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Action of serotonin on the laryngeal airway in anaesthetized cats

Małgorzata Szereda-Przestaszewska and **Beata Kopczyńska**

Department of Neurophysiology, PAS Medical Research Centre,
5 Pawiński St., 02-106 Warsaw, Poland

Abstract. The pulmonary chemoreflex induced by an intravenous injection of serotonin (5-hydroxytryptamine) in cats consists of prompt apnoea, bradycardia and hypotension, followed by rapid, shallow breathing. The present study had two purposes (1) to compare the effect of 5HT on ventilation and laryngeal resistance in cats and (2) to assess the role of laryngeal afferents in these responses. The effects of an intravenous injection of serotonin at a dose of 0.05 mg per kg of body weight were studied in eighteen anaesthetized cats, breathing spontaneously *via* a tracheal cannula. In eleven cats the larynx was isolated in situ to measure laryngeal resistance. In post-serotonin apnoea, the expiratory laryngeal resistance rose four-fold. This coincided with the increased afferent activity of the superior laryngeal nerve. In the initial phase of resumed shallow breathing, the increase in the expiratory laryngeal resistance was coupled with reduced tidal volume. Bilateral section of the superior laryngeal nerve failed to affect laryngeal constriction and the ventilatory response to serotonin. Thus laryngeal afferents running within the superior laryngeal nerve are not essential for the respiratory phenomena induced by serotonin.

Key words: control of breathing, cat, serotonin, upper airways, laryngeal afferents, laryngeal resistance

INTRODUCTION

Serotonin is an endogenous autacoid contained within the lungs in the neuroepithelial bodies (Lauweryns et al. 1986) and blood platelets (Cohen 1988). It might be released by various stimuli such as hypoxia (Christian et al. 1989) in concentration sufficient to activate cardiopulmonary vagal afferents. When introduced into the right side of the circulation in experimental animals including cats, serotonin evokes the pulmonary chemoreflex, consisting of prompt apnoea, bradycardia and hypotension, followed by rapid shallow breathing (Comroe et al. 1953). The respiratory and cardiovascular responses to serotonin are attributed to stimulation of pulmonary vagal C-fibre afferents, possibly endowed with 5-HT_2 and 5-HT_3 receptors (Kay and Armstrong 1991, Yoshioka et al. 1992b). These C-fibres endings are located in the lung parenchyma in the area between right atrium and aortic valves, directly accessible to serotonin injected into the pulmonary circulation.

In cats and rats serotonin has been shown to cause bronchoconstriction (Comroe et al. 1953, Yoshioka et al. 1992a). Relatively little attention has been paid to the effects of serotonin on the upper airways. The present study was designed to examine the effect of exogenous serotonin on laryngeal resistance in vagally intact cats during and following the post-serotonin respiratory arrest and trying to define how much they interact with the early and late ventilatory changes following the apnoeic episodes. These conditions do not seem to have been studied previously by direct measurements of laryngeal resistance, although Stránsky et al. (1973) have investigated the effect of phenyldiguanide, an agonist of 5-HT_3 receptors. This finding implied that the challenge with an exogenous serotonin, presumed to release it also from the endogenous stores, may evoke a potent laryngeal constriction. In addition, by recording integrated neural activity of the superior laryngeal nerve (SLN), we looked at the contribution of laryngeal input to the respiratory response to serotonin. There is only one report showing no effect of serotonin on SLN activity in paralyzed, artificially ventilated rats (Yoshioka et al. 1994). So far as we are aware there has been no such study in cats. A preliminary report of some of these findings has previously appeared (Wypych and Szereda-Przestaszewska 1994).

METHODS

Eighteen adult cats of either sex (weight 3.0-3.5 kg) were anaesthetized with an intraperitoneal (i.p.) injection of 30 mg kg^{-1} of sodium pentobarbitone. Supplemental doses of alpha-chloralose (16 mg kg^{-1}) were administered intravenously (i.v.) to maintain possibly constant level of surgical anaesthesia. Ethical approval for the experimental procedures used in this study was obtained from the local committee. Cats were placed supine on an operating table, breathing spontaneously room air. A femoral vein and femoral artery were catheterized for further injections and to monitor blood pressure, respectively.

