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SPECIAL REPORT

Release of 5-hydroxytryptamine by hypoxia from epithelioid cells of chicken thoracic aorta

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Epithelioid cells in the chicken thoracic aorta are shown to contain 5-hydroxytryptamine (5-HT) in immunocytochemical studies. To determine whether these cells act as chemoreceptors, as do type I cells of the carotid body, we examined the effects of hypoxia and acidosis on the release of 5-HT from the chicken thoracic aorta. Hypoxia caused the output of 5-HT in incubation medium. A reduction of pH to 6.8 failed to evoke 5-HT release. The response to hypoxia was inhibited by the removal of extracellular Ca^{2+} and by nifedipine and ω-conotoxin GVIA. These results suggest that epithelioid cells in the chicken thoracic aorta are chemoreceptors which sense a decrease in P_{O_2} and then release 5-HT by Ca^{2+} influx through voltage-dependent L- and N-type Ca^{2+} channels. The epithelioid cells in the chicken aorta may be a useful model for pharmacological and physiological studies of 5-HT-containing cells.

Keywords: Chick; aortic body; epithelioid cells; 5-HT-containing cells; 5-HT release; chemoreceptor; hypercapnia; hypoxia

Introduction There are two main chemoreceptor organs in the peripheral tissue: the carotid body located in the carotid bifurcation and the aortic body located in the wall of the aorta. It is generally accepted that chemoreceptor cells of the carotid body, sensing changes in plasma P_{O_2}, P_{CO_2} and pH, release transmitters which activate the sensory nerve endings of the carotid sinus nerves. Catecholamines such as dopamine and/or noradrenaline contained in chemoreceptor cells are putative transmitters in various species (Gonzalez *et al*., 1994).

There is little knowledge to date about the characteristics of chemoreceptor cells in the aortic body. It has been reported that the epithelioid cells in the luminal wall of the chicken thoracic aorta contain 5-hydroxytryptamine (5-HT) (Yamamoto *et al*., 1989) that aggregated into clusters and formed a band about 1 mm in width (Miyoshi *et al*., 1995). The aim of the present experiment was to determine whether 5-HT-containing epithelioid cells are aortic chemoreceptors. For this purpose, we examined the effects of hypoxia and acidosis on the release of 5-HT from the chicken thoracic aorta.

Methods Male chicks (day 14–18 after hatching) were anaesthetized with ether and then decapitated. The thoracic aortae were removed and freed from surrounding tissues. The aortic strips (3–4 mm in length) containing epithelioid cells were cut longitudinally to open their lumen.

Two physiological solutions of the following composition were used (mM): a HEPES-buffered solution: NaCl, 144; KCl, 4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; glucose, 5.5; HEPES, 5. (pH, 7.3 or 6.8 with NaOH), and a HCO_3-CO_2-buffered solution: NaCl, 127; KCl, 4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; glucose, 5.5; $NaHCO_3$, 22.5; HEPES, 5 (pH 7.3 with 5% CO_2 or pH 6.8 with 20% CO_2). The solutions were aerated with 100% O_2, 20% CO_2/80% O_2 or 5% CO_2/95% O_2 under oxygenated condition. Under hypoxic condition, the 5% CO_2/95% O_2 was replaced with 5% CO_2/95% N_2. In Ca^{2+}-free solution, $CaCl_2$ was omitted and 0.5 mM EGTA was added. The aortic strips were put into physiological solution (0.1 ml) on ice and the air phase of the sample tube was filled with appropriate gas. The strips were then incubated at 37°C for 10 min to release 5-HT. The secretory responses were terminated by the placement of the tubes on ice. The medium was acidified with perchloric acid (PCA; 0.4 N) after the removal of the aortic strip. The strip was treated with 0.4 N PCA to measure the amount of 5-HT left in the tissue. These acidified media were centrifuged and treated with K_2HPO_4 (290 mM). After centrifugation, each supernatant underwent high-performance liquid chromatography (HPLC; JASCO Corp., Hachiouzi, Japan). 5-HT and its metabolite, 5-hydroxyindole acetic acid (5-HIAA) were separated with an ODS-column (Catecholpak, JASCO Corp.) and detected by an electrochemical detector (Eicom, Kyoto, Japan). The composition of the mobile phase was KH_2PO_4-H_3PO_4 buffer, 100 mM (pH 3.5); EDTA, 40 μM; sodium octasulfonic acid, 1.16 mM and methanol, 15–17%. All data are expressed as mean ± s.e.mean. Statisitical analysis of the data was performed by an unpaired Student's t test. Differences with $P<0.05$ were considered significant.

