then taken for determination of amylase released from the tissue, and the tissue pieces were homogenized in the remaining incubation medium, to allow determination of the total amylase present. Amylase was assayed using Remazolbrilliant Blue-labelled starch[5].

The composition of the standard Krebs solution was (mM): NaCl, 106; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13; NaH_2PO_4, 1.15; $NaHCO_3$, 25; glucose, 2.8; Na fumarate, 2.7; Na pyruvate, 4.9; Na glutamate, 4.9. Low Ca^{2+} Krebs was similar to standard Krebs except that it contained only 0.1 mM $CaCl_2$. Solutions were bubbled with 95% O_2, 5% CO_2. In the experiments using colchicine (10^{-4} M) or cytochalasin B (1.1×10^{-6} M), these agents were present during the washing and incubation periods. Bethanechol chloride, colchicine and cytochalasin B were purchased from Sigma Chemical Co. (USA). Remazolbrilliant Blue was obtained from Calbiochem (USA).

Results and discussion. Bethanechol at high concentrations ($\geq 10^{-4}$ M) was a more effective stimulant of amylase secretion when the [Ca^{2+}] in the medium was low (fig. 1), indicating that Ca^{2+}, the 2nd messenger for muscarinic receptor-induced amylase secretion, can have toxic effects when excessive amounts become available. Savion and Selinger suggested that disruption of the terminal web of the acinar cells was responsible for the biphasic dose-response curve[1]. We have attempted to confirm the involvement of microfilament disruption in this inhibitory response by measuring amylase secretion in the presence of a low concentration of cytochalasin B. Maximum amylase output was reduced by cytochalasin B ($p < 0.05$, Student's t-test), but the anti-microfilament agent did not potentiate the toxic effects of Ca^{2+} seen with high concentrations of bethanechol (fig. 2). These results do not support the idea that microfilament disruption is involved in the decreased secretion produced by high [Ca^{2+}]. Ethanol, the vehicle for cytochalasin B, in the concentrations used in these experiments had no effect on amylase secretion (results not presented).

Microtubules may be regulated by the cytoplasmic [Ca^{2+}][6]. However, colchicine did not remove the inhibitory effect of Ca^{2+} on amylase secretion at high secretagogue concentrations (fig. 2). The uptake of colchicine from a 10^{-4} M solution by mouse pancreas is almost complete within the 30-min wash period used for our experiments, although in the period of our experiments, 1 h, colchicine does not cause disruption of pre-formed microtubules[7]. Vinblastine, used under conditions which cause the disappearance of microtubules, does not change the biphasic nature of the dose-response curve for bethanechol-induced amylase secretion[7]. Thus, microtubules do not seem to be involved in this inhibitory action of Ca^{2+}.

Elucidation of the pathway through which Ca^{2+} acts to inhibit secretion will provide information on stimulus-secretion, by identifying structures which must be intact for a normal secretory response.

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The effects of phenoxybenzamine on the aortic pressure-diameter relationship in dogs[1]

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Summary. The normalized diameter ($D/D_{13.3}$ where $D_{13.3}$ equals D at 13.3 kPa under control conditions) was measured at selected pressure levels under different hemodynamic conditions. Hemorrhage caused the normalized diameter to decrease (−3.3%) when compared to control values at a given pressure. Volume expansion and α-blockade with phenoxybenzamine caused $D/D_{13.3}$ to increase (+3.3% and +8.5% respectively).

During the last decade it has become clear that changes in circulating blood volume cause reflex changes in the aortic pressure-diameter relationship. It was shown in anesthetized dogs that hemorrhage causes a decrease in the thoracic aortic diameter for any given pressure[3], while volume expansion causes an increase in the aortic diameter for any given pressure[4]. These responses are mediated by the aortic smooth muscle cells which are under sympathetic nervous control. Indeed, stimulation of sympathetic efferents to the thoracic aorta caused the aortic diameter to decrease at any chosen pressure[5]. In the abdominal aorta, this diameter reduction was found to be directly related to the stimulation frequency[6]. It was also observed that the aortic response to hemorrhage was abolished after α-blockade with phenoxybenzamine[7] or after elimination of the sympathetic input to the aorta via spinal cord transection[8]. As a result of α-blockade or spinal cord transection, the aortic diameter normally increased when compared to the control diameter determined at the same pressure. These diameter changes have not been studied in detail. It was the purpose of this research to quantitate the diameter changes after α-blockade and to compare them with the changes observed after hemorrhage or volume expansion.

Methods. Experiments were performed on 6 adult male mongrel dogs, weighing between 24 and 28 kg. The animals were premedicated with morphine sulfate (2 mg/kg i.v.) and anesthetized with sodium pentobarbital (20 mg/kg i.v.). The trachea was intubated and the dogs were ventilated with a positive-pressure respirator. A continuous slow infusion (150 ml/h) of Krebs-Ringer solution was administered i.v. for the duration of the experiment. A left thoracotomy was performed at the level of the 4th intercostal space and 2 piezoelectric crystals (4 mm diameter) were attached across the thoracic aorta using cyanoacrylate glue. The transit time for a burst of ultrasound to pass from one crystal to the other was measured by means of a sonomicrometer. The voltage output of this instrument was proportional to the transit time[9] and thus also to the external aortic diameter. The sonomicrometer was calibrated at the end of each experiment.

