ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 889-896

Respiratory effects of serotonin challenge to pulmonary and laryngeal circulation in anaesthetized cats

Małgorzata Szereda-Przestaszewska and **Beata Wypych**

Department of Neurophysiology, PAS Medical Research Centre, 3 Dworkowa St., 00-784 Warsaw, Poland

Abstract. Administration of serotonin (5-HT) to pulmonary circulation elicits prompt apnoea, followed by subsequent tachypnoea. The present study was designed to ascertain whether 5-HT challenge into the laryngeal artery will evoke the full constellation of this chemoreflex and to examine the role of laryngeal sensory input and importance of vagal afferents in the respiratory sequelae.The experiments were done on 10 anaesthetized, spontaneously breathing cats.Laryngeal artery injections of 5-HT, similarly to intravenous challenge, caused apnoeas, which were significantly diminished by the section of cervical vagal trunks. Breathing frequency increased in all condtions on intravenous injection but only prior to vagotomy, when administered into laryngeal artery. With resumed breathing,the peak inspiratory airflows were significantly increased in the neurally intact, those treated by bilateral section of the superior laryngeal nerves (SLNs-cut) and vagotomized cats, with no difference between them and independent of the route of injection. The results show that serotonin chemoreflex could evolve from the laryngeal vascular bed and that laryngeal afferents do not contribute to the respiratory arrest.

Key words: apnoea, control of breathing, serotonin (5-HT), laryngeal afferents

INTRODUCTION

The ventilatory sequence of the pulmonary chemoreflex induced by serotonin (5-hydroxytryptamine) in cats includes an expiratory apnoea followed by tachypnoeic breathing. Serotonin excites the smallest sensory fibres: non-myelinated C-fibres (Coleridge and Coleridge 1984) and central transmission of this input leads to reflex activation of autonomic pathways. There is now considerable evidence that afferent responses are mediated through activation of peripheral 5-HT_2 and 5-HT_3 receptors on vagal C-fibres (Armstrong and Kay 1990, Kay and Armstrong 1991, Meller et al. 1991, 1992). There are some data showing that the laryngeal airway includes, too, C-fibre system (Tsubone et al. 1991).

Activation of laryngeal sensory endings has an inhibitory effect on respiration resulting in protracted apnoea (Widdicombe 1986). Previous work has shown that midcervical vagotomy markedly reduces the occurrence of post-serotonin apnoea (Comroe et al. 1953, Ginzel and Kottegoda 1954, Jacobs and Comroe 1971). Following this neurotomy however, injection of serotonin into laryngeal vascular bed failed to evoke the apnoeic response, still affecting the tidal component of the breathing pattern (Szereda-Przestaszewska and Wypych 1995).

This finding implied that serotonin enhanced afferent input from the laryngeal airway may modify the apnoeic spells prior to vagotomy.

We have therefore tried to see how reflexes from the lung C-fibre interact with those from the larynx in the overall ventilatory response to serotonin.

Some of our results have been described briefly elsewhere (Szereda-Przestaszewska and Wypych 1993).

METHODS

Ten adult cats of both sexes (weight 2.7-4.0 kg) were anaesthetized with an intraperitoneal injection of 30 $mg \cdot kg^{-1}$ sodium pentobarbitone (Sagatal, May and Baker Ltd), later supplemented with 16 $mg \cdot kg^{-1}$ of intravenous alpha-chloralose (Fluka AG). Ethical approval for the experimental procedures used in this study was obtained from the local commitee. The animals were placed supine on a heated operating table, breathing spontaneously room air. A femoral vein and femoral artery were catheterized for further injections and to monitor blood pressure, respectively. A laryngeal branch, usually of the right cranial thyroid artery was catheterized anterogradely.

The trachea was divided below the larynx and the cannula inserted into the caudal end was connected to a pneumotachograph. The two recurrent laryngeal nerves were identified and spared. The C_4-C_5 root of the right phrenic nerve was cleared, cut, desheathed and prepared for recording.

The superior laryngeal nerves (SLNs) as well as the cervical vagus nerves were separated, isolated and prepared for division later in the experiment.

