Autonomic nervous control of venous pressure and secretion in submandibular gland of anesthetized dogs

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Lung, Mary A. Autonomic nervous control of venous pressure and secretion in submandibular gland of anesthetized dogs. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G331–G341, 1998.—In dogs anesthetized with pentobarbital sodium, hilar venous pressure (P_{hv}) and secretion were measured from the submandibular gland receiving spontaneous blood flow or vascular perfusion at the normal resting flow rate. Parasympathetic nerve stimulation and ACh-induced secretion increased P_{hv} and its pulse pressure; P_{hv} also showed an obvious arterial (or perfusion pressure)-like waveform. Vasoactive intestinal polypeptide (VIP) exerted also showed an obvious arterial (or perfusion pressure)-like waveform. Vasoactive intestinal polypeptide (VIP) exerted similar effects on P_{hv} but produced negligible secretion. Sympathetic nerve stimulation, phenylephrine, and clonidine did not induce secretion and had no significant action on P_{hv}, whereas isoproterenol provoked secretion and changed P_{hv} as with parasympathetic stimulation. Background or superimposed sympathetic nerve stimulation reduced the parasympathetic nerve-induced responses; the sympathetic inhibition was abolished by phentolamine and yohimbine but not by prazosin and propranolol. The results suggest a direct relationship between P_{hv} and secretion during parasympathetic salivation: the elevation in P_{hv} was primarily independent of the concurrent blood flow response, mediated via muscarinic and peptidergic mechanisms, and related to an opening of arteriovenous anastomoses. Sympathetic inhibition of parasympathetic salivation may be related to prevention of an increased P_{hv} exerted primarily via the α_{2}-adrenergic mechanism.

parasympathetic salivation; α- and β-adrenergic mechanisms; muscarinic and peptidergic receptors; arteriovenous anastomoses

PARASYMPATHETIC NERVE stimulation causes profuse secretion and an increase in blood flow in salivary glands of experimental animals (3, 16). We have found in the dog submandibular gland that salivary flow in response to a short period of parasympathetic nerve stimulation over a wide range of stimulus frequencies is independent of the change in blood flow, indicating that the concomitant blood flow response may not be necessary for supporting the secretory response of an actively secreting gland (12). It is now well known that electrolyte transport in one form or another in the acinar cells is fundamental to the formation of primary saliva (23, 30). However, we cannot rule out the fact that the actively secreting acinar cells must have an adequate amount of fluid supply. Fluid movement across the capillary walls depends on the total transcapillary pressure, including both hydrostatic and oncotic pressure; net filtration requires hydrostatic pressure to be larger than oncotic pressure (19). Fluid efflux from a capillary normally results in an increase in oncotic pressure along the vessel. It is therefore crucial to know the mechanism that maintains an adequate hydrostatic pressure in the capillaries for net fluid filtration during short periods of profuse salivary secretion when the oncotic pressure is concurrently increasing. Recent anatomic studies have shown that arteriovenous anastomoses are present in dog submandibular glands and that they are open when the glands are actively secreting (13). We have also found that parasympathetic nerve stimulation increases the venous pressure of the gland, irrespective of whether the gland receives spontaneous blood flow or controlled vascular perfusion at the normal resting flow rate. This study describes the action of autonomic nerves on venous pressure and salivary secretion in the dog submandibular gland as a first step in elucidating the vascular mechanism for maintaining a high hydrostatic pressure across the walls of blood vessels during copious salivary secretion.

METHODS
The study was approved by the Committee on the Use of Live Animals for Teaching and Research of The University of Hong Kong (CULATR no. 29–93 and 95–94). The experimental animals were supplied by the Laboratory Animal Unit of The University of Hong Kong. Mongrel dogs (17–20 kg body wt; n = 50) of either sex were anesthetized with intravenous administration of pentobarbital sodium (30 mg/kg); supplemental doses (10 mg·kg^{-1}·h^{-1}) were given when necessary. Body temperature (rectal) was maintained at 37°C by means of an electric heating pad placed beneath the animal. Ventilation was monitored via a pneumotachograph. A femoral artery was cannulated for measurement of systemic arterial pressure. Heparin (2,000 U) was introduced via a cannulated femoral vein before the perfusion system was connected, and 1,000 U/h of heparin were given thereafter. The doses given were within the recommended range for initial dose (50–150 U/kg) and supplementary doses (25–400 U·kg^{-1}·h^{-1}) for total body perfusion in open heart surgery (29). There was no sign of blood clotting or bleeding due to inappropriate administration of heparin.

Measurement of submandibular arterial flow, venous flow, hilar venous pressure, pressure in the extraglandular and segment of the outflow vein, and external jugular venous pressure. The glandular branch of the facial artery is the major artery supplying the dog submandibular gland (Fig. 1A). To determine arterial flow, an ultrasonic flow sensor (25, Transonic System) was placed around the facial artery just proximal to the origin of the glandular artery (12). The facial artery that was distal to the origin of the glandular artery was retrogradely cannulated for intra-arterial administration of drugs into the gland.

