Influence of Ouabain on the Tracheal Musculature of the Dog

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Abstract—The effects of ouabain on the smooth muscles of the airway were investigated in anesthetized, paralyzed and artificially ventilated mongrel dogs. Ouabain (30 μg/kg, i.v.) caused a constriction of the tracheal smooth muscle which was followed by bradycardia. When ouabain was infused at a rate of 2 μg/kg/min (i.v.), the tracheal constriction was induced by a total dose of 45.0±5.5 μg/kg, while the bradycardia appeared with a total dose of 54.4±6.1 μg/kg. The ouabain-induced tracheal constriction was inhibited by bilateral vagotomy. The tracheal constriction induced by i.a. infusion of 10 μM ouabain into the bilateral cranial thyroid arteries was inhibited by bilateral vagotomy, but it was not completely blocked. With bilateral vagotomy, the tracheal constriction induced by i.a. infusion of ouabain was unaffected by 3 μM hexamethonium, but it was significantly inhibited by 1 μM atropine. These results suggest that ouabain may induce tracheal constriction by a neurogenic action in addition to its action via the augmentation of the vagal reflex, and the neurogenic action of ouabain may be related, in large part, to the release of acetylcholine from the presynapses of vagus nerves in dogs.

Cardiac glycosides, which are known to inhibit specifically the Na⁺,K⁺-ATPase activity of cell membranes, are used most frequently to increase the adequacy of the circulation in patients with congestive heart failure. Inhibition of Na⁺,K⁺-ATPase activity also induces contractions in various types of isolated smooth muscles (1–3), including airway smooth muscle (4, 5). In situ, therapeutic amounts of cardiac glycosides are known to enhance efferent vagal activity to the heart by means of the following mechanisms: 1) increase in the frequency of discharges from carotid sinus nerves (6); 2) effects on nodose ganglia and the central vagal nucleus (7); and 3) alterations in the excitability of vagal efferent nerve fibers (8).

Present evidence indicates that the vagal reflex-induced constriction of the airway plays an important role in asthmatic attacks (9–11). It is therefore satisfactory to consider that cardiac glycosides should be used cautiously in patients with asthma. Although the potentiating effect of cardiac glycosides on the efferent vagal activity in the heart is recognized as an established fact, there are few reports of investigations that relate to the airway. In the present study, we examined the effects of ouabain on the tracheal musculature of the dog, using this preparation to measure the vagal reflex-induced tracheal response, as described previously (12).

Materials and Methods
Thirty-five male mongrel dogs weighing between 8 and 21 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Experiments were carried out with the preparation used for the evaluation of the vagal reflex-induced responses of the airway, as described previously (12). Briefly, the cervical trachea was exposed and then transected at a site about 7 cm caudal to the larynx with the membranous wall left intact. Care was taken not to obstruct the recurrent laryngeal nerves. The membranous wall at the transected site was ligated with a thread to interrupt the blood flow across the wall. A tracheal cannula was inserted into the caudal side of the transected trachea. The animals breathed or were ven-
tilated through this cannula. The left femoral artery was cannulated. The systemic arterial blood pressure was measured from a femoral arterial catheter with a pressure transducer (Nihon Kohden, MPU-0.5). The heart rate was measured with a tachometer (Nihon Kohden, RT-5), using the systolic blood pressure as the trigger.

The animals were immobilized with decamethonium bromide (initial dose of 0.4 mg/kg, i.v., and supplemental doses of 0.2 mg/kg, i.v., at hourly intervals) and ventilated artificially with room air through the tracheal cannula connected to an artificial respirator (Shinano, SN-480-4) at a constant volume and a frequency of 20 breaths/min. End-tidal concentrations of CO₂ and O₂ were continuously monitored with an expired-gas monitor (San-Ei, 1H21) and were maintained at an optimal ventilation level under the resting condition.