Arterial pressure was measured with pressure manometer (C.K. 01 Mera-Tronik) and blood pressure monitor (MCK 40 11S). Airflow signals were recorded from pneumotachograph (Electrospirometer CS6, Mercury). End-tidal CO_2 was measured with a capnograph (Engström Eliza plus). Action potentials of the phrenic nerve were amplified (Tektronix 3A3) and integrated (Medipan type 464). The time constant of the integrator was 100 msec. All recordings were registered with Honeywell Omnilight Recorder 8M 36. Rectal temperature was maintained between 37-39°C throughout the experiment.

Cats were normoxic. End-tidal CO_2 concentration was 4.3% ± 0.38 on average. Mean arterial pressure remained at about 145 mm Hg throughout the experiments.

At the begining of the experiment and after each denervation step, 0.3 ml bolus of physiological saline was administered to serve as a volume control on either route of injection.Serotonin (Serotonin hydrogenoxalat, Fluka AG, Buchs SG) in a dose of 0.05 $mg \cdot kg^{-1}$ dissolved in 0.9% sodium chloride solution was injected as a bolus through the catheter placed in the right femoral vein or in the right laryngeal artery. All drug administrations were followed by a flush of 0.3 ml of physiological saline.

The respiratory effects of serotonin were recorded in all ten cats while (1) intact, (2) following section of the superior laryngeal nerves, and (3) after subsequent division of the cervical vagi.

Inspiratory time (T_I) and expiratory time (T_E) were determined from the start and the peak of the phrenic neurogram, and breathing frequency was computed. Prolongation of the T_E was measured as the ratio of maximal T_E during post-serotonin apnoea or expiration ($T_{E\ test}$) to control expiration ($T_{E\ control}$), $T_{E\ test}/T_{E\ control}$.

The ventilatory responses were assessed by comparing the mean of five breaths during rapid, shallow breathing following serotonin injection to the mean of five preceding breaths (control = pre-challenge values) and expressed as absolute changes. Results are quoted as the mean ± 1 SEM.

Inspiratory airflow ($\dot{V}_I$), expiratory airflow ($\dot{V}_E$) and respiratory rate (f) were analysed by repeated measures of 3-way ANOVA with injection mode (lar.art. or i.v.), drug treatment (physiological saline or serotonin) and denervation status (intact, SLNs-cut and (SLNs+vagi)-cut) as independent variables. $T_{Etest}/T_{Econtrol}$ data were analysed by 2-way ANOVA with injection mode (lar.art. or i.v.) and denervation status as independent variables. The significance of differences between individual groups was determined by contrast analysis. In all cases, a $P< 0.05$ was considered significant.

RESULTS

Intravenous and close laryngeal injections of serotonin produced similar effects on respiratory variables occurring after the apnoeic phase. Post--serotonin apnoea was usually associated with bradycardia and hypotension prior to midcervical vagotomy.

A typical response to injection of serotonin *via* laryngeal artery in the intact cat is illustrated in Fig. 1. The expiratory arrest of breathing emerged prior to the drop in blood pressure.

On laryngeal artery injection of serotonin the expiratory apnoea appeared in four out of ten cats and in the same four cats with sectioned superior laryngeal nerves, and lasted 13.0 ± 1.7 s and 9.4 ± 1.4 s, respectively (n=4). Following midcervical vagotomy, this challenge of serotonin provoked apnoea in one cat only.