Results In the PCA extract of the chicken thoracic aorta, 5-HT was dominant. Noradrenaline, dopamine and 5-HIAA were less than 5-HT at all days tested (Figure 1a). The content of 5-HT increased gradually from day 7 to 13 after hatching and then attained a plateau (Figure 1b). The aortic strip was incubated with solution containing 5-HT (1 μM) at 37°C for various times to determine the metabolic rate for 5-HT in the presence of the tissue. The amount of exogenous 5-HT decreased and that of 5-HIAA increased to 5–9% of 5-HT at the end of a 10-min incubation period. Therefore, the amount of 5-HT released from the aorta was expressed as the sum of amounts of 5-HT and 5-HIAA in the subsequent experiments.

When the aortic strips were incubated with bicarbonate-containing solution at pH 7.3 for 10 min at 37°C under the oxygenated condition (Figure 2), a spontaneous output of 5-HT occurred, which was decreased by the remvoval of extracellular Ca^{2+} ($P<0.05$). A decrease in the pH to 6.8 failed to evoke a singificant 5-HT output. In the bicarbonate-free, HEPES-buffered solution with 100% O_2 acidosis did not elicit 5-HT output (pH 7.3, 8.66 ± 0.56%, $n=20$; pH 6.8, 6.05 ± 0.52%, $n=14$). In contrast, hypoxia at pH 7.3 produced a significant release of 5-HT. The secretory responses to hypoxia were abolished by the removal of extacellular Ca^{2+} and were partly reduced by nifedipine (1 μM) or ω-conotoxin GVIA (1 μM) but not by ω-agatoxin IVA (1 μM). The secretory response was greatly inhibited by nifedipine and ω-conotoxin GVIA in combination.

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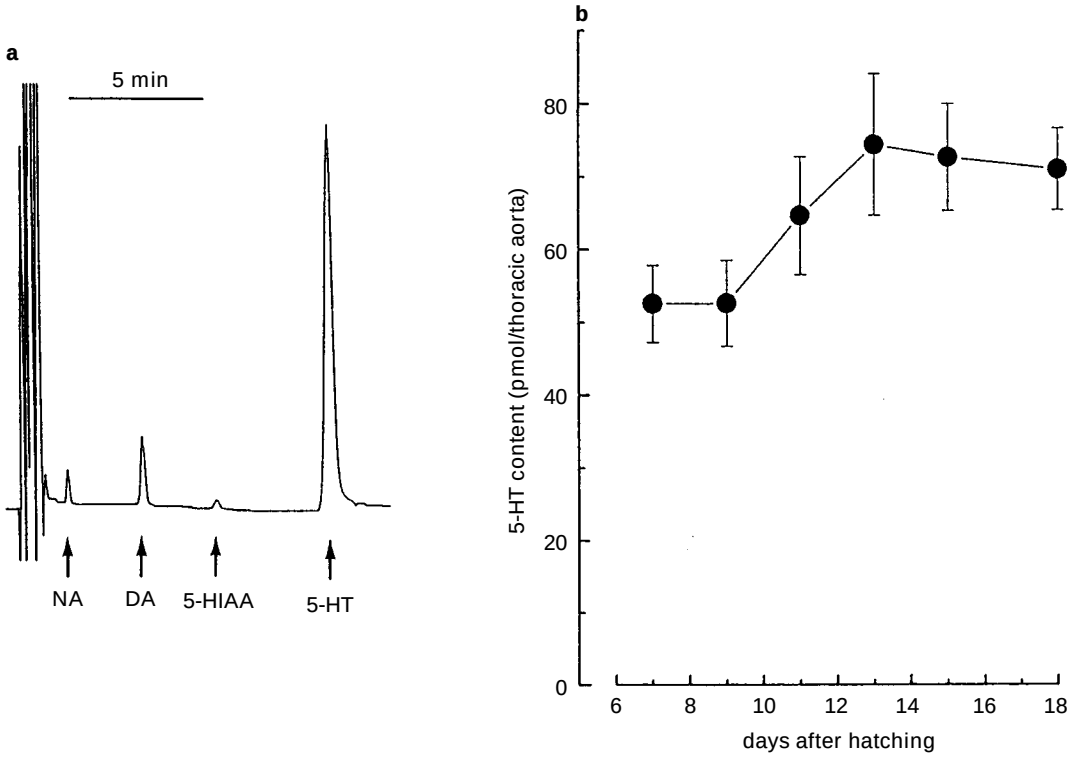

Figure 1 The HPLC profile of the perchloric acid extract of the chicken thoracic aorta (a) at day 18 after hatching and (b) changes in 5-HT content in the thoracic aorta from day 7 to 18 after hatching. 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindole acetic acid; DA: dopamine; NA: noradrenaline. Each symbol indicates the mean ± s.e.mean ($n = 9-22$).

