the respiratory drive during apnoeic spells should be attributed to stimulation of 5HT₃ receptors within the nodose ganglia (26). This assumption is consistent with the ability of i.v. 5HT₃ receptor antagonist HGR38032F to decrease the occurrence of post-5HT apnoea in the rat (27), preclusion of all ventilatory effects of 5HT challenge by i.v. application of the selective 5HT₃ receptor antagonist MDL 72222 in the cat (28), and — most importantly — abolition of all respiratory effects of 5HT by supranodose vagotomy in both these species (6, 16, 27).

In conclusion, this study shows the importance of carotid body input in determining the direction of tidal volume response to i.v. nicotine in cats with vagal feedback removed. Present results suggest also that the apnoea and increase in the respiratory rate induced by 5HT under the same experimental conditions may be carried out beyond the carotid afferents.

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