Responses of the tracheal musculature were measured as the change in the intratracheal pressure of the air-filled balloon introduced into the rostral side of the transected trachea. The air-filled balloon was connected to a pressure transducer (Nihon Kohden, LPU-0.1) through polyethylene tubing. The volume of air in the balloon was adjusted initially to give a resting intraluminal pressure of 50 mmH₂O. Recordings were made on a polygraph (Nihon Kohden, RM-85). One hour was allowed for stabilization of the preparation after completion of the operation.

Drugs were administered intravenously through the cephalic vein or intra-arterially into the bilateral cranial thyroid arteries. For intravenous administration, ouabain was administered by single injection or was infused at a rate of 2 μg/kg/min with an infusion pump (Natume, KN-202). To administer the drugs directly at the site where the tracheal constriction was measured, we used the preparation for perfusion in situ of the upper trachea, as described previously (13). The upper cervical region was incised at the midline, and the bilateral cranial thyroid arteries, the main arteries supplying the upper portion of the trachea, and their branches were exposed and carefully dissected free. The muscular, pharyngeal and cricothyroid branches of the bilateral cranial thyroid arteries were all ligated, with the cranial thyroid arteries left intact. The bilateral cranial thyroid arteries were cannulated and perfused with arterial blood delivered from the right femoral artery with a micro-tube pump (Tokyo Rikakikai, MP-1011). The perfusion pressure was measured between the pump and the perfused artery with a pressure transducer (Nihon Kohden, MPU-0.5). A constant-flow perfusion was carried out and the flow was adjusted at the beginning of each experiment so that the perfusion pressure was approximately equal to the systemic arterial blood pressure. Just before the start of the perfusion, the animal was given heparin sodium, 800 units/kg, i.v.; and 200 units/kg of heparin sodium were additionally given i.v. at hourly intervals. A bilateral vagotomy was performed by section of the bilateral vagus, superior laryngeal and recurrent laryngeal nerves.

Drugs used were ouabain octahydrate (Sigma), hexamethonium dihydrochloride (Wako) and atropine sulfate (Sigma). All doses are expressed as final concentrations in terms of the respective base. All drugs were dissolved in saline solution. Solutions of ouabain, hexamethonium and atropine were infused into the bilateral cranial thyroid arteries at a rate of 50 μl/min to give final concentrations of 10, 3 and 1 μM, respectively. Infusions of hexamethonium and atropine were begun 10 min before infusion of ouabain.

The results shown in the figures and in the text are expressed as mean values±S.E. Multiple comparisons were performed using Scheffe’s test. The differences were considered significant at P<0.05.

Results

Effects of ouabain administered by intravenous injection and by infusing on the tracheal musculature: Intravenous injection and infusion of saline solution had no effect on the measured parameters. Typical recordings of the changes in systemic blood pressure, heart rate and intratracheal pressure after a single intravenous injection of ouabain are shown in Fig. 1. Ouabain at the doses of 10 and 20 μg/kg (i.v.) caused a slight increase in blood pressure, but it had no effect on the heart rate and intratracheal pres-
sure. A dose of 30 μg/kg (i.v.) of ouabain increased the blood pressure and intratracheal pressure, and these increases were accompanied by bradycardia and arrhythmia. The bradycardia occurred after a brief lag period subsequent to the tracheal constriction. This

Fig. 1. Effects of ouabain on systemic blood pressure (B.P.), heart rate (H.R.) and intratracheal pressure (I.P.). Ouabain was injected i.v. at a dose of 10, 20 and 30 μg/kg into the cephalic vein.

Fig. 2. Effects of the infusion of ouabain on systemic blood pressure (B.P.), heart rate (H.R.) and intratracheal pressure (I.P.). Ouabain was infused i.v. at the rate of 2 μg/kg/min into the cephalic vein. Tracheal constriction and bradycardia occurred at total doses of 45.0±5.5 μg/kg (n=5) and 54.4±6.1 μg/kg (n=5), respectively.
experiment was performed in a total of five animals.