In the dog submandibular gland, venous blood from the acini and the ductal system drains first into the intralobular venules and then into the interlobular venules. The interlobular venules in the hilar region are connected by venovenous anastomoses (1–2 mm in diameter), thereby forming a ring-like structure. Two to three outflow veins (2–3 mm in
diameter), arising from the ringlike structure, drain to the periphery of the gland and finally into the external jugular vein (13). In some experiments, venous outflow was monitored by placing an ultrasonic flow sensor (2S, Transonic System) around the largest glandular outflow vein (Fig. 1B).

For measuring the hilar venous pressure of the gland, two methods were attempted: 1) using a fluid-filled narrow catheter (0.6 mm OD; Portex) or 2) using an ultraminiature catheter-tip pressure transducer (1 mm OD; Millar 2F). An incision was made in the jugular vein. Either the narrow catheter or the catheter-tip pressure transducer was inserted via the incision and pushed retrogradely into the largest glandular outflow vein until its tip reached the venous ringlike structure in the hilar region of the gland. The incision was closed with a purse-string suture (Fig. 1B). The position of the narrow catheter or the catheter-tip pressure transducer was checked with postmortem examination.

In some experiments, the pressure in the extraglandular segment of the glandular outflow vein was monitored by pulling the inserted ultraminiature catheter-tip pressure transducer backwards until the tip was positioned in the venous segment just outside the fibrous capsule of the gland. External jugular venous pressure was monitored by further pulling the catheter-tip pressure transducer until the tip was positioned in the external jugular vein.

Vascular perfusion of the submandibular gland. The facial artery that was just distal to the origin of the glandular artery was retrogradely cannulated with a catheter (1.2 mm ID;
Vasculature. The facial artery proximal to the origin of the glandular artery was closed with a snare. The glandular artery was then perfused by means of a peristaltic pump, via the facial arterial catheter, with blood from a reservoir (50 ml) that was continuously replenished from the femoral artery via an inserted catheter (2 mm ID; Portex). The perfusion pressure was measured by means of a pressure transducer from tubing (1.6 mm ID; Portex) connected to the side arm of a four-way stopcock that was placed between the facial arterial catheter and the tubing (2 mm ID; Norprene) of the peristaltic pump. The perfusion rate was adjusted to give a perfusion pressure approximating the systemic arterial pressure (12) (Fig. 1A).

Measurement of salivary secretion. The submandibular duct was retrogradely cannulated and the catheter was connected to a bottle in which the secreted saliva displaced a saline solution. Drops of saline, 0.025 ml in volume, displaced from the bottle were measured by means of a drop counter (92–100–70; E & M); salivary flow was calculated from the time interval between falling drops (12) (Fig. 1A).

Electrical stimulation of the autonomic nerves. In the dog, the preganglionic parasympathetic fibers to the submandibular gland follow the chorda tympani nerve and then the ramus communicans to synapse in the superior cervical ganglion. The ramus communicans to the submandibular ganglion, which courses along the submandibular duct, was exposed. The sympathetic trunk just cranial to the caudal cervical sympathetic ganglion was also exposed. The tied peripheral ends of both nerves were stimulated separately by bipolar platinum electrodes with varying frequency at fixed supramaximal voltage (5 V for parasympathetic nerve and 20 V for sympathetic nerve) and pulse duration (1 ms) according to the experimental protocol (12).

Drugs. Drugs were dissolved in saline solution and given intra-arterially by bolus injection in a volume of 0.1 ml at an infusion rate of 0.1 ml/min into the gland, via either the inserted facial arterial catheter in preparations of spontaneous blood supply or the perfusion circuit in preparations with constant-flow vascular perfusion. The submandibular gland was exposed. The submandibular gland follow the chorda tympani nerve and then the ramus communicans to the submandibular ganglion located in the hilum of the gland. The preganglionic sympathetic fibers pass to the cervical sympathetic trunk to synapse in the superior cervical ganglion. The sympathetic fibers pass to the cervical sympathetic trunk to synapse in the superior cervical ganglion. The ramus communicans to the submandibular ganglion, which courses along the submandibular duct, was exposed. The sympathetic trunk just cranial to the caudal cervical sympathetic ganglion was also exposed. The tied peripheral ends of both nerves were stimulated separately by bipolar platinum electrodes with varying frequency at fixed supramaximal voltage (5 V for parasympathetic nerve and 20 V for sympathetic nerve) and pulse duration (1 ms) according to the experimental protocol (12).

Experimental protocol. Insertion of a narrow catheter or an ultraminiature catheter-tip pressure transducer into the hilar venous system may not cause significant hindrance to the total venous outflow or an increase in hilar venous pressure under normal or low blood flow conditions, as the venous vessels are compliant and venous blood can drain easily via other outflow veins (Fig. 1B). However, at times of high blood flow, there may be an increase in hilar venous pressure as the venous vessels become less compliant, and the presence of a narrow catheter or a catheter-tip pressure transducer may exaggerate the increase in hilar venous pressure caused by the high blood flow. To estimate the magnitude of elevation of hilar venous pressure under different blood flow conditions when pressure was being monitored by either the narrow fluid-filled catheter or the catheter-tip pressure transducer in the present preparation, the relationship between the hilar venous pressure and blood flow was studied in glands under vascular perfusion (n = 6). We found that the normal resting blood flow to the submandibular gland was 0.5 ± 0.03 ml·min⁻¹·g⁻¹ (n = 50), and blood flow at a high level of parasympathetic nerve stimulation (16 Hz) was increased to 3 ± 0.23 ml·min⁻¹·g⁻¹ (n = 8). Hence, the control perfusion blood flow rate was set at 0.5 ml·min⁻¹·g⁻¹. Blood flow was then altered by varying the perfusion flow rate in steps of 0.5 ml·min⁻¹·g⁻¹ until a flow rate of 3 ml·min⁻¹·g⁻¹ was achieved. Each perfusion flow rate was maintained for a period of 1 min for recording the steady-state response of the hilar venous pressure.