Intravenous injection of serotonin provoked in all ten cats while intact and following division of the superior laryngeal nerves the expiratory apnoea of

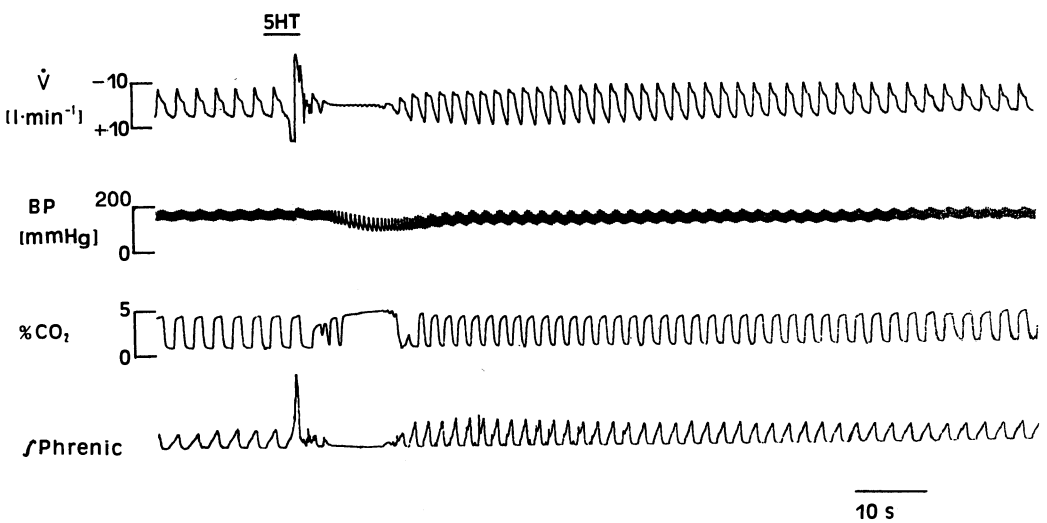

Fig. 1. Response to injection of serotonin (5-HT) into laryngeal artery in the intact cat. Traces from above down: lower tracheal airflow ($\dot{V}$), blood pressure (BP), end-tidal CO_2 (% CO_2), integrated neurogram of the phrenic nerve (Phrenic). Injection of serotonin at the signal causes apnoea, preceded by augmented breath and followed by tachypnoea, moderate hypotension and bradycardia.

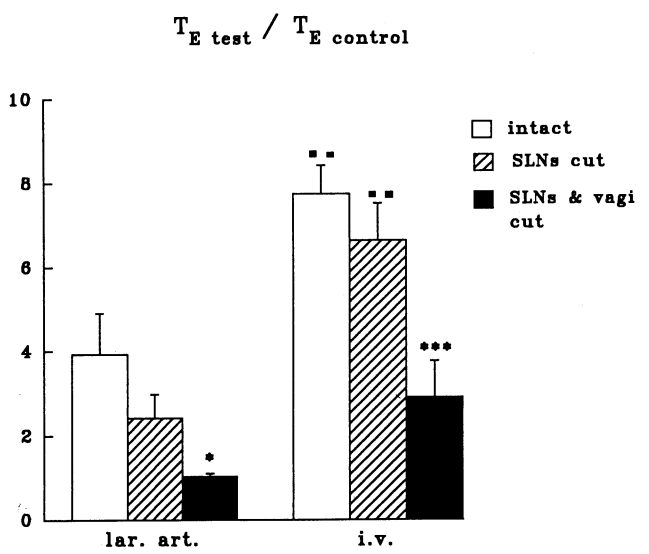

Fig. 2. Mean prolongation of T_E measured as the ratio of post--serotonin apnoea or expiration (T_{Etest}) to control expiration ($T_{Econtrol}$) subsequent to an intraarterial (lar. art.) and intravenous (i.v.) administration of serotonin in intact animals and those with SLNs and vagal trunks cut. Data are presented as means ± SEM. *** $P<0.001$, * $P<0.05$ compared to intact stateat each route of administration. Rectangles, $P<0.01$ intravenous vs. lar. art injection. (2-way ANOVA) $n = 10$.

mean duration of 16.2 ± 2.2 s and 14.7 ± 1.5 s, respectively (n=10). In six out of ten cats treated by midcervical vagotomy the mean duration of apnoea was 8.5 ± 2.2 s (n=6).