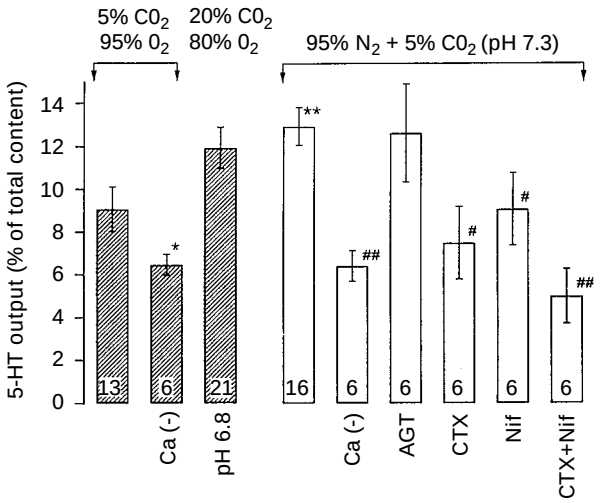

Figure 2 Summary of the output of 5-HT from the chicken aortic strips under various conditions. 5-HT output is expressed as a percentage of the total content of 5-HT in the tissue. Striped and open columns indicate responses under oxygenated and hypoxic conditions, respectively. Gas mixtures used are shown above the columns. The pH of the solution was 7.3 when 5% CO_2 was used. The pH was decreased to 6.8 by 20% CO_2. Ca (−) represents the removal of Ca^{2+} from the medium. ω-Agatoxin IVA (AGT), ω-conotoxin GVIA (CTX) or nifedipine (Nif) was added to the incubation medium at 1 μM or in combination. Numbers in the columns indicate the number of experiments. Symbols near the columns represent significant differences from the control at pH 7.3 under the oxygenated condition ($^{*}P<0.05$, $^{**}P<0.01$) and the control at pH 7.3 under the hypoxic condition ($^{\#}P<0.05$, $^{\#\#}P<0.01$).

Discussion

The present results demonstrated that epithelioid cells in the chicken thoracic aorta are capable of releasing 5-HT in response to hypoxia. This suggests that 5-HT-containing epithelioid cells play a role as chemoreceptors in the chick corresponding to chemoreceptor cells in the mammalian aortic body. In chemoreceptor cells of the rat carotid body, it has been shown that L-type Ca^{2+} channels are activated by hypercapnic acidosis (Buckler & Vaughan-Jones, 1994), and both L- and N-type Ca^{2+} channels have been seen in voltage-clamp experiments (eSilva & Lewis, 1995). The release of 5-HT in response to hypoxia seems to depend upon depolarization which activates Ca^{2+} influx through voltage-dependent L- and N-type Ca^{2+} channels in epithelioid cells of the chicken aorta. Acidic stimulation has been shown to release ^{3}H-dopamine from the chemoreceptor cells in the carotid body, which is more effective in the presence of bicarbonate than in its absence (Rigual *et al*., 1991). However, acidic stimulation failed to release 5-HT from aortic epithelioid cells in the chick. It has been reported that afferent stimulation of the aortic nerve does not modify phrenic nerve discharges in the anaesthetized dog (Hopp *et al*., 1991). It therefore seems likely that the aortic chemoreceptor cells have a different function from the carotid chemoreceptor cells.

Compared with our knowledge about the pharmacology of catecholamine turnover such as synthesis, release and uptake, less is known about 5-HT turnover. It is difficult to investigate 5-HT turnover quantitatively because 5-HT-containing cells such as enterochromaffin cells lie scattered in the gastrointestinal tract. In the central nervous system, the complicated neural network makes it difficult to evaluate the characteristics of 5-HT-containing neurons. Therefore, the epithelioid cells in the thoracic aorta of the chick may provide a great advantage for pharmacological and physiological studies of 5-HT-containing cells.

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