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Calcium causes the biphasic dose-response curve for pancreatic amylase secretion

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Summary. High concentrations of bethanechol (10^{-4} to 10^{-3} M) were effective stimulants of amylase secretion from the mouse pancreas if incubations are performed in low [Ca^{2+}] (0.1 mM) solutions but not if normal Krebs solution (2.56 mM Ca^{2+}) was used. This inhibitory effect of Ca^{2+} at high secretagogue concentrations did not appear to be mediated through the microtubules or microfilaments.

Agonists which act on muscarinic or cholecystokinin receptors of pancreatic acinar cells stimulate amylase secretion when their concentration is low, but are not such effective secretagogues when they are used at higher concentrations[1-3]. This decrease in secretion with high concentration of agonists is not due to tachyphylaxis or some other event in receptor activation, but is a post-receptor phenomenon[1,3]. Savion and Selinger showed that damage occurs to the apical area of the acinar cells when they are exposed to high concentration of secretagogues and they suggest that this leads to an impairment of secretion[1]. Williams[3] suggested that Ca^{2+} is the 2nd messenger responsible for inhibition of secretion, but it is possible that a Ca^{2+}-independent phenomenon, such as membrane changes resulting from secretagogue-induced phosphatidylinositol hydrolysis[4], may explain the impaired secretory response to high concentrations of agonists. The experiments described in this paper were undertaken in an attempt to elucidate the mechanisms underlying this biphasic dose-response curve.

Methods. Male mice were starved for 16 h (with water ad libitum) and then killed by cervical dislocation. Pancreata were removed and cross cut (0.6 mm) with a McIlwain tissue chopper. Tissue pieces were washed in Krebs solution for 2 × 10 min (extended to 3 × 10 min in experiments using colchicine), and incubated for 30 min at 37 °C in 5 ml of Krebs which contained bethanechol at appropriate concentrations. A sample of the incubating medium was

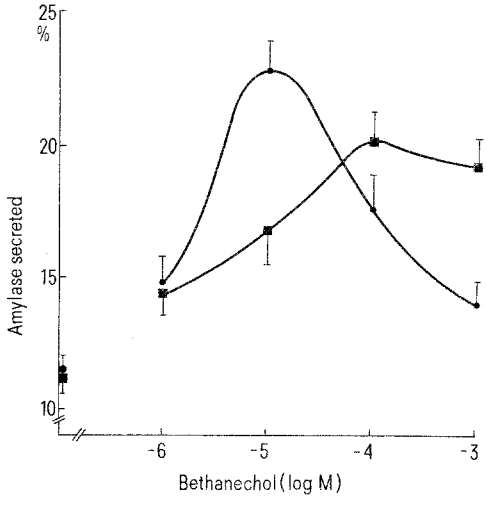

Figure 1. Effect of extracellular calcium concentration on amylase secretion by fragments of mouse pancreas. Tissues were incubated in Krebs solution containing either 0.1 mM $CaCl_2$ (■) or 2.56 mM $CaCl_2$ (●). Each point represents the mean ± SE for 8 or 9 samples. Amylase secretion is expressed as a percentage of total amylase present in the tissue.

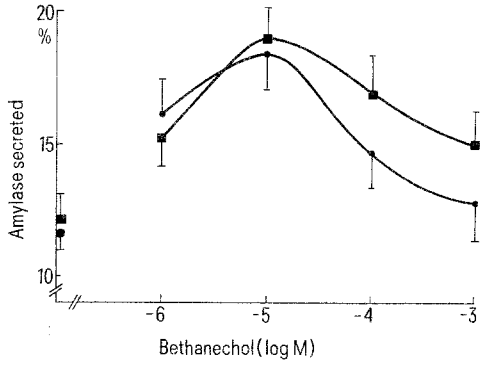

Figure 2. Effect of cytochalasin B and colchicine on amylase secretion by the mouse pancreas. Tissues were pre-washed and incubated in the presence of either 1.1×10^{-6} M cytochalosin B (■) or 10^{-4} M colchicine (●). Each point represents the mean ± SE for 8 or 9 samples.

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.