The experiments were carried out in two different protocols. In the first experimental group (11 cats) the trachea was sectioned below the larynx and each end cannulated with a tracheal tube. The caudal tube was connected to a pneumotachograph to measure tidal volume (VT). Air warmed to body temperature and saturated with water vapour was passed through the rostral tube and through the larynx at a constant rate of 2 l min^{-1}. The pharyngeal wall was opened widely on the right, rostral to the hyoid bone and the epiglottis was fixed with a suture. Translaryngeal pressure was measured as the pressure difference between the rostral tube and the pharyngeal opening. The pressure drop across the isolated larynx was measured from the infralaryngeal region, referenced to atmospheric pressure. Laryngeal resistance was calculated as the ratio of translaryngeal pressure (difference between upper tracheal and atmospheric pressures) to constant laryngeal airflow, as previously described (Stránsky et al. 1973). The two recurrent laryngeal nerves were identified and spared. The superior laryngeal nerves were separated, isolated and prepared for division later in the experiment. The following variables were calculated from the VT tracings: total duration of the respiratory cycle (TTOT), respiratory rate (f = 60/TTOT) and minute ventilation (V = VT x f).

The responses of ventilatory variables to serotonin were assessed by comparing the mean of five breaths in restored respiration 30 and 60 s after the challenge to the mean of five control breaths and expressed as absolute values. Laryngeal resistance was measured in control conditions, during the expiratory apnoea (apnoeic phase), 30 and 60 s following serotonin injection.

In the second experimental protocol we used 7 cats, breathing through the tracheal cannula, where tidal airflow was recorded. A superior laryngeal nerve (SLN), usually the left, was identified, isolated and cut 2 cm from the larynx. The peripheral cut end was desheathed and whole nerve activity was monitored by a pair of silver electrodes. The contralateral SLN was prepared with

loose ligatures for division later in the experiment. The integrated electroneurogram recorded from the left superior laryngeal nerve was analysed in terms of its peak activity and expressed in arbitrary units as previously described for nerve recordings (Hwang et al. 1984a). The peak height of the SLN integral was measured for five breaths prior to serotonin administration (control), during apnoea and during five breaths with resumed activity at 30 and 60 s after the challenge. Comparisons were made between control and test conditions.

Arterial blood pressure was measured with a pressure transducer (C.K.01 Mera Tronik) and blood pressure monitor (40ll, MCK). Volume or flow signals were recorded from pneumotachograph (Electrospirometer CS6, Mercury). Translaryngeal pressure was measured using a differential capacitance manometer (Hilger, I.R.D.6). End-tidal CO_2 concentration was measured with a capnograph (Engstrom Eliza plus, Gambro). Action potentials of the peripheral cut end of the SLN (second protocol) were amplified (Tektronix 3A3) and integrated with a leaky integrator (Medipan type 464). The time constant of the integrator was 100 ms. All recordings were recorded with an Omnilight Recorder 8M36 (Honeywell). Constant body temperature, monitored with a rectal thermometer was maintained with a heating pad between 37-39°C throughout the experiment. Cats were normoxic. End-tidal CO_2 concentration was 5.2% on average. Mean arterial blood pressure remained at about 19 kPa throughout the experiments. The respiratory effects of serotonin were recorded prior to and following section of the superior laryngeal nerves (protocol 1) and prior to and following section of the contralateral SLN (protocol 2).

Serotonin (serotonin - hydrogenoxalat) in a dose of 0.05 mg kg^{-1} (0.188 µmol kg^{-1}) dissolved in 0.9% saline was injected as a bolus through the catheter placed in the right femoral vein. At the beginning of experiment a 0.3 ml bolus of the saline was administered to serve as a volume control. All drug administrations were delivered in volume of 0.4 ml and followed by a flush of 0.4 ml saline.

