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6. 6-Hydroxydopamine hydrobromide (Regis Chemical) was used. Rats received 20 μl of either 200 μg of 6-hydroxydopamine (the dose given is of the free base) or the vehicle (0.9 percent NaCl, 0.1 percent ascorbic acid) into the cerebrospinal fluid by way of the left lateral ventricle. Three days later the same injections were delivered by way of the right lateral ventricle of each rat.
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Glucagon Release: Paradoxical Stimulation by Glucose During Calcium Deprivation

Abstract. *During calcium deprivation, the rate of glucagon release by the isolated perfused rat pancreas is positively related to the glucose concentration of the perfusion medium. It is suggested that such a paradoxical behavior, which is reminiscent of the abnormality of glucagon secretion recently disclosed in diabetic subjects, results from a perturbation in the normal structural and functional bridging between pancreatic alpha and beta cells.*

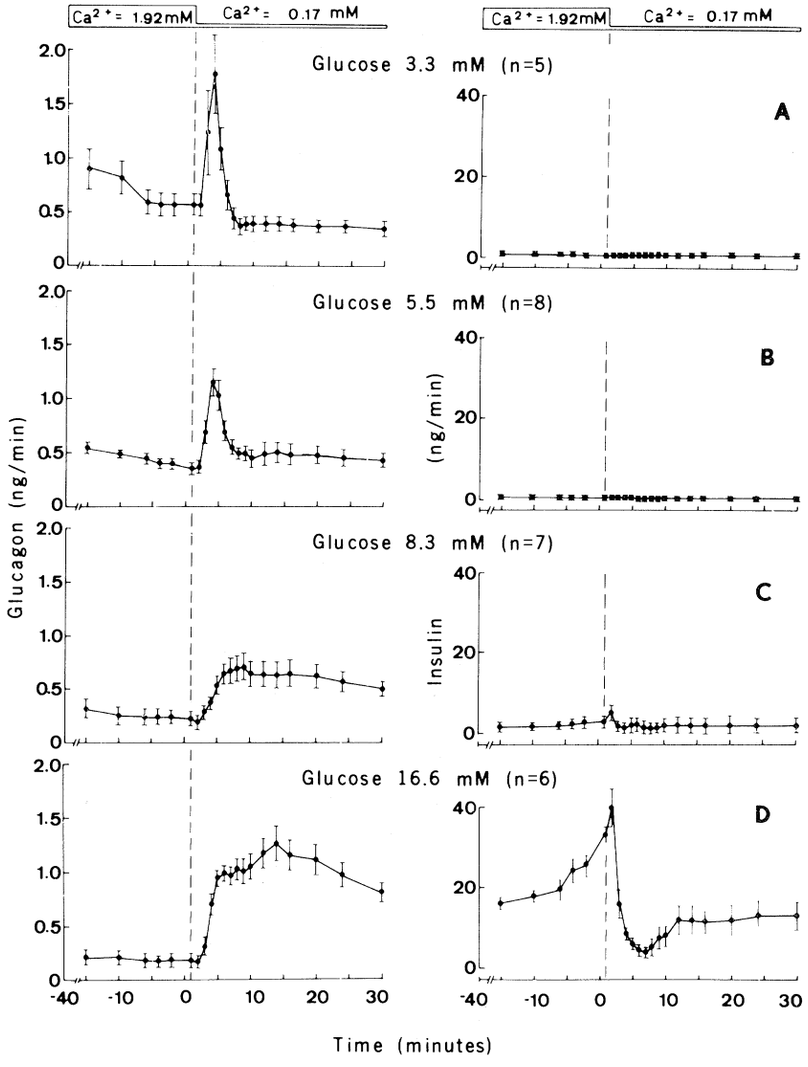
Whereas calcium is an essential requirement for most exocrine and endocrine secretory processes (*1*), the release of Parathormone (*2*) and, more recently, that of glucagon (*3*) were shown to be enhanced during calcium deprivation. The latter finding was documented by incubating pieces of pancreas obtained from duct-ligated rats in media containing a fixed concentration of glucose (8.3 m*M*). In the experiments reported here, we further investigated the effect of lowered calcium levels on the secretion of glucagon, using the more sensitive and dynamic technique of perfusion of the rat pancreas and performing the experiments at various glucose concentrations (3.3, 5.5, 8.3, and 16.6 m*M*). This procedure led us to discover a paradoxical stimulation of glucagon release by glucose during calcium deprivation. It is proposed that such a phenomenon, which is reminiscent of the situation encountered in human diabetes mellitus, may shed light on the significance of the structural and presumably functional coupling between different types of endocrine pancreatic cells, as recently revealed by the freeze-etching technique (*4*).