Such a large difference in the apnoea on two routes of injection of serotonin is reflected in the mean prolongation of T_E expressed as the ratio of $T_{E\ test}$ to $T_{E\ control}$. Two-way ANOVA revealed significant effects of both injection routes and denervation status on the $T_{E\ test}/T_{E\ control}$ ratio ($F_{1,9}$=29.38, P=0.0004, and $F_{2,18}$=31.11, P=0.000001, respectively). There was no interaction between the two main factors ($F_{2,18}$=2.021, P=0.16). As shown in Fig.2 on intraarterial and intravenous routes of injection of serotonin prolongation of T_E was significantly diminishesd following midcervical vagotomy ($F_{1,9}$=8.72, P=0.02 and $F_{1,9}$=41.468, P=0.00012, respectively). The duration of the apnoea after intravenous injection of the drug was significantly longer than that after laryngeal artery injection in the intact cats ($F_{1,9}$=13.86, P=0.005) and subsequently treated by the division of the superior laryngeal nerves ($F_{1,9}$=19.812, P=0.002). Following midcervical vagotomy there was no statistical difference between the two challenges ($F_{1,9}$=4.741, P=0.057).

After reinitiation of breathing the mean maximum ventilatory responses were recorded within the first minute following injection. Three minutes after injection the respiratory variables reverted to near control values.Three-way ANOVA revealed significant effects of serotonin challenge ($F_{1,9}$=23.25, P=0.001) and denervation status ($F_{2,18}$=34.03, P=0.000001) on respiratory rate and significant interactions between injection route and drug treatment ($F_{1,9}$=7.874, P=0.02), and between drug treatment and denervation status ($F_{2,18}$=8.638, P=0.003). Contrast analysis showed significant differences in denervation state x drug treatment both in cats given serotonin *via* intraarterial ($F_{2,18}$=5.481, P=0.014) and intravenous route ($F_{2,18}$=3.711, P=0.045). Serotonin given *via* laryngeal artery increased significantly the respiratory rate prior to vagotomy. On intravenous serotonin challenge there was a significant increase in respiratory rate for each denervation state (Fig. 3).

With the onset of tachypnoea the respiratory airflow increased. Analysis of variance showed signi-

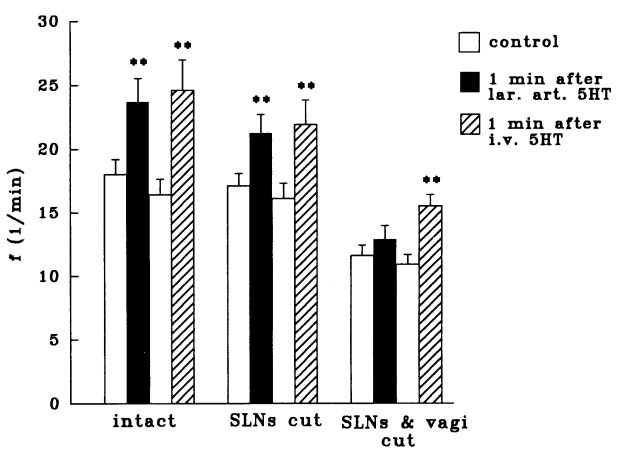

Fig. 3. Mean increases in respiratory rate (f) subsequent to laryngeal artery and intravenou administration of serotonin in control conditions and 1 min following injection in intact, SLNs-cut and vagotomized animals. ** $P<0.01$ compared with pre-serotonin values. (3-way ANOVA), $n = 10$.

ficant effect of serotonin challenge ($F_{1,9}$=27.98, P=0.0005), a trend toward significant injection route effect ($F_{1,9}$=4.093, P=0.074) but no effect of denervation status ($F_{2,18}$=1.186, P=0.33) on inspiratory airflow. There was a significant drug treatment x denervation status interaction ($F_{2,18}$=3.897, P=0.04). However, contrast analysis showed no significant differences in drug treatment x denervation status in the cats given serotonin *via* intravenous and intraarterial route analysed separately ($F_{2,18}$=1.677, P=0.215, and $F_{2,18}$=1.137, P=0.343, respectively). Mean changes in the peak inspiratory airflow within the first minute following intraarterial and intravenous injection of serotonin are shown in Fig. 4. In each condition, the peak airflows were significantly increased from the corresponding control values.