Tidal volume (VT), respiratory rate (f), ventilation (VE), inspiratory (RI) and expiratory (RE) laryngeal resistance, and SLN peak amplitude height data were analysed by repeated measures 2-way ANOVA with time after serotonin injection (0, apnoeic phase, 30 s and 60 s) and denervation status (intact, SLNs-cut) as independent variables. Contrast analysis was used to analyse the effect of post-serotonin time in two arms of the experimental design (intact cats, SLNs-cut cats) separately. The significance of differences between individual experimental states was determined by post-hoc Scheffé's

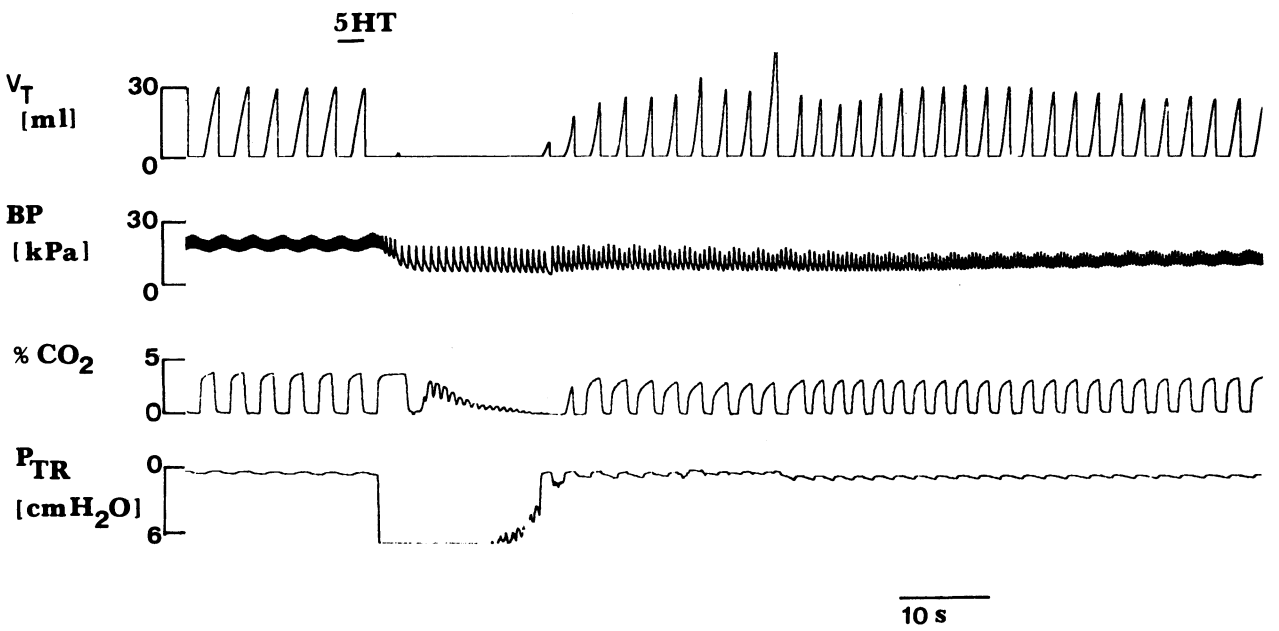

Fig. 1. Experimental record illustrating the respiratory response to an intravenous injection of serotonin (5-HT) in the SLN intact cat. 5-HT injection represented by the horizontal bar. Note the expiratory apnoea coupled with the large increase in translaryngeal pressure. VT, tidal volume; BP, systemic blood pressure; %CO_2, end-tidal CO_2; PTR, translaryngeal pressure.

test. Results are quoted as the mean ± SEM. In all cases, a $P<0.05$ was considered significant.

RESULTS

Serotonin (5-HT) produced consistent and repeatable effects. When an equal volume of 0.9% saline was given, it failed to produce any respiratory changes.

