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INFLUENCE OF THE STIMULATION OF CENTRAL CHEMORECEPTORS ON THE GASTRIC MUCOSAL BLOOD FLOW IN ARTIFICIALLY VENTILATED AND SPONTANEOUSLY BREATHING RATS

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Respiratory failure coincides frequently with the occurrence of gastric ulceration. In advanced respiratory insufficiency hypoxemia is often accompanied by hypercapnia, which is the stimulus for central chemoreceptors as well as for carotid body chemoreceptors. The purpose of the work was to investigate the reflex effect of stimulation of central chemoreceptors on gastric mucosal blood flow (GMBF) in the rat. Central chemoreceptors were stimulated by a gas mixture composed of 10% carbon dioxide, 50% oxide and 40% nitrogen. In artificially ventilated and spontaneously breathing animals, the stimulation of central chemoreceptors caused a significant increase in gastric mucosal vascular resistance, accompanied by a marked decline in blood flow. We hypothesize that in patients with respiratory insufficiency accompanied by hypercapnia, the reflex impairment of GMBF may contribute to gastric ulceration.

Key words: central chemoreceptors, carotid body chemoreceptors, gastric mucosa, gastric mucosal blood flow, gastric ulcer.

INTRODUCTION

The higher risk of gastric ulcerations and upper gastrointestinal bleeding in patients with respiratory insufficiency is widely recognized (1, 2). Hypercapnia is a common feature of advanced respiratory failure. The main mechanism responsible for cardiovascular and respiratory adaptation to hypercapnia originates from both arterial and central chemoreceptors. The latter are located at
the surface of the ventral lateral medulla (VLM) (3,4,5). In contrast to all other neurons of the CNS, the neurons of the above region are activated by hypercapnia. Stimulation of central chemoreceptors causes an increase in ventilation and activation of sympathetic nervous system (6).

The purpose of this study was to find out if central chemoreceptor stimulation influences gastric mucosal blood flow in artificially ventilated and spontaneously breathing rats.

MATERIALS AND METHODS:

Experiments were performed on 30 Wistar Glaxo male rats (280 g - 320 g) obtained from the Animal Facilities of the Institute of Pharmaceutical Industry (Warsaw, Poland). Rats were kept 4 per standard laboratory cage at a controlled 12 hours dark and light phase with food and water available ad libitum. Rats were anaesthetized with chloral hydrate (Sigma - Aldrich Chemie GmbH, Deisenhofen, Germany) at a dose of 32 mg/kg i.p. the left femoral artery was cannulated for blood pressure measurements and the femoral vein for drug and fluid application. Blood pressure measurements were obtained using a blood pressure transducer which is a part of the Minograf 7 system (Siemens - Elema, Solna, Sweden). Tracheotomy was performed in all rats. Mechanically ventilated rats were paralyzed with pancuronium (Pavulon, Organon Teknika B.V, Boxtel, Holland) at a dose of 0,005 mg/kg b.w. and connected to a Harvard pump for small animals (Harvard Apparatus, Rodent Ventilator 683, South Natick, Massachusetts, USA). The tidal volume and frequency of the respirator were adjusted under the control of arterial blood gasometry (Automatic Blood Gas System 995L, AVL, Graz, Austria) to maintain values of pH, pCO₂ and pO₂ within normal limits. After laparotomy a small incision (6 mm) was made in the anterior curvature of forestomach using microcautery. Stomach contents were lavaged with warm saline. To avoid any effect of surgery on gastric mucosal blood flow 15 minutes were allowed to pass before any measurements were taken. Gastric mucosal blood flow was measured using a Laser Doppler Flowmeter (ALF 21, Transonic, System Inc., Ithaca NY, USA). An N type laser flow probe was placed in a balance arm holder (Kanatec, Transonic System Inc., Ithaca NY, USA) and positioned above the gastric mucosa. Mean blood pressure (MAP) and gastric mucosal blood flow (GMBF - ml/min/100g) were recorded continuously on a Siemens - Elema chart recorder. Values of gastric mucosal vascular resistance (GMVR) were calculated from GMBF and MAP (mmHg/ml/min/100g). Rectal temperature was kept constant at 38.0° C during the experiment.