Three-way ANOVA revealed significant effects of both serotonin challenge ($F_{1,9}$=7.486, P=0.023) and denervation status ($F_{1,9}$=18.70, P=0.00004), but no effect of injection route ($F_{1,9}$=3.064, P=0.11) on expiratory airflow. Moreover, there was significant drug treatment x denervation status interaction ($F_{2,18}$=6.918, P=0.006). Contrast analysis showed significant difference in drug treatment x denervation status in the cats given intravenous serotonin ($F_{2,18}$=4.321, P=0.029) and a trend towards such a difference in cats injected intrarateriallly with serotonin. As illustrated in Fig. 5 the peak expiratory airflows after intraarterial administratiom of serotonin increased significantly except for nonsignificant increase in the intact animals. On intravenous challenge the peak expiratory airflows tended to increase prior to midcervical vagotomy and achieved statistical significance following vagotomy.

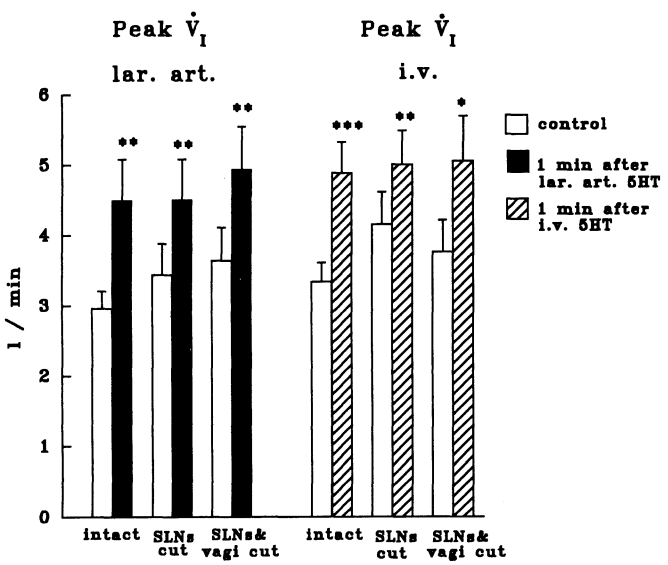

Fig. 4. Mean effect of laryngeal artery and intravenous injections of serotonin on peak inspiratory airflows in each experimental stage. ** P<0.01 compared with corresponding control. (3-way ANOVA), n = 10.

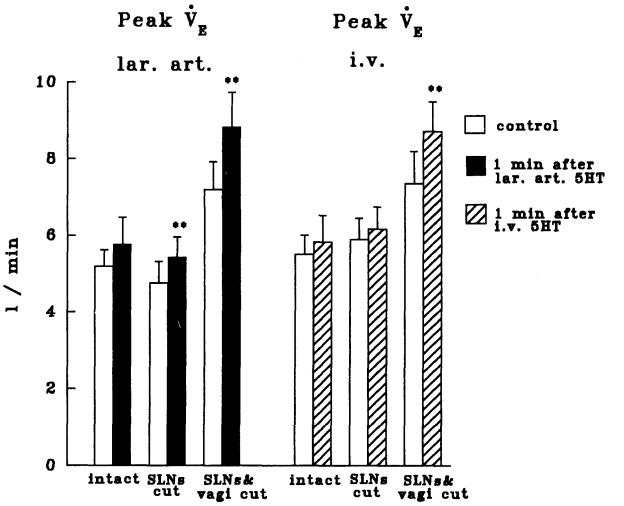

Fig. 5. Mean increases in the peak expiratory airflows on an intraarterial and intravenous administration of serotonin in intact, SLNs cut and vagotomized animals. ** P< 0.01 compared with pre-challenge values. (3-way ANOVA), n = 10.