Parasympathetic nerve stimulation and sympathetic nerve stimulation were performed at various stimulation frequencies (1–16 Hz) in submandibular glands receiving spontaneous blood flow (n = 8). Glandular arterial and venous blood flows, hilar venous pressure, and salivary flow responses were recorded continuously during the period of stimulation. The steady-state response of all measured variables was achieved within 30 s. Stimulation was given for a period of 1 min, always after complete recovery from the effects of the previous stimulation. The experiments were repeated in submandibular glands under controlled vascular perfusion at the normal resting flow rate (n = 6). The drug was given singly or in combination intra-arterially, always after complete recovery from the effects of the previous injection. The steady-state response of the measured variables was recorded. The experiments were repeated in submandibular glands receiving controlled vascular perfusion at the normal resting flow rate (n = 6).

In the dog, the preganglionic parasympathetic fibers to the submandibular gland follow the chorda tympani nerve and then the ramus communicans to synapse in the superior cervical ganglion. The sympathetic fibers pass to the cervical sympathetic trunk to synapse in the superior cervical ganglion. The ramus communicans to the submandibular ganglion, which courses along the submandibular duct, was exposed. The sympathetic trunk just cranial to the caudal cervical sympathetic ganglion was also exposed. The tied peripheral ends of both nerves were stimulated separately by bipolar platinum electrodes with varying frequency at fixed supramaximal voltage (5 V for parasympathetic nerve and 20 V for sympathetic nerve) and pulse duration (1 ms) according to the experimental protocol (12).

Data recording and analysis. All pressure and flow variables were recorded on magnetic tape (Store 14; Racal) and an oscillographic chart recorder (2800S; Gould). Gould P23ID transducers were used for arterial pressure measurement. All pressure transducers were zeroed to the atmospheric pressure and set at the level of the midchest. The flow sensors, which were precalibrated by the manufacturer, were connected to an ultrasonic flowmeter (T206; Transonic System). The zero baseline of the flow sensors was determined using stagnant saline solution before and at the end of each experiment. All values are given as means ± SE. Student's t-test for paired or unpaired data was used to test the statistical differences between two means. P < 0.05 indicated significant difference.
RESULTS

Under resting conditions, the mean systemic arterial blood pressure was 110 ± 1.7 mmHg (n = 50), submandibular gland arterial inflow was 0.5 ± 0.01 ml·min⁻¹·g⁻¹ (n = 50), and glandular venous outflow (measured from the largest outflow vein) was 0.4 ± 0.02 ml·min⁻¹·g⁻¹ (n = 14). Mean hilar venous pressure was 12 ± 0.2 mmHg (n = 50), hilar venous pulse pressure was 0.6 ± 0.01 mmHg (n = 50), pressure in the extraglandular segment of the outflow vein was 6 ± 0.6 mmHg (n = 6), and pulse pressure in the extraglandular segment of the outflow vein was 0.5 ± 0.01 mmHg (n = 6). Mean external jugular venous pressure was 5 ± 0.5 mmHg (n = 6), external jugular venous pulse pressure was 0.5 ± 0.01 mmHg (n = 6), and salivary secretion was absent. The resting values of the submandibular arterial inflow and venous outflow were not affected by the presence of a narrow catheter or an ultraminiature catheter-tip pressure transducer in the hilar venous system.

Hilar venous pressure and blood flow. Increases in blood flow (via changes in the vascular perfusion rate) were found to elevate the mean hilar venous pressure, whereas the hilar venous pulse pressure was not significantly affected. Figure 2 shows the relationship between the mean hilar venous pressure and blood flow in glands with vascular perfusion and with venous pressure monitored by two different methods, i.e., by the narrow catheter or the catheter-tip pressure transducer. The two parameters were found to be highly correlated (r = 0.99, P < 0.001) in both preparations.

The regression coefficient for the change in blood flow on mean hilar venous pressure was also similar, 2.6–2.7 mmHg·ml⁻¹·min⁻¹·g⁻¹.