Fed female albino rats, with a mean body weight of 220 g, were used. The pancreases were dissected under sodium pentobarbital anesthesia (48 mg per kilogram of body weight, intraperitoneally) by the procedure described by Loubatières *et al.* (*5*); all of the adjacent organs, including the duodenum, were excluded. The pancreases were perfused in situ, through the coeliac and superior mesenteric arteries by means of a cannula inserted in the aorta, the effluent being collected without recycling from the portal vein. The flow rate ranged between 1.7 and 2.0 ml/min. The perfusion medium was a Krebs-Ringer bicarbonate buffer (*p*H 7.4) containing bovine albumin (4 g/100 ml; Pentex, fraction V, Miles Laboratories), equilibrated against a mixture of O_2 and CO_2 (95 : 5), constantly filtered (pore size, 1.2 μm), and warmed to 37°C at the entrance of the pancreas (*6*). Glucose (3.3, 5.5, 8.3, or 16.6 m*M*) was present throughout the 80 minutes of perfusion, the first hormonal measurements being performed 25 minutes after the onset of the perfusion (– 15 minutes in Figs. 1 and 2). Calcium was added to the perfusion medium by a sidearm syringe to attain a theoretical concentration of 2 m*M* during the initial 40-minute equilibration period. Calcium deprivation was induced by stopping this calcium infusion (Fig. 1). Upon assay, the total calcium concentration averaged 1.92 ± 0.21 m*M* (n = 14) during the equilibration period and 0.17 ± 0.01 m*M* (n = 14) during calcium deprivation (*7*). The effluent was collected every minute in chilled tubes containing 2000 kallikrein inhibitor units of Trasylol (*8*). Glucagon and insulin were estimated by radioimmunoassay, with beef and pork glucagon and rat insulin as standards (*9*).

Glucose in high concentration (8.3 and 16.6 m*M*) stimulated insulin release at the high calcium level (the equilibration period), this stimulant action being inhibited during the period of calcium deprivation (Fig. 1, C and D). The true degree of inhibition of insulin release at the two highest glucose concentrations was more marked than that suggested in Fig. 1; indeed, in control experiments in which the high calcium level was maintained throughout, the rate of insulin release evoked by glucose progressively increased during the second part of the experiment (Fig. 2). The output of glucagon during the equilibration period, that is, at the high calcium level, was inversely related to the glucose concentration of the perfusion medium (Fig. 1; Table 1, minutes – 15 to 1). These findings are in agreement with the known effects of glucose and calcium on the pancreatic beta

Table 1. Mean glucagon output by the perfused rat pancreas during the control period and the early and late periods of calcium deprivation, at various glucose concentrations; n, number of experiments.

Glucose (m*M*)	n	Mean glucagon output (pg/min)		
		Control period	Calcium deprivation	
		Minutes – 15 to 1 (mean ± S.E.M.)	Minutes 2 to 7 (mean ± S.E.M.)	Minutes 8 to 30 (mean ± S.E.M.)
3.3	5	694 ± 128	1049 ± 183	377 ± 57
5.5	8	451 ± 35	804 ± 74	470 ± 81
8.3	7	256 ± 68	459 ± 55	615 ± 104
16.6	6	203 ± 62	704 ± 52	1048 ± 125