As shown in Fig. 2, ouabain was also infused intravenously. The systemic blood pressure began to increase gradually immediately after the infusion of ouabain at the rate of 2 μg/kg/min. Tracheal constriction occurred at a total dose of 45.0±5.5 μg/kg (n=5) of ouabain. When the total dose of ouabain reached 54.4±6.1 μg/kg (n=5), a decrease in heart rate occurred.

The tracheal constriction induced by ouabain was inhibited by about 50% by bilateral vagotomy. In contrast, ouabain caused no decrease in heart rate after the bilateral vagotomy.

Effects of ouabain administered by close i.a. infusion into the bilateral cranial thyroid arteries on the tracheal musculature: Typical recordings of the changes in intratracheal pressure by i.a. infusion of 10 μM ouabain into the bilateral cranial thyroid arteries are shown in Fig. 3. Ouabain infusion caused an increase in intratracheal pressure. The maximum magnitude of the tracheal constriction induced by 10 μM ouabain was 320.0±30.6 mmH₂O (n=5). An increase in systemic blood pressure, bradycardia and arrhythmia occurred after a brief lag period subsequent to the tracheal constriction, but the bradycardia was a weak response as compared with that observed when ouabain was administered intravenously.

Bilateral vagotomy inhibited the maximum magnitude of the tracheal constriction induced by ouabain infusion (i.a.) by about 50% (Figs. 3 and 4). The increase in systemic blood pressure and the arrhythmia were unaffected by the bilateral vagotomy, while the bradycardia disappeared completely (data not shown). The tracheal constriction that remained after bilateral vagotomy was unaffected by an i.a. infusion of 3 μM hexamethonium (Fig. 5). In contrast, an i.a. infusion of 1 μM atropine almost abolished the tracheal constriction induced by ouabain after bilateral vagotomy (Fig. 5). Infusion of neither hexamethonium nor atropine had any significant effect on the measured parameters.

Discussion

The i.v. injection of ouabain resulted in a sustained constriction of the tracheal smooth muscle, accompanied by bradycardia. Furthermore, in the case of i.v. infusion of ouabain, there was a differential tendency with respect to the total dose of ouabain between the appearance of tracheal constriction and bradycardia. In a previous report (12), we demonstrated that inhalation of histamine, over a range of concentrations from 0.000625–0.0025%, caused a vagal reflex-induced tracheal constriction before there was a measurable change in blood pressure and heart rate. Although intracerebroventricular administration of histamine induced the vagally mediated tracheal constriction in guinea pigs (14) and dogs (15), there were no
Fig. 4. Effects of bilateral vagotomy on the tracheal constriction induced by infusion of ouabain. Ouabain was infused i.a. at a final concentration of 10 μM. Bilateral vagotomy was performed by sectioning of the bilateral vagus, superior laryngeal and recurrent laryngeal nerves. Each column shows the mean value with S.E. of results from five experiments. The change is significant at **P<0.01 when compared to the value of the control.

Fig. 5. Effects of hexamethonium (C₆) and atropine on the tracheal constriction induced by ouabain after bilateral vagotomy. Infusions of C₆ and atropine were begun 10 min before infusion of ouabain. Ouabain was infused i.a. at a final concentration of 10 μM. Each column shows the mean value with S.E. of results from five experiments. The change is significant at ***P<0.001 when compared to the value of the control.
changes in blood pressure and heart rate. These findings indicate that a difference in sensitivity to vagal activity exists between the smooth muscle of the airway and the heart.