Parasympathetic stimulation. In dog submandibular gland with spontaneous blood flow or controlled vascular perfusion at the normal resting flow rate, parasympathetic nerve stimulation, apart from eliciting salivary secretion and an increase in spontaneous blood flow (or a decrease in parasympathetic pressure), caused an increase in the hilar venous pressure of the gland (Fig. 3). The responses of the salivary flow and spontaneous blood flow (or the decrease in the perfusion pressure) were similar to results previously reported when measurements were made in glands without the insertion of a narrow catheter or a catheter-tip pressure transducer (12, 16). Figure 4 summarizes the steady-state response (at 60 s) of the mean hilar venous pressure and salivary secretion to short periods (1–2 min) of parasympathetic nerve stimulation (1–16 Hz) under both blood flow conditions and with hilar venous pressure monitored by two different methods, i.e., by the narrow catheter or the catheter-tip pressure transducer. The magnitude of all responses is in direct proportion to the frequency of stimulation. There was no significant difference between the group with spontaneous blood flow and the group with controlled blood flow with regard to the increase in salivary flow and hilar venous pressure in response to parasympathetic nerve stimulation. Similar results were obtained irre-
In preparations in which the hilar venous pressure was measured by means of a catheter-tip pressure transducer, the hilar venous pulse pressure was found to increase significantly from its resting value at moderate and high levels of parasympathetic nerve stimulation (4–8 Hz). In glands with spontaneous blood supply, the hilar venous pulse pressure was raised to 7 ± 0.6 mmHg (n = 8; P < 0.05) at 4 Hz and to 11 ± 0.7 mmHg (n = 8; P < 0.05) at 8 Hz. For glands with controlled vascular perfusion with normal blood flow, the venous pulse pressure was raised to 6 ± 0.5 mmHg (n = 8; P < 0.05) at 4 Hz and to 9 ± 0.6 mmHg (n = 8; P < 0.05) at 8 Hz. The contour of the venous pressure was found to closely resemble that of the systemic arterial pressure in glands with spontaneous blood flow or that of the perfusion pressure in glands under controlled vascular perfusion (Fig. 3). Changes in hilar venous pulse pressure were not detected when measurements were made by means of the fluid-filled narrow catheter.

The pressure in the extraglandular segment of the outflow vein was not significantly affected by parasympathetic nerve stimulation at all levels of stimulation (1–16 Hz), during either spontaneous blood flow or controlled vascular perfusion at the normal flow rate. There was no obvious change in the pulse pressure and waveform of this extraglandular venous pressure at all levels of parasympathetic nerve stimulation in both groups of glands.

Intra-arterial infusion of ACh (0.1–1 µg·min⁻¹·kg⁻¹) or VIP (0.01–0.1 µg·min⁻¹·kg⁻¹) was found to increase blood flow in glands with spontaneous blood supply or to decrease perfusion pressure in glands with controlled vascular perfusion at the normal blood flow rate. Both agents caused dose-dependent increases in the mean hilar venous pressure and the hilar venous pulse pressure, although ACh induced copious secretion dose dependently, whereas VIP elicited negligible secretion. The magnitude of changes in all measured variables was not statistically different in the two groups of glands (Fig. 6). ACh caused an abrupt increase in venous pressure followed by a slight decline to a steady-state value, whereas VIP elicited an initial gradual increase in venous pressure and finally reached steady state by 30–60 s. Combined infusion of ACh and VIP caused a bigger change in hilar venous pressure and venous pulse pressure than infusion of either agent alone. The contour of the venous pressure was found to show a close resemblance to the waveform of the systemic arterial pressure in glands with spontaneous blood flow or to the perfusion pressure in glands with controlled vascular perfusion during the infusion of both agents.

Sympathetic stimulation. Sympathetic nerve stimulation was found to cause very little salivary secretion whether the gland received spontaneous blood flow or controlled vascular perfusion at the normal flow rate. In glands with spontaneous blood supply, although blood flow was significantly decreased as previously reported (12), the hilar venous pressure was not significantly affected, as in glands with constant-flow vascular perfusion (Fig. 7).
Intra-arterial bolus injection of phenylephrine (0.1–1 µg/kg) or clonidine (0.1–1 µg/kg) was found to decrease blood flow in glands with spontaneous blood supply and to increase perfusion pressure in glands under controlled vascular perfusion. Neither agent caused salivary secretion, and neither had a significant effect on the hilar venous pressure or pulse pressure in the two groups of glands (Fig. 6). Intra-arterial bolus injections of isoproterenol were found to increase blood flow in glands with spontaneous blood supply or to decrease perfusion pressure in glands with controlled vascular perfusion at the normal blood flow rate. Isoproterenol also caused dose-dependent increases in the hilar venous pressure, hilar venous pulse pressure, and salivary secretion; the magnitude of changes in these variables was not statistically different in the two groups of glands (Fig. 6). The contour of the hilar venous pressure showed an obvious resemblance to the waveform of the systemic arterial pressure in glands with spontaneous blood flow and a resemblance to the perfusion pressure in glands with controlled vascular perfusion.