Intravenous injection of serotonin in 10 intact animals and in 11 with divided superior laryngeal nerves evoked prompt apnoea of mean duration of 13.5 ± 5.9 s and 15.1 ± 4.9 s, respectively, followed by rapid breathing. Post-serotonin apnoea was usually associated with bradycardia and hypotension in both intact and SLN-sectioned animals. Figure 1 depicts a representative record of the response to an i.v. injection of serotonin in the intact cat. The arrest of breathing was accompanied by raised expiratory translaryngeal pressure. After resumption of breathing changes in translaryngeal pressures during inspiration were inconsistent, whereas those observed in expiration invariably showed a moderate increase. Two-way ANOVA revealed significant effect of serotonin challenge on tidal volume ($F_{2,20} = 21.245$, $P<0.001$) but no effect of denervation and serotonin challenge x denervation interaction. Contrast analysis showed significant effect of post-serotonin time in the intact ($F_{2,20} = 30.341$, $P<0.001$) and SLNs-sectioned cats ($F_{2,20} = 10.330$, $P<0.001$). Mean changes in tidal volume during control and post-serotonin breathing are shown for 11 cats in Table I.

Tidal volume fell significantly at the initial phase of resumed breathing (30 s after 5-HT administration) compared to the control values but reverted to baseline conditions within 60 s prior to and following SLNs section.

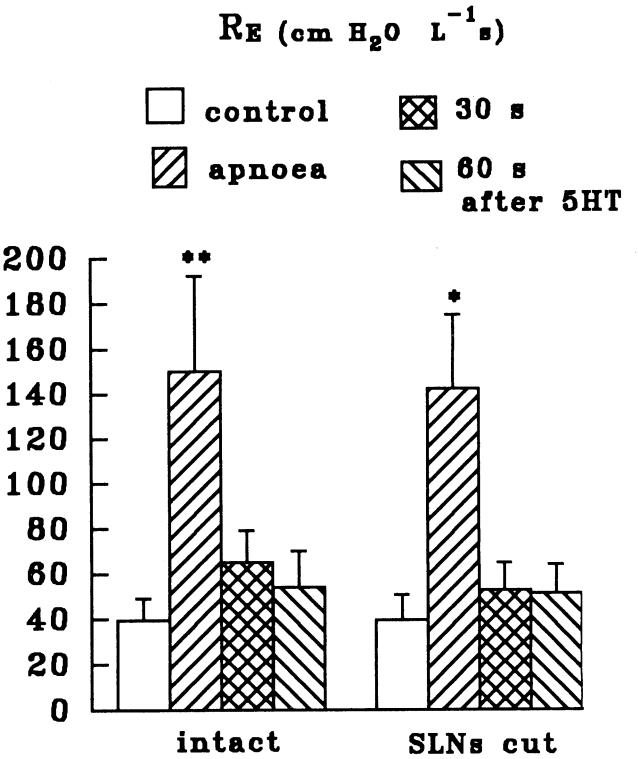

Fig. 2. Mean values ± SEM of expiratory laryngeal resistance (RE) and the effect of serotonin administration in the SLN intact and neurotomized cats. Note the large increase in the expiratory laryngeal resistance during apnoea. $^{**}P<0.01$, $^{*}P<0.05$ compared to control (Scheffé's test); $n = 11$.

Analysis of variance yielded significant effect of serotonin administration on minute ventilation ($F_{2,20} = 10.741$, $P<0.001$) but no effect of denervation and serotonin challenge x denervation interaction. Contrast analysis exposed significant effect of post-serotonin time in the intact ($F_{2,20} = 9.064$, $P<0.01$) and SLNs-neuro-

TABLE I

Ventilatory effects of an intravenous serotonin challenge in the SLN intact and neurotomized cats

	VT (ml)			VE (ml/min)		
	control	30 s	60 s	control	30 s	60 s
intact	33.3 ± 3.0	20.3 ± 3.4***	33.5 ± 3.6	574.4 ± 76.8	471.6 ± 98.3	732.8 ± 124.3**
SLNs cut	32.7 ± 3.4	21.9 ± 3.5***	32.4 ± 3.8	552.4 ± 87.6	473.5 ± 87.7	729.6 ± 115.4**