The tracheal constriction induced by ouabain was significantly inhibited by bilateral vagotomy, but it was not completely blocked. The bilateral vagotomy was performed by sectioning not only of the bilateral cervical vagus nerves but also the bilateral superior laryngeal and recurrent laryngeal nerves. In a previous report, we showed that a complete inhibition of vagal reflex-induced tracheal constriction was not observed with a bilateral vagal blockade alone (12). It is well-known that afferent fibers exist in the superior laryngeal nerve. Several investigators have reported the existence of afferent fibers in the recurrent laryngeal nerve (16-18). We found previously (12) that the reflex tracheal constriction is completely blocked by the combination of a vagal blockade and sectioning of the superior laryngeal nerves, indicating that the extravagal pathway plays a role in some part of the afferent pathway of the vagal reflex responses of the airway. In the present study, therefore, the bilateral cervical vagus, superior laryngeal and recurrent laryngeal nerves were cut to effect a complete vagal blockade.

To investigate further the characteristics of the residual tracheal constriction after the bilateral vagotomy, we infused ouabain directly at the site where the tracheal constriction was measured. Infusion of ouabain induced a sustained constriction of the trachea, accompanied by an increase in systemic blood pressure, bradycardia and arrhythmia. The ouabain-induced tracheal constriction was, in part, inhibited by bilateral vagotomy, while the increase in systemic blood pressure and arrhythmia remained. The inhibited constrictive response and increase in systemic blood pressure and arrhythmia observed after a brief lag period subsequent to the tracheal constriction may be due to a systemic effect of accumulated ouabain, because the bradycardia disappeared after bilateral vagotomy.

The tracheal constriction that remained after bilateral vagotomy may be due to a direct action of ouabain. It has been reported that ouabain induces contraction in isolated smooth muscle from the airway of humans and other species (5). Moreover, it has been shown that perturbations in the activity of the Na⁺ electrogenic pump can readily affect the contractile state of airway smooth muscle, with relaxation and contraction being typically elicited under conditions associated with stimulation and inhibition of the electrogenic pump, respectively (19, 20). Although it is known that an active electrogenic pump is present in the smooth muscle of the guinea pig and bovine airway (19), it remains to be determined whether the Na⁺ electrogenic pump is present in the canine trachea. It has been reported that a K⁺-free solution has no effect on the isolated canine trachea (5). In the present study, the tracheal constriction that remained after bilateral vagotomy was almost completely abolished by atropine. Therefore, the tracheal constriction induced by ouabain bilateral vagotomy seems not to be related, or to be related to a lesser extent, to the Na⁺ electrogenic pump in the canine trachea, and it may be involved in the effect of acetylcholine released from cholinergic terminals.

In the vascular smooth muscle of many species, ouabain causes an increase in the spontaneous release of norepinephrine (21, 22). In contrast, ouabain enhances the release of acetylcholine from Auerbach's plexus of the guinea pig ileum (23). In the airway system, the influence of ouabain on the release of acetylcholine is not clear. In the present study, atropine almost completely abolished the tracheal constriction induced by ouabain after bilateral vagotomy, while the tracheal constriction induced by ouabain was unaffected by hexamethonium. The site of action of ouabain may, therefore, be a presynaptic site of a cholinergic terminal. Although it is necessary to extend these investigations to the in vitro situation, it is possible that ouabain may release acetylcholine from cholinergic presynapses.

Canine tracheal smooth muscles are innervated by cholinergic excitatory and adrenergic inhibitory systems (24, 25), but not by non-adrenergic and non-cholinergic systems (26–28). In the present study, the effect of ouabain on the release of norepi-
nephrine was not investigated. The tracheal smooth muscle in the dog is thought to have mainly β-receptors, but there is evidence that α-receptors are also present. The α-receptors that subserve tracheal constriction should have been revealed by a blockade of β-receptors by a β-blocker. Therefore, it appears that constriction mediated by α-receptors is not involved in the present study.

In conclusion, these findings suggest that ouabain may induce tracheal constriction by some neurogenic action in addition to its augmentation of the vagal reflex, and the neurogenic action of ouabain may be related, in large part, to the release of acetylcholine from the presynapses of the vagus nerves.

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