Interaction between parasympathetic and sympathetic stimulation. In glands with spontaneous blood flow or controlled vascular perfusion at the normal resting flow rate, superimposed sympathetic nerve stimulation (20 Hz for 1 min) was found to inhibit the steady-state response (at 30–60 s) of both salivary secretion and the increase in hilar venous pressure caused by parasympathetic nerve stimulation (2–8 Hz). Against a background of continuous sympathetic nerve stimulation (20 Hz), salivary secretion and the increase in hilar venous pressure induced by parasympathetic nerve stimulation (2–8 Hz) were also found to be significantly reduced (Fig. 8). Even under conditions of superimposed sympathetic nerve stimulation or continuous background sympathetic discharge, the relationship between salivary secretion and hilar venous pressure during parasympathetic nerve stimulation still remained highly correlated, although the regression coefficient for the change in hilar venous pressure on salivary secretion was lower than the normal value (Fig. 9).
The sympathetic inhibition of the parasympathetic nerve-induced salivary secretion and the increase in hilar venous pressure was abolished by phentolamine (0.1 mg/kg ia; \( P < 0.05, n = 8 \)) and yohimbine (0.025 mg/kg ia; \( P < 0.05, n = 8 \)) and was not significantly affected by prazosin (0.025 mg/kg ia; \( P = \text{not significant (NS)}, n = 8 \)) or propranolol (0.1 mg/kg ia; \( P = \text{NS}, n = 8 \)) (Fig. 8).

**DISCUSSION**

This is the first study to monitor the in situ intraglandular venous pressure at the hilum region of the submandibular gland in experimental animals and to study its relationship to salivary flow during active secretion. Previous anatomic studies have suggested that arteriovenous anastomoses are present in the submandibular gland of the dog and that they are open during copious secretion caused by parasympathetic stimulation or application of a secretagogue (13). Shunting of arteriovenous anastomotic flow into the postcapillary venous segment would not only increase the mean venous pressure but would also impose a pulsatile wave onto the venous pressure. When venous pressure is measured by means of a conventional fluid-filled narrow catheter, the pulsatile nature of the venous pressure, if present, is significantly dampened.

Our finding shows that when the hilar venous pressure is measured by a narrow catheter it is nonpulsatile not only at rest but even at a high level of parasympathetic nerve stimulation. To monitor the nondampened pulsatile wave of the venous pressure, in situ measurement of the hilar venous pressure is essential, and hence an ultraminiature catheter-tip pressure transducer, the smallest available at present for small animal research (1 mm OD), was chosen.

Does the presence of a narrow catheter or an ultraminiature catheter-tip pressure transducer affect the hilar venous pressure response to parasympathetic nerve stimulation in glands with controlled vascular perfusion at the normal flow rate? According to Bernoulli’s principle on fluid energetics, if the total energy remains constant throughout a tube the total pressures at wide and narrow sections will not be different but the lateral pressure in the narrow section will be smaller than the lateral pressure in the wide section.

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**Fig. 7.** Effects of sympathetic nerve stimulation on \( P_{hv} \) (measured by a catheter-tip pressure transducer) and \( Q_{ss} \) in canine submandibular glands. \( \nabla \) and \( \bigcirc \), \( P_{hv} \) and \( Q_{ss} \), respectively, in glands with spontaneous blood flow at 30–60 s of nerve stimulation. \( \nabla \) and \( \bullet \), \( P_{hv} \) and \( Q_{ss} \), respectively, at 30–60 s of nerve stimulation in glands with controlled vascular perfusion at a normal resting flow rate; \( n = 8 \) animals in each group. *\( P < 0.05 \) compared with corresponding control.

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**Fig. 8.** Effects of continuous background and superimposition of sympathetic nerve stimulation as well as adrenergic antagonists on the steady-state parasympathetic nerve-induced response in \( P_{hv} \) (measured by a catheter-tip pressure transducer) and \( Q_{ss} \). A: glands with spontaneous blood flow. B: glands under constant-flow vascular perfusion with a normal resting flow rate. N, normal response; cs, continuous background sympathetic stimulation (20 Hz); ss, superimposed sympathetic stimulation (20 Hz of 1-min duration); ph, phentolamine (0.1 mg/kg ia); yo, yohimbine (0.025 mg/kg ia); pra, prazosin (0.025 mg/kg ia); pro, propranolol (0.1 mg/kg ia); \( n = 8 \) experiments in each group. *\( P < 0.05 \) compared with normal response.
Fig. 9. Effects of continuous background (CS; 20 Hz) and superimposition (SS; 20 Hz of 1-min duration) of sympathetic nerve stimulation on QSS and Phv (measured by a catheter-tip pressure transducer) during parasympathetic nerve-induced salivation in canine submandibular glands. In glands with spontaneous blood flow, □ shows normal relationship with a slope of the regression line for the change in Phv on QSS of $3.4 \times 10^{-3}$ ml·min$^{-1}$·g$^{-1}$·mmHg$^{-1}$ ($r = 0.97$, $P < 0.001$) at 30–60 s of parasympathetic nerve-induced salivation, ■ shows relationship after CS with the slope of the regression line for the change in Phv on QSS of $4.5 \times 10^{-3}$ ml·min$^{-1}$·g$^{-1}$·mmHg$^{-1}$ ($r = 0.98$, $P < 0.001$), and open circles with an x inside show the relationship after SS having a slope of regression line for the change in Phv on QSS of $5.7 \times 10^{-3}$ ml·min$^{-1}$·g$^{-1}$·mmHg$^{-1}$ ($r = 0.98$, $P < 0.001$). In glands with controlled vascular perfusion at a normal resting flow rate, □ shows the normal relationship with a slope of the regression line for the change in Phv on QSS of $3.8 \times 10^{-3}$ ml·min$^{-1}$·g$^{-1}$·mmHg$^{-1}$ ($r = 0.95$, $P < 0.001$), and open circles with an x show the relationship after SS having a slope of regression line for the change in Phv on QSS of $4.4 \times 10^{-3}$ ml·min$^{-1}$·g$^{-1}$·mmHg$^{-1}$ ($r = 0.99$, $P < 0.001$).