DISCUSSION

Serotonin given into the right side of circulation in cats evokes pulmonary chemoreflex including apnoea, bradycardia and hypotension followed by acceleration of respiratory movements. The purpose ot this study was to determine the extent of the chemoreflex response to serotonin challenged into laryngeal circulation.In agreement with our previous report (Szereda-Przestaszewska and Wypych 1995), the results indicate that locally administered serotonin invariably triggers an extenuated chemoreflex with inconsistent and less profound arrest of breathing as compared to classical pulmonary

chemoreflex subsequently induced in the same animals. Because we wanted the results of our study to be applicable to the responses reported in the preceding paper (Szereda-Przestaszewska and Wypych 1995), we have examined carefully the pattern of respiratory response to close laryngeal challenge of serotonin in different system of neurotomies.

In three out of four cats treated by intraarterial challenge of serotonin the expiratory apnoea occurred well before the end of injection and was preceded by one or two deep breaths (Fig. 1). These last were observed in the rest of six cats undergone close laryngeal injection, which did not generate the arrest of breathing. Less frequent and transient apnoeas on intralaryngeal artery administration of serotonin, as described in the results and shown in Fig. 2 indicate that serotonin given locally affects the respiratory pattern differently from that evoked on an intravenous route.

It is of note that arrival of the drug at the laryngeal airway through the thin catheter in the laryngeal artery lasted longer than the bolus injection into the femoral vein and comprised a relatively small area. Moreover, the large dosage of serotonin we have applied on either challenge (50 $\mu g.kg^{-1}$) was more concentrated reaching directly the laryngeal site and could have evoked localized and intensive stimulatory effects. Presumably only half of the larynx was challenged and the laryngeal reflex could not be fully developed.

Prompt occurrence of the augmented breaths evoked by close laryngeal challenge of serotonin might be due to stimulation of laryngeal afferents. In view of the known actions of serotonin on vagal receptors (Coleridge and Coleridge 1984) we interpret these findings as indicating that stimulation of laryngeal afferents might potentiate the reflex expiratory inhibition. Evidence from the present study does not argue clearly for the latter suggestion. Section of the superior laryngeal nerves showed tendency to diminish the expiratory inhibition evoked by close laryngeal administration of serotonin (Fig. 2), thus not completely corroborating the inhibitory contribution of laryngeal afferents to breathing.

Neurotomy of the superior laryngeal nerves had no effect on the duration of the respiratory arrest of the pulmonary chemoreflex (Fig. 2). The bolus of serotonin injected intravenously traverses the pulmonary circulation with its enormous surface area thus inducing massive activation of cardiopulmonary afferents (intrapulmonary C-fibres). In such circumstances the involvement of laryngeal afferents can not come into light.

There is general agreement that interruption of vagally mediated afferent input reduces the respiratory effects of serotonin (Reid 1952, Comroe et al. 1953, Ginzel and Kottegoda 1954, Jacobs and Comroe 1971). Our own study on the contribution of vagal afferents to post-serotonin apnoea (Szereda-Przestaszewska and Wypych 1995) as well as the experiments of the present research show that mid-cervical vagotomy substantially attenuates the expiratory arrest of breathing in the pulmonary chemoreflex evoked by administration of serotonin to the right side of circulation and in the laryngeal chemoreflex. It appears likely that excitation of the laryngeal afferents run in the superior laryngeal nerves elicited by local treatment with serotonin needs to be reinforced by the vagal input into the nodose ganglion to generate the apnoeic spells. Ablation of the latter prevents the post-serotonin apnoea (Jacobs and Comroe 1971, Yoshioka et al. 1992, Szereda-Przestaszewska and Wypych 1995).

Opening of the vagal loop precluded the increase in the respiratory rate on close laryngeal challenge of serotonin (Fig. 3) and this finding again agrues for the importance of infranodose vagi in mediating the laryngeal input. The ventilatory changes in the late phase of resumed breathing (1 min after intraarterial and intravenous challenges) consisted of increased inspiratory airflows (Fig. 4). The effects on expiratory airflows were less consistent (Fig. 5).

In keeping with the classical description, pulmonary chemoreflex is clearly dependent on afferent vagal pathways (Dawes and Comroe 1964). Given that vagal afferents mediate the timing component of the breathing cycle in serotonin-induced laryngeal chemoreflex, the effects on tidal component occur beyond the vagal loop (Figs. 4 and 5).