Abbreviations are provided in the text. Numbers are mean ± SEM; $n = 11$. ***$P<0.001$, **$P<0.01$ compared to control values detected by Scheffé test.

tomized cats ($F_{2,20} = 9.143$, $P<0.01$). As shown in Table I, during the initial phase of restored breathing (30 s after 5-HT) minute ventilation showed a tendency to decrease but without statistical significance in the intact and SLNs-cut animals. Within 60 s it was appreciably increased compared to control conditions. In general during resumed breathing serotonin did not affect the inspiratory laryngeal resistance (ANOVA, $P=0.08$).

Two-way ANOVA disclosed significant effect of serotonin challenge on expiratory laryngeal resistance ($F_{3,30} = 15.527$, $P<0.001$) but no effect of denervation and serotonin challenge x denervation interaction. Contrast analysis disclosed significant effect of post-serotonin time in the intact ($F_{3,30} = 6.252$, $P<0.01$) and SLNs-cut animals ($F_{3,30} = 14.661$, $P<0.001$). Figure 2 presents changes in expiratory laryngeal resistance, which rose appreciably in post-serotonin apnoea compared to control values in the intact cats ($P<0.01$) and those with divided SLNs ($P<0.05$). After reappearance of breathing (30 s) the expiratory laryngeal resistance declined but exceeded the control level. In the later phase of resumed breathing (60 s) the resistance returned to a value not significantly different from the baseline.

In general agreement with previous workers (Mathew et al. 1984) the afferent activity of the superior laryngeal nerve prior to serotonin injection showed clear inspiratory modulation (Fig. 3). During apnoea neural activity of the superior laryngeal nerve ceased and then the expiratory activity appeared when breathing resumed. These changes were observed with the contralateral SLN intact or cut.

Analysis of variance exposed significant effect of serotonin injection on SLN peak amplitude height ($F_{3,18} = 21.453$, $P<0.001$), no effect of denervation, but significant serotonin challenge x denervation interaction ($F_{3,18} = 5.688$, $P<0.01$). Contrast analysis indicated significant effect of post-serotonin time in the intact ($F_{3,18} = 20.34$, $P<0.001$) and SLNs-ablated cats ($F_{3,18} = 8.91$, $P<0.001$). Figure 4 represents the post-serotonin increase in peak SLN activity. During the arrest of breathing the amplitude of the SLN neurogram rose in both neural states ($P<0.001$, $P<0.05$, respectively). With restored breathing, peak respiratory activity of the SLN remained elevated above the control level at 30 s after serotonin injection in each condition ($P<0.001$, $P<0.05$, respectively) and at 60 s in unilaterally neurotomized cats.