In the group of artificially ventilated animals (15 rats), GMBF and MAP were measured before and one minute after the respirator was connected to the gas mixture (carbon dioxide, 50% oxygen and 40% nitrogen).

In order to denervate carotid body chemoreceptors, carotid bifurcations were bilaterally exposed and sinuses nerves were identified and cut under 25 x magnification. In order to identify efferent mechanisms responsible for GMBF changes an a-receptor antagonist, phenolamine, was applied at a dose 2,5 mg/kg i.v. (Regitine, Ciba, USA). Vagus nerves were cut bilaterally and atropine was used at a dose 0,5 mg/kg (Athropinum Sulfuricum, Polfa, Poland). In spontaneously breathing animals (15 rats) a hypercapnic - hyperoxic gas mixture was applied through a tube of 1 cm diameter placed over the tracheostomic cannula. Care was taken to keep the pressure of inspiratory gas between 1 - 2 cm H2O.

All data are presented as means ± SEM. As the effect of the given stimulus on the measured parameters was significantly higher than individual variability within the groups, instead of paired data analysis we used Student t-test for unpaired data.

The study was accepted by the local ethics committee.
RESULTS

Artificially ventilated rats

Use of the hypercapnic - hyperoxic gas mixture (n=15) caused a decrease in GMBF from 29.46 ± 0.82 ml/min/100g (Fig. 1) to 23.3 ± 0.97 ml/min/100g (p<0.001). Changes in blood gas values are presented in Table 1. MAP rose from 91.4 ± 3.5 mmHg to 110.1 ± 4.24 mmHg (p<0.01). The calculated GMVR rose from 3.1 ± 0.098 mmHg/ml/min/100g to 4.9 ± 0.029 mmHg/ml/min/100g. Hypercapnia - hyperoxia caused similar changes in rats (n=7) after bilateral sinus nerve dissection to those observed in intact nerves (Fig. 1).

Following the phentolamine infusion (n=7), the hypercapnic - hyperoxic gas mixture caused an increase in GMBF from 36 ± 2.33 ml/min/100g to 45.2 ± 3.1 ml/min/100g (p<0.01) (Fig. 2). At the same time MAP decreased from 70.3 ± 3.26 mmHg to 62.4 ± 4.0 mmHg (p<0.05). GMVR also decreased from 1.95 ± 0.3 mmHg/ml/min/100g to 1.38 ± 0.27 mmHg/ml/min/100g (p<0.01). Bilateral cutting of vagus nerves (n=8) did not cause changes in GMBF in normoxic conditions 32.1 ± 3.0 ml/min/100g vs. 28.94 ± 2.5 ml/min/100g n.s. Blocking of muscarinic receptors did not evoke changes in GMBF and GMVR in control conditions either. Hypercapnia - hyperoxia after bilateral vagotomy caused a decrease in GMBF from 28 ± 1.5 ml/min/100g to 22 ± 2.4 ml/min/100g (Fig. 3). A similar situation occurred when atropine was injected.

Spontaneously breathing animals

In spontaneously breathing animals, similar changes in GMBF and GMVR were observed during stimulation of central chemoreceptors with hypercapnic-hyperoxic gas mixture. GMBF decreased from 29.5 ± 2.1 ml/min/100g to 24.1 ± 1.8 ml/min/100g (p<0.01) respectively. At the same time MAP increased from

![Fig. 1 Influence of hypercapnia and hyperoxia on GMBF in artificially ventilated rats. A) GMBF in control conditions (blank); B) GMBF during hypercapnia and hyperoxia (shadowed); C) GMBF in normoxic and normocapnic conditions after the sinus nerve was severed (hatched); D) GMBF after the sinus nerve dissection (crosshatched). * p < 0.01 compared to A,C.](image-url)
Table 1 pH, pO₂, pCO₂ value changes in artificially and spontaneously ventilated rats. Central chemoreceptors were stimulated using a hyperoxic-hypercapnic gas mixture * p < 0.05.