narrow catheter or a catheter-tip pressure transducer will decrease the cross-sectional area of the part of the venous system where it is located. This may be taken as equivalent to the narrow section of a tube. In glands with constant-flow vascular perfusion, i.e., the same total energy in the hilar venous system, the value of the hilar venous pressure measured when either the narrow catheter or the catheter-tip pressure transducer is present should be the same (for total pressure) or even smaller (for lateral pressure) than the value obtained in their absence. Hence, the presence of a narrow catheter or a catheter-tip pressure transducer would not affect the measurement of hilar venous pressure in glands with constant-flow vascular perfusion. It seems unlikely that the presence of either catheter will exaggerate the response of the hilar venous pressure to parasympathetic nerve stimulation in glands with controlled vascular perfusion at a normal resting flow rate.

In glands with spontaneous blood flow, parasympathetic nerve stimulation induces a frequency-dependent increase in blood flow (12). As mentioned in METHODS, the presence of a narrow catheter or a catheter-tip pressure transducer in the hilar venous system may exaggerate the rise in venous pressure under conditions of high blood flow. Figure 2 shows that the regression coefficient for the change in blood flow on hilar venous pressure is the same (2.6–2.7 mmHg·ml$^{-1}$·min$^{-1}$·g$^{-1}$) whether the narrow catheter or the catheter-tip pressure transducer was used. Figure 4 also shows that the response of the hilar venous pressure to parasympathetic nerve stimulation is the same whether measurement was made by the narrow catheter or the catheter-tip pressure transducer. The findings suggest that the catheter-tip pressure transducer, which is bigger than the narrow catheter, does not significantly enhance the response of the hilar venous pressure to changes in blood flow or parasympathetic nerve stimulation compared with the narrow catheter. Normal resting blood flow to the submandibular gland is 0.5 ml·min$^{-1}$·g$^{-1}$ on average, and blood flow during high levels of parasympathetic nerve stimulation (16 Hz) is −6 mmHg, which is small compared with the change in hilar venous pressure (35–45 mmHg) that actually occurred (Fig. 4). Hence, it seems unlikely that the presence of a narrow catheter or a catheter-tip pressure transducer would have significantly exaggerated the response of the hilar venous pressure to parasympathetic nerve stimulation in glands with spontaneous blood flow.

Parasympathetic nerve stimulation causes, in a frequency-dependent manner, parallel increases in hilar venous pressure and salivary secretion, irrespective of whether the blood flow to the gland was allowed to increase spontaneously or under control by vascular perfusion at the normal flow rate (Figs. 3 and 4). The regression coefficient for the change in hilar venous pressure on salivary secretion was found to be similar under both blood flow conditions (Fig. 5). The result therefore suggests the existence of a direct relationship between salivary secretion and venous pressure in the submandibular gland during parasympathetic salivation. The finding also indicates that the concurrent blood flow response to parasympathetic nerve stimulation is not an absolute necessity for the change in venous pressure. Under resting conditions, the hilar venous pulse pressure was found to be small and almost nonpulsatile, as in the systemic veins. During moderate-to-high levels of parasympathetic nerve stimulation, the hilar venous pulse pressure was significantly increased, with a magnitude similar to that of the pulse pressure in small systemic arteries, e.g., 6–12 mmHg in cat mesenteric arteries 50–80 µm in diameter (32), and the waveform of the pulse pressure showed an obvious resemblance to that of the systemic arterial pulse pressure in glands with spontaneous blood flow or to that of the perfusion pulse pressure in glands with controlled vascular perfusion (Fig. 3). Parasympathetic nerve stimulation causes vasodilatation, and the changes in venous pressure were also observed in glands with controlled vascular perfusion. Is it possible that the high venous pressure and its arterial-like waveform are related to a direct transmis-
sion of the arterial pressure and its pulse pressure through the dilated arterioles and open capillaries? It is well known that the capillary network of any vascular bed contributes greatly to the degradation of hydrostatic pressure by virtue of the narrow caliber and large number of vessels. The pulsatile nature of the pressure waveform is greatly attenuated as the blood moves through the arteriolar and precapillary branching, and the capillary pulse pressure in most vascular beds is normally very small, e.g., 1–4 cmH2O in cat mesenteric capillaries (26, 31). The presence of obvious pressure oscillations in the veins may reflect some form of arteriovenous shunting (32). Fromek and Zweifach (7) studied the changes in hydrostatic pressure of the microvessels in response to systemic vasodilatation in skeletal muscle microcirculation, where arteriovenous shunt vessels are not commonly observed (28, 32). They found that, with maximal dilatation induced by papaverine, the increase in pressure in the smallest venules (8–15 µm in diameter) is ~4 mmHg and is negligible in veins larger than 80 µm in diameter (7). Maspers et al. (18) have also shown that the increase in postcapillary venular pressure (measured from a venule <10 µm in diameter) with maximal metabolic vasodilatation (induced by muscle exercise) is ~15 mmHg from the control value prevailing at normal intrinsic tone. We found in this study that high levels of parasympathetic nerve stimulation (8–16 Hz) were able to raise the hilar venous pressure, which was monitored from a venous vessel ~2–3 mm in diameter, by 30–40 mmHg (Fig. 4). In addition, the venous pulse pressure was increased by a magnitude of 8–12 mmHg (Fig. 3). Hence it is doubtful that such large increases in the mean pressure and pulse pressure in a large (hilar) vein could be caused by a direct transmission of pressure and pulse pressure from the arterial side to the venous side via dilated arterioles and open capillaries. However, further experiments on the measurements of micropressures in the salivary gland are required to justify this point.

