LARYNGEAL EFFECTS OF SEROTONIN IN RABBITS

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Abstract. Changes in laryngeal resistance to airflow have been measured in rabbits during spontaneous breathing, while larynx was isolated "in situ". Intravenous injections of serotonin resulted in variable apnoea followed by rapid, shallow breathing, coupled with increases in laryngeal inspiratory and expiratory resistances. These laryngeal responses persisted after cutting vagi in the chest. The "central" actions of serotonin seem to be responsible for stimulatory laryngeal effects.

INTRODUCTION

Serotonin, a naturally occurring substance, is widely distributed in mammalian tissues — in enterochromaffin cells of gastrointestinal tract, in blood platelets, mast cells, spleen and the central and peripheral nervous system. Respiratory and cardiovascular actions of injected 5 HT are well established. Bronchoconstrictive, vasoconstrictive effects (on pulmonary vessels) as well as hypotensive and bradycardiac influences were described in various species. The respiratory effects consist mainly of a variably occurring apnoea followed by rapid, shallow breathing. This has been confirmed for cats (7, 13, 18, 20, 21, 23, 26), dogs (2, 17), and cattle (1). Stimulation of breathing was described as hyperpnoea in dogs (2, 5, 9) and man (22). Rabbits were found to produce rapid, labored breathing (31). Bronchoconstriction was shown in isolated lung of dogs (24), guinea-pigs (4) and by measurement of lung mechanics in dogs (2, 3, 17, 19) and cats (6, 7). Respiratory effects of 5 HT are present whether vagi are intact or cut (1, 7, 9, 13, 18, 23).
Serotonin is released from the blood platelets in anaphylactic shock in rabbits (15, 25, 31) and could be responsible for respiratory deterioration in this pathological state. It has been found previously (28) that the laryngeal constriction in anaphylactic rabbits is more pronounced after midthoracic vagotomy. Serotonin was one of putative factors responsible for that effect. To analyse this possibility we have tried to relate the respiratory changes induced by serotonin to quantitative measurements of laryngeal resistance to airflow in rabbits.

METHODS

The experiments were performed on 16 adult male rabbits, weighing 2.5–3.6 kg, anaesthetized with the mixture of urethane + chloralose (0.8 g/kg + 35 mg/kg of body weight, 3/4 of the dose i.v. and 1/4 i.m.). The animals breathed spontaneously through a cannula inserted in the low cervical trachea. A second, rostrally directed tracheal cannula, was placed just below the cricoid cartilage. The motor innervation of the larynx was preserved. Right suprahyoid pharyngotomy was performed and the epiglottis was retracted with a suture. A constant stream of humidified warm air was passed through the upper cannula and the larynx at the constant rate (1–2 l/min) and the ratio of inflow pressure to flow rate provided a measure of laryngeal resistance. The air was leaving the respiratory tract by the pharyngeal hole and possibly through the mouth and nose.

Upper tracheal pressure was measured with capacitance manometer (Hilger I.R.D.). Blood pressure was recorded from a catheter in the right femoral artery by strain gauge transducer (type S.E. 4–82, S.E. Laboratories) and carrier amplifier (type 3 C 66 Tektronix). The electrical activity of the C3 root of the right phrenic nerve was amplified (Tektronix 3 A 3 amplifier) and “integrated” with a diode pump and smoothing circuit (16). End-tidal CO2 was determined using a rapid infrared absorption meter (Godart capnograph KK). All the variables were displayed on an oscilloscope (Tektronix 565) and photographed.