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<th>Artificially ventilated rats</th>
<th>Spontaneously breathing rats</th>
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<tr>
<td></td>
<td>control group</td>
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<tr>
<td>pH</td>
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<tr>
<td>7.39 ± 0.03</td>
<td>7.18 ± 0.04 *</td>
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<td>pO₂</td>
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<td>92.6 ± 3.8</td>
<td>394.3 ± 7.9 *</td>
<td>88.2 ± 3.9</td>
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<td>pCO₂</td>
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<td>42.6 ± 2.5</td>
<td>69.9 ± 3.4 *</td>
<td>37.8 ± 3.1</td>
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Fig. 2 Influence of hypercapnia and hyperoxia on GMBF following phentolamine administration. A) GMBF in control conditions before phentolamine administration; B) GMBF in normoxic and normocapnic conditions after phentolamine infusion; C) GMBF during hypercapnia and hyperoxia after phentolamine * p < 0.01 compared to A, # p < 0.01 compared to B.

81.2 ± 4.2 mmHg to 102.6 ± 3.9 mmHg (p<0.01). The calculated GMVR increased from 2.75 ± 0.35 mmHg/ml/min/100g to 4.25 ± 0.23 mmHg/ml/min/100g (p<0.01). Sinus nerve severence, phentolamine, vagus nerve dissection and atropine evoked the same changes as observed in artificially breathing animals.
DISCUSSION

In the past decade gastric mucosal blood flow became recognized as an important and intensively investigated factor in the pathophysiology of gastric mucosal damage (7, 8, 9). It was shown that the stimulation of sympathetic fibers decreases gastric blood flow whereas the opposite is true in the case of stimulation of efferent vagal fibers. In 1993 Kimura et al. (10) demonstrated that carotid body stimulation causes activation of sympathetic and parasympathetic fibers innervating the stomach. Siński et al. (11, 12) found that the stimulation of carotid body chemoreceptors in artificially ventilated rats results in a profound decrease in gastric mucosal blood flow and in an increase in gastric mucosal vascular resistance.

The substitution of a hypercapnic - hyperoxic gas mixture for room air is recognized as a selective stimulus for central chemoreceptors. Results of the present study indicate that stimulation of central chemoreceptors evokes constrictions of mucosal vessels in either spontaneously or artificially ventilated animals. Reaction is abolished by $\alpha$-receptor blocking but not by vagotomy. It is worth to note that changes in GMBF during the stimulation of central chemoreceptors were not dependent on the ventilation type (spontaneous or artificial). This finding is different from what we found during the stimulation of carotid body chemoreceptors (12). The impairment of gastric mucosal blood flow was observed only in artificially ventilated animals, whereas in spontaneously breathing rats hypoxemia caused the opposite effect, namely a increase in GMBF. Stimulation of pulmonary stretch receptors was designated as a causative factor responsible for the observed differences between spontaneously and artificially breathing animals.

In respiratory failure arterial hypercapnia is always accompanied by hypoxemia. Therefore both central and peripheral chemoreceptors are activated.

Fig. 3 Influence of hypoxia and acid saline solution injection on GMBF after atropine vagotomy. A) GMBF in control conditions before vagotomy; B) GMBF in normoxia and normocapnia after vagotomy; C) GMBF after vagotomy and during hypercapnia and hyperoxia. * $p < 0,001$ compared to A, B.
The question arises: why during reflex hyperventilation due to hypercapnia in spontaneously ventilated rats no pulmonary mechanoreceptor derived dilation of mucosal arteries was observed like it was found in the case of stimulation of carotid body chemoreceptors (12)? The most probable mechanism responsible for this finding is that hypercapnia decreases the sensitivity of slowly adapting pulmonary mechanoreceptors (13, 14).

If the obtained results apply to humans as well, we can form a hypothesis that in patients with respiratory insufficiency accompanied by hypercapnia, the reflex impairment of GMBF may contribute to gastric ulceration.

REFERENCES


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