Recent anatomic studies have confirmed the presence of arteriovenous anastomoses in the dog submandibular gland and demonstrated their opening during profuse salivaion (13). Most of the arteriovenous anastomoses are seen draining into venules (<100 µm in diameter) devoid of valves (13). Fluid exchange is found to occur not only in the capillaries but also in the permeable postcapillary venules in some vascular beds, e.g., in the diaphragm and frog pial microvessels (22, 25). Measurements of hydraulic conductivity of walls of single mammalian capillaries, e.g., in rat intestine and cat mesentery, have shown that vessels at the venous end of the microcirculation usually have a higher hydraulic conductivity than those at the arterial end (19). If the postcapillary valveless venules of the salivary gland are permeable and have a higher hydraulic conductivity than the microvessels at the arterial end, as in other vascular beds, the pressure toward the venous end would then be the most important factor for fluid exchange. It is highly probable that during copious secretion, arteriovenous anastomoses open, allowing rapid transmission of the arterial pressure into the venules, and this immediately elevates the venular pressure, enhancing filtration. Arteriovenous anastomoses in some vascular beds have been shown to have a richer supply of cholinergic and peptidergic nerves than arteries of comparable size (21), suggesting that parasympathetic nerve stimulation may induce a larger dilatatory action on arteriovenous anastomoses than on the arterioles. If this happens, the predominant dilatatory response of arteriovenous anastomoses will not only result in a shifting of blood flow through arteriovenous anastomoses but also a direct transmission of the perfusion pressure to the venules, even in glands with controlled vascular perfusion at a normal resting flow rate. Hence, it is probable that the increases in venous pressure and pulse pressure and the arterial-like waveform observed during parasympathetic nerve stimulation are related to a direct transmission of the arterial pressure and pulse pressure through open arteriovenous anastomoses, irrespective of whether the gland receives spontaneous blood supply or controlled vascular perfusion. However, further studies on the measurements of the fenestral density and hydraulic conductivity of the postcapillary venules and arteriovenous anastomotic flow of the salivary gland, as well as their changes during parasympathetic salivation, are required to verify this point.

The arterial pressure and pulse pressure that are transmitted through open arteriovenous anastomoses would be rapidly lost in the highly compliant venous vessels if there were no mechanism present for their rapid transmission and preservation in the venous system. The mean pressure and the pulse pressure of the extraglandular segment of the outflow vein were not affected during parasympathetic nerve stimulation, implying that the arterial pressure and pulse pressure transmitted through the opened arteriovenous anastomoses had already been lost in the extraglandular venous segment. Hence, the mechanisms responsible for preserving the transmitted pressures must be located within the gland. Histological studies have shown that smooth muscle cells are scarce in the venous blood vessels (13), suggesting that venoconstriction is unlikely to play an important role in preserving the transmitted arterial pressure and pulse pressure. However, dense connective tissue is found to enclose the ductal system and its accompanying structures (blood vessels, lymphatic vessels, and nerves), being most abundant in the hilum and diminished aborally (13). Moreover, the gland is encased by a strong fibrous capsule. On leaving the gland, all outflow veins must penetrate the fibrous capsule. Hence, the mechanisms responsible for preserving the high intraglandular venous pressure are probably the dense connective tissue surrounding the venous vessels and the fibrous capsule. This may explain why the pressure in the extraglandular segment of the outflow vein was not significantly affected during parasympathetic nerve stimulation, as the venous segment lies outside the fibrous capsule and is no longer surrounded by dense connective tissue.
ACh and VIP were found to induce not only an arterial-like waveform onto the hilar venous pressure but also to increase its level and the size of its pulse pressure in a dose-dependent manner, as with parasympathetic nerve stimulation (Fig. 6). It is interesting to note that ACh acts rapidly to cause an abrupt increase in venous pressure, whereas VIP acts slowly to raise this pressure. However, combined infusion of both agonists provoked a larger and more sustained change in venous pressure, indicating that the agonists act synergistically to bring about a very rapid and maintained increase in filtration pressure for copious salivary secretion. Parasympathetic nerve fibers supplying the arteriovenous anastomoses in many vascular beds have been shown to be positive for ACh and immunoreactive for VIP (9, 11). Hence, both parasympathetic neurotransmitters increase the venous pressure of the salivary gland, probably by activating dilatation of the arteriovenous anastomoses.