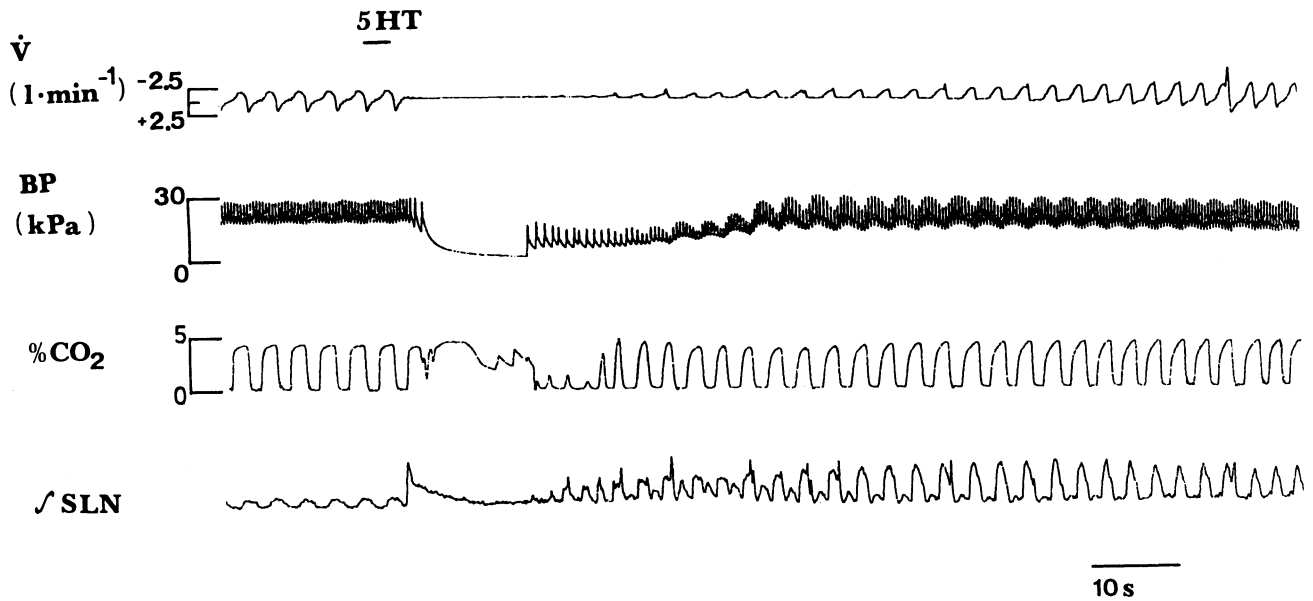

Fig. 3. Experimental record illustrating the response of the SLN integral to an intravenous injection of serotonin (5-HT) with the contralateral SLN intact. Note the expiratory apnoea coupled with the fall in systemic blood pressure and an increase in SLN electroneurogram. Rapid,shallow breathing which followed the apnoea is associated with large increase in the inspiratory and the appearance of expiratory activity of the SLN. V, respiratory airflow; BP, systemic blood pressure; % CO_2, end-tidal CO_2; SLN, integrated neurogram of the superior laryngeal nerve.

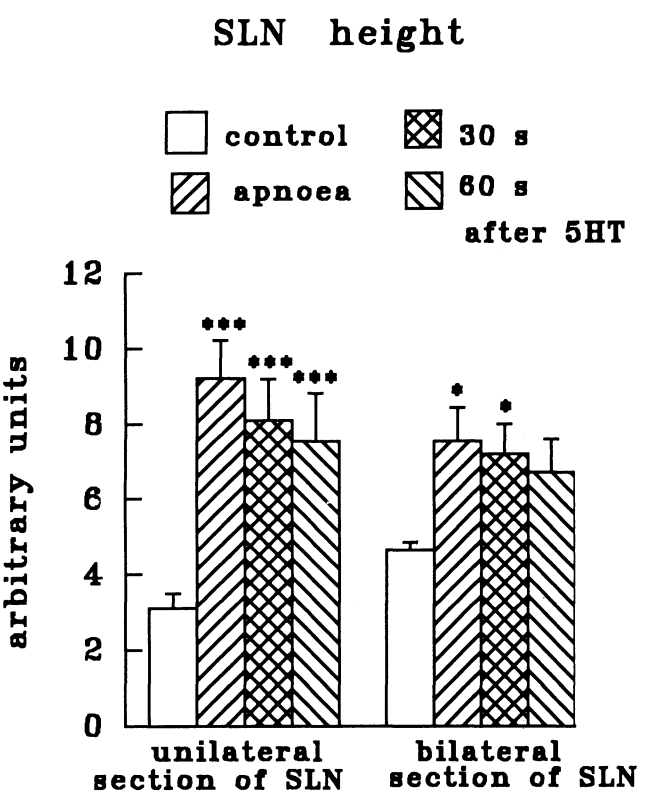

Fig. 4. Mean values ± SEM of peak afferent SLN activity before and after an intravenous injection of serotonin in intact and SLNs cut animals. $^{***}P<0.001$, $^{*}P<0.05$ compared to control (Scheffé's test); $n = 11$.