We subsequently opened the chest on both sides (with artificial ventilation) and cut the vagi below the recurrent laryngeal nerves (the main motor supply to the larynx). The chest was then closed and spontaneous breathing was restored. Serotonin (Serotonin-hydrogenoxalot, Fluka AG, Buchs SG) in a dose of 0.05 mg/kg of body weight dissolved in 0.9% NaCl was injected through a catheter placed in the right femoral vein. The experimental protocol was to register the respiratory effects of serotonin in the intact and vagotomized rabbits.
RESULTS

Intact rabbits. Control values and changes in respiratory rate and in laryngeal resistance are given in Table I. The values shown for laryngeal resistance are the maximum expiratory and minimum inspiratory resistance data. Intravenous injection of serotonin in a dose of 0.05 mg/kg of body weight induced in all 16 rabbits rapid, shallow breathing. In 6 out of 16 rabbits the stimulation of breathing was preceded by a period of expiratory apnoea of 10.6 s of average duration. The mean value for the increase in laryngeal expiratory resistance during apnoea was not statistically significant. The respiratory effects of serotonin were observed in 0.75–1 s after intravenous injection. They coincided with a drop in blood pressure and variable bradycardia. The increase in breathing frequency was associated with a decrease in end-tidal CO₂ and an increase in the frequency of phrenic discharges (Fig. 1 A).

Table I

<table>
<thead>
<tr>
<th>Animals</th>
<th>n</th>
<th>Breathing frequency</th>
<th>Laryngeal resistance</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>in inspiration</td>
<td>in expiration</td>
</tr>
<tr>
<td>Intact</td>
<td>16</td>
<td>32.4±12.8</td>
<td>31.3±24.3</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td>73.2±36.0</td>
<td>39.4±29.6</td>
</tr>
<tr>
<td>Vagotomized</td>
<td>16</td>
<td>P = 0.0004</td>
<td>P = 0.04</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>P = 0.0004</td>
<td>P = 0.02</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td>29.4±7.8</td>
<td>25.1±19.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52.1±20.6</td>
<td>41.7±35.9</td>
</tr>
</tbody>
</table>

Fig. 1. Effects of intravenous injection of 0.05 mg of serotonin/kg of body weight into a rabbit with the larynx isolated “in situ”. A, intact rabbit; B, same rabbit after vagotomy. From above down: blood pressure (BP); integrated phrenic nerve activity (Int. Phrenic); end-tidal CO₂ (%CO₂) and translaryngeal pressure (P_TR). The dot above the blood pressure record shows the moment of injection. Note a drop in blood pressure, increase in breathing frequency, drop in end-tidal CO₂, and an increase in translaryngeal pressure. Note the apnoea followed by increased expiratory laryngeal pressure during rapid breathing (B).
control value for the respiratory rate was 32.4 ± 12.8 breaths/min, during rapid, shallow breathing the mean value reached 73.2 ± 36.0 breaths/min. The control values for inspiratory and expiratory laryngeal resistance were 31.3 ± 24.3 cm H₂O/l/s and 36.7 ± 26.0 cm H₂O/l/s respectively. Both values are higher than previously published control values for rabbits, but the airflow was greater and the pressure/flow relationship of the larynx is alinear (27). There was an increase in inspiratory and expiratory laryngeal resistances on serotonin injection. The inspiratory resistance increased much less than expiratory (39.4 ± 29.6 and 53.7 ± 33.7 cm H₂O/l/s respectively).

**Vagotomized rabbits.** In seven out of 16 rabbits the injection of serotonin induced an expiratory apnoea of average duration of 8.5 s. The mean value for laryngeal expiratory resistance during apnoea was 154.4 ± 136.9 cm H₂O/l/s compared to mean control value in these 7 rabbits of 43.7 ± 45.0 cm H₂O/l/s (P = 0.03). The respiratory effects of serotonin occurred in 1-2 s after an injection. During rapid, shallow breathing the respiratory rhythm raised from 29.4 ± 7.8 breaths/min (control level) to 52.1 ± 20.6 breaths/min. A decrease in end-tidal CO₂ and an increase in the frequency of phrenic nerve discharges were also observed (Fig. 1, B). Blood pressure changes were variable. The control values for laryngeal resistance in vagotomized animals differed from those for the intact animals. The inspiratory laryngeal resistance was lower (25.1 ± 19.2 cm H₂O/l/s) and the expiratory was much higher (54.6 ± 40.5 cm H₂O/l/s). This more distinct difference in inspiratory/expiratory resistance values in vagotomized animals may depend on slowing and deepening of breathing. There were increases in both inspiratory and expiratory laryngeal resistances in response to serotonin. The increase in inspiratory resistance achieved similar values (41.7 ± 35.9 cm H₂O/l/s) as that in the intact rabbits, but was much higher as compared to the control value in vagotomized animal. The rise in expiratory resistance was more than twofold (135.3 ± 85.9 cm H₂O/l/s) compared to control value and to the increase observed in the intact rabbits after serotonin (53.7 ± 33.7 cm H₂O/l/s).