Aortic pressure was measured in the cross-section of the crystals by means of a catheter-tip transducer (Millar Instruments, PC-360). This instrument was inserted via the right femoral artery and correctly positioned with the aid of a fluoroscope. Aortic pressure and diameter were digitized at a rate of 1000 samples per sec and displayed on a digital oscilloscope (Nicolet Explorer III). This oscilloscope was capable of storing the digitized wave forms on magnetic disks for later analysis. In order to obtain aortic pressure and diameter in a wide pressure range, a sinusoidal piston pump was attached to the abdominal aorta by means of a cannula inserted into the left femoral artery. When operating, this pump produced slow oscillations in aortic pressure and diameter with a cycle period of 5 sec (fig. 1). In all 6 experiments, recordings were made under the following conditions: under control conditions, after hemorrhage (−15% of the estimated blood volume), after reinfusion (new control conditions), after volume expansion (+15% of the estimated blood volume) and 30 min after α-blockade with phenoxybenzamine (5 mg/kg). For each condition, the pump was operated for a least 10 sec. During this period, the pressure and diameter signals were recorded on magnetic disk. At a later time, the diastolic pressure-diameter relationship was analyzed by determining the aortic diameter at 0.5 kPa pressure intervals. In each experiment the diameter values were normalized by dividing them by $D_{13.3}$ which was the diameter value at 13.3 kPa (100 mmHg) obtained in that experiment under control conditions. This normalization procedure eliminated the variability in the aortic diameter of the different animals.

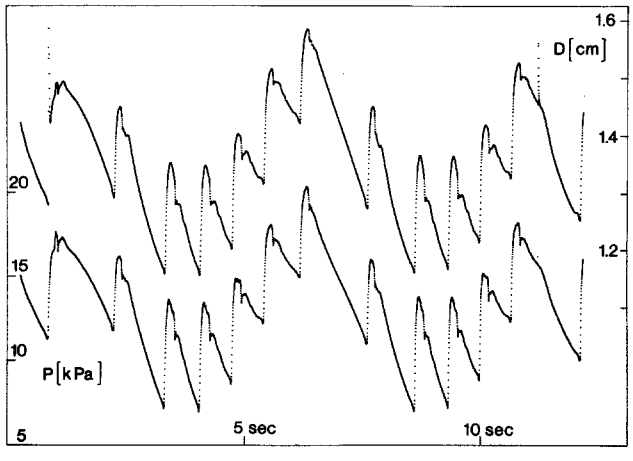

Figure 1. Aortic pressure (lower trace) and diameter during 2 complete pumping cycles.

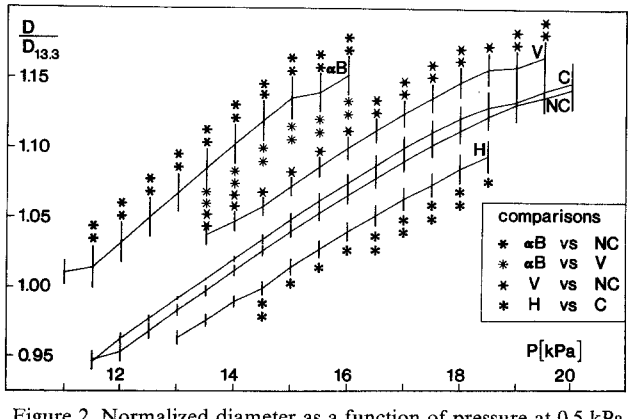

Figure 2. Normalized diameter as a function of pressure at 0.5 kPa intervals for 5 different conditions. The vertical bars indicate the SE of the mean. C, control; H, after hemorrhage; NC, new control; V, after volume expansion; αB, after α-blockade. 1 asterisk indicates significance at the 0.05 level, 2 asterices indicate significance at the 0.01 level.

Results. Under control conditions, the average of the mean arterial blood pressure for all 6 animals was equal to 15.3 kPa (115 mmHg). The average blood pressure values after hemorrhage, after reinfusion and after volume expansion were not significantly different from this control value. After α-blockade the blood pressure was decreased to 14.5 kPa (109 mmHg) ($p < 0.05$, paired t-test). The average of the normalized diameter values obtained in all 6 experiments under the different conditions are shown in figure 2. Hemorrhage caused the normalized diameter to decrease by an average of 3.3% when compared to the control values at the same pressure. Volume expansion and α-blockade caused the normalized diameter to increase respectively by an average of 3.3% and 8.5% when compared to the new control values. A 1-way analysis of variance with repeated measurements was performed at each pressure level, followed by the Newman-Keuls post-test[10] to compare the normalized diameters under the different conditions. The results of these post-tests are depicted in the figure by means of asterices.

Conclusions. The changes in the normalized diameter values after hemorrhage and after volume expansion, obtained here with the sonomicrometer technique agree with the results obtained earlier with the Pieper pressure-diameter gauge[3,4]. Bergel indicated the need for caution in using the Pieper gauge because the diameter sensing braces exert a small distending force on the wall[11]. A confirmation of these earlier results using the sonomicrometer is therefore of value.

It was found in this study that α-blockade with phenoxybenzamine results in an increased normalized diameter at each pressure level varying between 7.02% (at 11.5 kPa) and 9.22% (at 15.0 kPa). These numbers represent the largest increase in diameter which is obtained by eliminating all sympathetic α-receptor input. Thus, the αB curve in figure 2 represents the upper limit of the normalized aortic diameter value unter α-adrenergic control.

The direction of the diameter changes observed after hemorrhage, after volume expansion and after α-blockade would also support the hypothesis that an increase in circulating blood volume decreases the sympathetic outflow to the aortic smooth muscle and that a decrease in circulating blood volume increases the sympathetic outflow to the aorta.

1 This work was supported in part by PHS grant HL-23239 and a grant from the Central Ohio Heart Chapter of the American Heart Association.
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