As noted in the introduction serotonin stimulates vagal C-fibre endings located in the lung parenchyma (Coleridge and Coleridge 1984, Kay and Armstrong 1991). There is a wealth of evidence demonstrating that afferents and somata of vagal primary C-fibre afferent neurones are endowed with 5-HT receptors (Higashi and Nishi 1982, Armstrong and Kay 1990, Meller et al. 1991, Meller et al. 1992, Mc Queen et al. 1993). Then serotonin given to pulmonary circulation might reach the receptor sites even on interrupted vagal trunks, however on condition, that supranodose vagus is preserved. Section of the latter substantially attenuates the response to serotonin (Sampson and Jaffe 1974).

These experiments do suffer from some limitations. To the best of our knowledge no studies are yet available on the effects of intravascular injection of serotonin on laryngeal receptor discharges. An indirect evidence has been brought forward in previous works, which showed that intravenous challenge of serotonin evokes laryngeal constriction in rabbits and cats (Szereda-Przestaszewska 1979, Wypych and Szereda-Przestaszewska 1994) and results in an appreciable increment of the integrated activity of the superior laryngeal nerve (Szereda--Przestaszewska 1993). In contrast to the latter it has not been found in rats (Yoshioka et al. 1994).

Perfusion with serotonin *via* the laryngeal vasculature might perhaps be expected to stimulate directly receptors distributed among the epithelial cells but also by exerting a multiplicity of actions on the vasculature to produce an additional effect on the receptor threshold.The question whether in cats serotonin activates receptors with myelinated fibres (Lee et al. 1987, Anderson et al. 1990) or scarce, with unmyelinated afferents (Miller and Loizzi 1974, Mei et al. 1980, Tsubone et al. 1990) goes beyond the scope of this study. The question of the nature of activation of laryngeal endings is not easily resolvable. Perhaps a more plausible possibility is to attribute it to 5-HT receptors present on vagal branches, which include the superior laryngeal nerves.

In conclusion: we provided evidence that afferent input from the larynx contributes merely to inhibition of breathing on close laryngeal challenge of serotonin. In renewed respiration, the timing and tidal components of the breathing pattern are mediated separately - within and beyond the vagal pathway, respectively.

ACKNOWLEDGEMENTS

We are grateful to Miss Monika Janisz for her excellent technical assistance. This work was greatly aided by the statistical advice of Dr S. Chrapusta.

REFERENCES

Anderson J.W., Sant'Ambrogio F.B., Orani G.P., Sant'Ambrogio G., Mathew O.P. (1990) Carbon dioxide - responsive laryngeal receptors in the dog. Respir. Physiol. 82: 217-226.

Armstrong D.J., Kay I.S. (1990) 5-hydroxytryptamine mediates the postembolic increase in respiratory rate in anaesthetized rabbits. Exp. Physiol. 75: 475-481.

Coleridge J.C.G., Coleridge H.M. (1984) Afferent vagal C fiber innervation of the lungs and airways and its functional significance. Rev. Physiol. Biochem. Pharmacol. 99: 1-110.

Comroe J.H.Jr., van Lingen B., Stroud R.C., Roncoroni A. (1953) Reflex and direct cardiopulmonary effects of 5-OH-Tryptamine (serotonin). Am. J. Physiol. 173: 397-386.

Dawes G.S., Comroe J.H., Jr. (1954) Chemoreflexes from the heart and lungs. Physiol. Rev. 34: 167-201.

Ginzel K.H., Kottegoda S.R. (1954) The action of 5-hydroxytryptamine and tryptamine on aortic and carotid sinus receptors in the cat. J. Physiol. (London) 123: 277-288.

Higashi H., Nishi S. (1982) 5-Hydroxytryptamine receptors of visceral primary afferent neurones on rabbit nodose ganglia. J. Physiol. (London) 323: 543-567.

Jacobs L., Comroe J.H., Jr. (1971) Reflex apnoea, bradycardia and hypotension produced by serotonin and phenyldiguanide acting on the nodose ganglia of the cat. Circ. Res. 29: 145-155.