**DISCUSSION**

Respiratory effects of intravenously injected serotonin in the rabbit include rapid, shallow breathing, preceded or not by a period of an expiratory apnoea. The increase in the respiratory rate in the intact

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1 All values are means and SD.
animals was coupled with an increase in inspiratory and larger increase in expiratory laryngeal resistances. These changes were not vagally dependent, which was established by their presence in vagotomized animals. It is well known that vagotomy slows down the breathing rate and increases the amplitude of respiratory movements. With greater tidal volumes the movements of the vocal cords are more intense, although in our experiments the airflow through the larynx was kept constant, the preserved motor innervation was subdue to the pattern of discharges of the respiratory centres. Rabbits, which have undergone midthoracic vagotomy, being deprived of hastening influence of the vagal nerves, presented lesser respiratory rate changes on serotonin injection, but the regulation of laryngeal lumen appeared to be more pronounced. The inspiratory laryngeal resistance increased to greater extent and constrictions of the larynx in expiration were more vigorous, showing an over twofold increase in expiratory resistance. This picture of laryngeal responses due to intravenous injection of serotonin resembles that of anaphylactic constriction of the larynx. The values for the increase in expiratory laryngeal resistance in anaphylactic shock were larger than values presented in this paper, but on both accounts they were not abolished, but then, better visible after midthoracic vagotomy (28). The lesser increase in laryngeal expiratory resistance after serotonin is believed to depend on the dose used. According to Waalkes et al. (31) the dose of 150 µg/kg of body weight of serotonin is needed to result in the concentration of this amine released in anaphylactic shock in the rabbit. Although the present work does not explain the mechanism, it adds some information on the respiratory serotonin effects in rabbits. It gives direct evidence on the behaviour of the laryngeal calibre: increase in inspiratory resistance — making laryngeal opening wider in inspiration, and enabling free ingress of air. The increase in expiratory resistance shows constriction of the larynx in expiration, which slows down the expiratory flow and prevents quick, emptying of the lungs. Larynx was found to be an important factor in determining the expiratory time and the respiratory frequency (12). Constrictions of the larynx have been described in other types of tachypnoea induced by phenylbiguanide, histamine and pneumothorax, but were shown to be lessened or abolished by midthoracic vagotomy (8, 27). Vagotomized animals displayed constrictions of the larynx in response to stimulation of peripheral and central chemoreceptors (8). Serotonin was shown to act independently of the vagal feedback and as it was found in the fine work of Jacobs and Comroe (18) — its respiratory effects are due to stimulation of chemosensitive structures within the nodose ganglia and the central structures. So any kind of vagotomy performed caudally
to these ganglia does not prevent the respiratory changes evoked by serotonin. In our experiments we have not switched off a great many of possible receptive fields stimulated by serotonin such as peripheral chemoreceptors, superior cervical ganglia, cranial nerves IX, XI and XII. This was done by Jacobs and Comroe (18) and it seems possible that strong laryngeal constrictions in vagotomized rabbits could be due to stimulating influence of serotonin on nodose ganglia, as shown for cats by these authors. There is no data describing the constrictive effects of serotonin on the striated muscles, which constitute the anatomical structure of the larynx.

In search for the mechanism responsible for the respiratory changes we cannot exclude the role of the central serotoninergic neurones. The mapping as well as the microelectrode studies provided data on serotonin neurones in the brain stem (10, 14), the site of central laryngeal neurones (11). Serotonin injected intravascularly does not penetrate the blood-brain barrier (29, 30), so there must exist a mechanism of central stimulation, which is not known as yet.

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