DISCUSSION

Serotonin given i.v. in cats induced a pulmonary chemoreflex. Earlier reports on the respiratory effects of intravenous injection in this species described apnoea followed by subsequent tachypnoea (Comroe et al. 1953, Schneider and Yonkman 1953). It is of note that report by Schneider and Yonkman (1953) describes the respiratory changes in artificially ventilated animals. We reported elsewhere (Szereda-Przestaszewska and Wypych 1995) the effects of serotonin on respiratory airflows, but neither this work nor any other performed in cats has examined the ventilatory changes in the early and later phase of post-apnoeic respiration.

In the current experiments we have measured tidal volume and computed minute ventilation in spontaneously breathing cats. Our results have shown that shallow tachypnoea without any significant change in minute ventilation takes place immediately from the resumption of respiration after the apnoeic pause. Later on (60 s after serotonin challenge), when tidal volume was restored, minute ventilation exceeded the control values (Table I). This may be due to activation of arterial chemoreceptors as demonstrated by the intracarotid administration of serotonin (Ginzel and Kottegoda 1954), although the resulting hyperpnoea has not been shown (Jacobs and Comroe 1971, Black et al. 1972).

Several records in the paper by Jacobs and Comroe (1971) show moderate decreases in tidal volume following intracarotid serotonin administration.

The question addressed by the experiments reported here was whether the ventilatory changes evoked by an intravenous challenge of serotonin are accompanied by the resistance posed by the laryngeal airway. In our preparation of the isolated larynx (protocol 1), the values for resistance were not influenced mechanically by changes in tidal airflow, and therefore reflect accurately the influence of variations in neuromuscular tone (Stránsky et al. 1973).

In the expiratory arrest of breathing induced by serotonin, the expiratory laryngeal resistance on average rose four-fold above the baseline values, which shows an appreciable narrowing of the laryngeal airway. This is the first demonstration of post-serotonin laryngeal constriction in cats. There was, however, considerable variability in the laryngeal response of individual cats. Few of them showed complete closure of the larynx, leading to an infinite resistance. Much the same laryngoconstriction was reported on intravenous injection of phenyldiguanide in cats (Stránsky et al. 1973). The similarity in laryngeal response to both drugs is reinforced by the fact, that phenyldiguanide (PDG) is an agonist of 5-HT_3 receptors (Kay and Armstrong 1990). Serotonin molecule is quite distinct from that of PDG, which is symmetrical (Kay and Armstrong 1990) and this may explain the potential differences in their pharmacological effects. Ventilatory and cardiovascular effects of PDG were shown to be abolished by mid-cervical vagotomy in the cat (Daly and Kirkman 1988), whereas those of serotonin are reduced but not eliminated by this neurotomy (Jacobs and Comroe 1971, Yoshioka et al. 1992a, Szereda-Przestaszewska and Wypych 1995).

The decrease in tidal volume during initial phase of resumed breathing was strictly coupled with increased expiratory laryngeal resistance (Fig. 2). Serotonin narrowed the laryngeal airway and limited the rate of airflow mainly in the expiratory phase of the respiratory cycle. During the apnoea there was an activation of the laryngeal constrictors which was protracted to post-ap-

noeic period of breathing. In resumed breathing the expiratory laryngeal resistance was apparently above the baseline level (Fig. 2) and these changes in the laryngeal calibre coincided with the increased tidal volume and enhanced ventilation 60 s after the challenge. In our experimental design afferents from the larynx were not essential for the respiratory phenomena induced by serotonin. Bilateral section of the superior laryngeal nerves did not affect either the control values of laryngeal resistances (Fig. 2) or of the ventilatory variables (Table I), which is consistent with earlier report in adult rabbits breathing with omission or through the upper airway (Citterio et al. 1985, Kamosińska and Szereda-Przestaszewska 1988). In our present work the effect of SLN section would be influenced by the fact, that the larynx was bypassed. Then the variety of afferent activity would be considerably reduced. In current experiments damage to this pathway did not affect the triad of the pulmonary chemoreflex induced by serotonin, which was shown to rely largely on vagal respiratory input (Jacobs and Comroe 1971, Yoshioka et al. 1992a, Szereda-Przestaszewska and Wypych 1995).