Kay I.S., Armstrong D.J. (1991) MDL (a selective 5-HT_3 receptor antagonist) prevents stimulation of intrapulmonary C-fibres by pulmonary embolization in anaesthetized rabbits. Exp. Physiol. 76: 213-218

Lee L.Y., Sant'Ambrogio F.B., Mathew O.P., Sant'Ambrogio G. (1987) Acute effect of cigarette smoke on laryngeal receptors. J. Appl. Physiol. 62: 1575-1581.

McQueen D.S., Dashwood M.R., Spyer K.M., de B. Daly M. (1993) Identification of 5-HT receptors associated with central and peripheral projections of cat carotid chemoreceptor afferents. Abstracts of the XXXII Congress of IUPS, Glasgow, 283. 1/P.

Mei N., Condamin M., Boyer A. (1980) The composition of the vagus nerve of the cat. Cell Tissue Res. 209: 423-431.

Meller S.T., Lewis J., Ness T.J., Brody M.J., Gebhart G.F. (1991) The peripherial nociceptive actions of intravenously administered 5HT in the rat requires dual activation of both $5HT_2$ and $5HT_3$ receptor subtypes. Brain Res. 561: 61-68.

Meller S.T., Lewis S.J., Brody M.J., Gebhart G.F. (1992) Vagal afferent-mediated inhibition of nociceptive reflex by i.v. serotonin in the rat. II. Role of 5-HT receptor subtypes. Brain Res. 585: 71-86.

Miller A.J., Loizzi R.F. (1974) Anatomical and functional differentiation of superior laryngeal nerve fibers affecting swallowing and respiration. Exp. Neurol. 42: 369-387.

Reid G. (1952) Circulatory effects of 5-hydroxytryptamine. J. Physiol. (London) 118: 435-453.

Sampson S.R., Jaffe R.A. (1974) Excitatory effects of 5-hydroxytryptamine, veratridine and phenyl diguanide on sensory ganglion cells of the nodose ganglion of the cat. Life. Sci. 15: 2157-2165.

Szereda-Przestaszewska M. (1979) Laryngeal effects of serotonin in rabbits. Acta Neurobiol. Exp. 39: 241-248.

Szereda-Przestaszewska M. (1993) Contribution of laryngeal afferents to the ventilatory response to serotonin. Eur. Resp. J. (Suppl.) 17: 402s.

Szereda-Przestaszewska M., Wypych B. (1993) Generation of post-serotonin apnoea is vagally conditioned in the anaesthetized cats. Abstracts of the XIXth Congress of the Polish Physiol Soc., Toruń, p. 482.

Szereda-Przestaszewska M., Wypych B. (1995) Effects of vagal and laryngeal afferents in apnoeic response to serotonin in cats. Respir. Physiol. 101: 231-237

Tsubone H., Sant'Ambrogio G., Anderson J.W., Orani J.P. (1991) Laryngeal afferent activity and reflexes in guinea-pig. Respir. Physiol. 86: 215-231.

Widdicombe J.G. (1986) Reflexes from the upper respiratory tract. In: Handbook of physiology. The respiratory system II. Part I (Eds. N. Cherniack and J.G. Widdicombe). Am. Physiol. Soc., Washington, p. 363-394.

Wypych B., Szereda-Przestaszewska M. (1994) Laryngeal patency in respiratory responses to serotonin in anaesthetized cats. Eur. Resp. J. Suppl. 18: p. 200s.

Yoshioka M., Goda Y., Togashi H., Matsumoto M., Saito H. (1992) Pharmacological characterization of 5-hydroxytryptamine-induced apnoea in the rat. J. Pharmacol. Exp. Ther. 260: 917-924.

Yoshioka M., Goda Y.,Togashi H.,Ushiki T., Saito H. (1994) Involvement of $5\text{-}HT_3$ receptors in the initiation of pharyngeal reflex. Am. J. Physiol. 266: R1652-R1658

Received 2 February 1996, accepted 12 September 1996