Sympathetic nerve stimulation was found to induce very little salivary secretion and had no significant effect on the hilar venous pressure whether the gland received spontaneous blood supply or controlled vascular perfusion at the normal flow rate (Fig. 7). Administration of phenylephrine and doxidine, $\alpha_1$ and $\alpha_2$ adrenergic agonists, respectively, did not provoke salivation and had no significant effect on the hilar venous pressure, whereas isoproterenol injection caused salivary flow and an elevated pulsatile venous pressure (Fig. 6). Hence, the salivary flow in response to sympathetic nerve stimulation is probably due to activation of the $\alpha_1$-adrenergic receptors of the secretory apparatus, as reported previously (6). We have previously shown that the paucity of salivary flow in response to sympathetic nerve stimulation is observed even in glands with controlled vascular perfusion at the normal resting flow rate, indicating that the phenomenon is unrelated to a reduced blood supply caused by vasoconstriction, as traditionally believed (12). In this study, we found that the hilar venous pressure was not significantly affected by sympathetic nerve stimulation in glands with controlled vascular perfusion, as in glands with natural blood supply. Sympathetic nerves can act directly on the acinar cells, provoking slight secretion. However, the paucity of salivary flow in response to sympathetic stimulation may be related to a certain extent to the concurrent low hilar venous pressure, a situation unfavorable for fluid filtration in the salivary gland microcirculation. Arteriovenous anastomoses have been found to possess a denser sympathetic innervation than the arteries and veins of the same vascular bed (10, 20, and 21). Sympathetic nerve stimulation or infusion of norepinephrine or methoxamine has been shown to cause constriction of the arteriovenous anastomoses, resulting in blood flow redistribution to the capillaries in the dog hindpaw and the sheep hindlimb (1, 8). Sympathectomy or infusion of phentolamine normally results in a redistribution of capillary blood flow to the arteriovenous anastomoses in the muscle and skin circulation of experimental animals (5, 8). In this study, the resting hilar venous pressure of the salivary gland was found to be rather nonpulsatile (Figs. 3 and 6), suggesting that, under normal resting conditions, most of the arteriovenous anastomoses in the salivary gland are probably closed, either by the basal sympathetic discharge or circulatory catecholamines, with only a small number remaining open. This may explain why sympathetic nerve stimulation did not exert a significant action on the already low resting hilar venous pressure, primarily because the number of open arteriovenous anastomoses available for closure is meager.

Continuous background or superimposed sympathetic nerve stimulation has been shown to inhibit steady-state parasympathetically induced salivary secretion via an $\alpha_2$-adrenergic mechanism (15). In this study, both modes of sympathetic stimulation were found to depress in a parallel fashion the parasympathetically induced elevation of venous pressure and salivary secretion (Fig. 8). It is therefore possible that the sympathetic inhibition of salivary secretion is related, to a certain extent, to the inhibition of the vascular response, i.e., prevention of increased venous pressure. Under sympathetic influence, the regression coefficient for the change in hilar venous pressure on parasympathetic salivary secretion was found to be smaller than the normal value (Fig. 9), suggesting a somehow altered relationship between venous pressure and salivary secretion. Hence we cannot rule out the possibility that the sympathetic inhibitory action on parasympathetic salivary flow may also be related to a direct inhibitory action on the secretory apparatus. The sympathetic inhibitory actions on both the salivary flow and the increase in venous pressure were not significantly affected by the selective $\alpha_1$-adrenergic antagonist prazosin but were abolished by the selective $\alpha_2$-adrenergic antagonist yohimbine (Fig. 8). Sympathetic control of the resistance of arteriovenous anastomoses is exerted through a combination of $\alpha_1$- and $\alpha_2$-adrenergic mechanisms in the dog hindpaw (2) but primarily by an $\alpha_2$-adrenergic mechanism in the human finger (4). In the dog submandibular gland, the sympathetic inhibition of parasympathetic salivation is very likely exercised primarily through the $\alpha_2$-adrenergic mechanism, acting directly on the secretory cells as well as indirectly via a closure of arteriovenous anastomoses.

This study has demonstrated for the first time that the volume of salivary flow during parasympathetic salivation is directly related to the venous pressure within the salivary gland. A plentiful supply of fluid to the secretory apparatus is a prerequisite for a copious and watery secretion. To ensure a massive fluid filtration across the walls of the microvessels in an actively secreting salivary gland when the oncotic pressure is concurrently increased because of fluid efflux, an extra high capillary hydrostatic pressure and/or an increased hydraulic conductivity is required. Studies on the rabbit submandibular gland have demonstrated that parasympathetic nerve stimulation does not lead to a change in capillary permeability (27). Whether this can be applied to the dog submandibular gland requires inves-
tigation. Studies in human skin have shown that a given increment in venous pressure will produce a much greater effect on capillary hydrostatic pressure than the same increment in arterial pressure (17, 24). If this is also true for the salivary gland, an increase in hydrostatic pressure at the venular end through opening of arteriovenous anastomoses will be more effective in elevating the capillary hydrostatic pressure than a similar increase in pressure at the arterial end through arteriolar dilatation. Of course both mechanisms may operate simultaneously to effect a high capillary hydrostatic pressure during copious secretion. The relative importance of each mechanism still awaits further investigation.

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