We have recorded integrated activity of the whole superior laryngeal nerve in seven cats (second protocol), breathing through a tracheostomy. The larynx was bypassed and since we used spontaneously breathing animals the larynx was subjected only to "drive" produced by the action of upper airway muscles related to ventilation (Mathew et al. 1984). Such muscle contractions and movements are known to affect activities of laryngeal receptors. It is supported by the observation of Yoshioka et al. (1994) that serotonin has no effect on SLN activity in paralyzed rats with the larynx bypassed where "drive" loses its respiratory modulation. Certainly, the tachypnoeic response to i.v. serotonin in spontanously breathing animals may well result in indirectly evoked changes in receptor discharge not occurring in paralyzed, artificially ventilated animals. In current experiments baseline recordings of the afferent activity of the superior laryngeal nerve presented a clear inspiratory modulation (Fig. 3), which is consistent with earlier research (Mathew et al. 1984). During post-serotonin apnoea, large positive pressure developed below the site of obstruction i.e. below the vocal folds is reflected on the superior laryngeal nerve integral by clear expiratory modulation. This fits with the observed expiratory laryngeal constriction and is in line with the expiratory augmenting pattern of SLN integral reported on positive pressure in rabbits (Mortola et al. 1985, Tsubone et al. 1987). These two factors: contraction of upper airway muscles and distending pressure activate the laryngeal receptors in rabbits (Tsubone et al. 1987) and cats (Hwang et al. 1984b). Bilateral section of laryngeal nerves failed to lower the rise in SLN integral during post-serotonin apnoea (Fig. 4). This could imply that elimination of the main afferent input from the larynx to the respiratory neurones can cause in turn increased efferent activity of the recurrent laryngeal nerve, which might affect the SLN activity. In breathing that followed the apnoeic pause we have noted, too, a substantial increase in the magnitude of the superior laryngeal nerve activity (Figs. 3 and 4) with the appearance of the expiratory activity, which might be related to laryngeal adductor muscle activity. Excitation of laryngeal afferents lasted longer than the expiratory constriction of the larynx. In view of the results of this study we could not exclude the possibility that serotonin stimulates afferent C-fibres within the larynx, likewise the ones in the lungs (Kay and Armstrong 1991). However a good deal of doubt exists whether increased SLN activity reflects direct effect of serotonin on laryngeal receptors or effects resulting from altered drive to the larynx. Experiments reported here can not settle the issue which is yet to be evaluated.

Serotonin was described to exert part of its respiratory effects through the SLN in the rat (Yoshioka et al. 1992a). It may well reflect species difference in response to serotonin. However, our present results discount the possibility that afferent activity emerging from the larynx contributes to the ventilatory effects of intravenous serotonin. This afferent path might be of importance in providing information on mechanical changes within the laryngeal airway during the arrest of breathing. Presumably with an intact airway the effect of serotonin on laryngeal receptors would be detected by SLN afferents.

Stimulation of vagal C-fibres by serotonin administered to the right side of circulation in cats results in afferent nerve conduction that elicits centrally mediated reflex change in parasympathetic and respiratory motor output (Solway and Left 1991). The latter consists of increased respiratory system resistance caused by airway smooth muscle constriction (Comroe et al. 1953, Spannhake et al. 1980, Yoshioka et al. 1992a) and immediate closure of the glottis revealed in the work reported here. The evidence provided here is consistent with our assumption that the decreased laryngeal patency interacts in the early ventilatory changes following the apnoeic episodes. In response to serotonin there is prolonged augmented ac-

tivity of the SLN in cats with the intact and deafferented laryngeal airway, which may show either stimulatory effect of serotonin on laryngeal receptors or effects of altered drive to the larynx. In our experimental design, the vagal feedback from the lungs was preserved to leave the main path for the occurrence of post-serotonin apnoea in cats (Szereda-Przestaszewska and Wypych 1995).

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