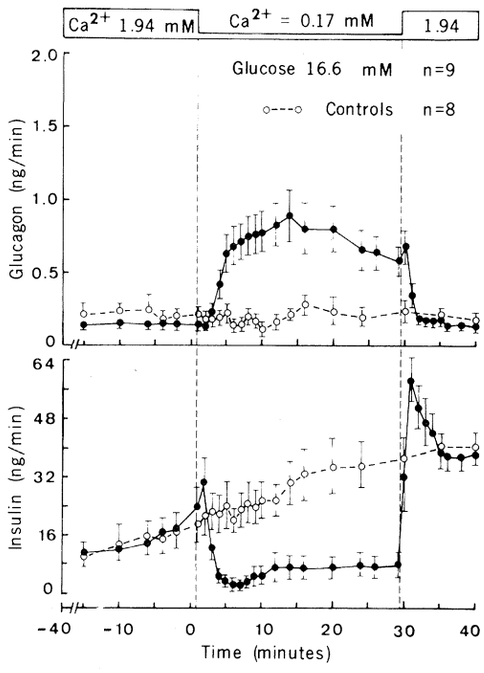


Fig. 1 (left). The effect of calcium deprivation on glucagon and insulin release by rat pancreas perfused in vitro at various concentrations of glucose. The vertical dashed line corresponds to the time at which the calcium-deprived media reached the pancreas, after a 40-minute equilibration period at the high calcium level. Mean values (± standard error of mean) are shown together with the number of experiments in each group (*n*). Fig. 2 (right). Reversibility of the changes in glucagon and insulin release evoked by calcium deprivation in the perfused rat pancreas. Glucose (16.6 m*M*) was invariably present throughout the perfusion, whether in the control experiments or during calcium deprivation. In the control experiments, the calcium concentration (1.70 ± 0.06 m*M*) was constant throughout.

cell (*1*), on one hand, and the classical negative feedback response of the alpha cell to glucose (*10*), on the other hand.

The reduction in calcium concentration consistently provoked an enhancement of glucagon secretion, confirming our earlier observations (*3*). The effect of calcium deprivation was reversible (Fig. 2). Incidentally, these results may appear at variance with those of Gerich *et al.* (*11*), who reported on the calcium dependency of glucagon secretion. However, the experimental conditions used by these investigators were different from ours, in that they studied the effect of an arginine stimulus on the endocrine response of the pancreas after a prolonged period of calcium deprivation.

The positive secretory response of the alpha cell to calcium deprivation in our experiments apparently consisted of two components. The first was characterized by an immediate, sharp, and short-lasting (approximately 4-minute) peak of secretion, which was most evident at the lowest concentration of glucose (Fig. 1A). This early peak decreased in amplitude when the glucose concentration was raised to 5.5 m*M* (Fig. 1B) and, possibly because of the contribution of the second component, was not easily identified at higher glucose levels (Fig. 1, C and D). Even so, the early glucagon response to calcium deprivation exhibited, to some extent, the classical inverse relationship to glucose concentration (Table 1, minutes 2 to 7).

In contrast, the second component, which might be referred to as a late secretory phase, exhibited a paradoxical positive relationship to the glucose concentration of the perfusion medium (Table 1, minutes 8 to 30). Indeed, at the lowest glucose concentration (3.3 m*M*), this phase was absent, the late output of glucagon merely representing a basal value (Fig. 1A). At 5.5 m*M* glucose, there was a trend toward a late secretory response (Fig. 1B). At higher glucose levels, the late phase was more marked, calcium deprivation now resulting in a long-lasting stimulation of glucagon release (Fig. 1, C and D).

The explanation for the paradoxical response of the alpha cell to glucose during calcium deprivation is unknown. The occurrence of such a response is, however, not an isolated finding. In diabetes mellitus also, glucose is unable to suppress the secretion of glucagon and may occasionally induce a paradoxical hyperglucagonemia (*12–14*). It was suggested that the abnormal response of the alpha cell to glucose is related to a concomitant insulin deficiency (*12*).

However, although insulin was able to correct the abnormal response of the alpha cell in experimental animals (*13*), this was not so in human diabetic subjects (*12*). Similarly, it seems unlikely that a deficiency in insulin secretion accounts for the positive response of the alpha cell to glucose seen during calcium deprivation in our experiments. Indeed, in the lower range of glucose values, the pattern of glucagon release in response to calcium deprivation was obviously influenced by the glucose concentration of the perfusion medium, although insulin secretion invariably occurred at a close to basal rate.

In view of the recent demonstration of a structural coupling between alpha and beta cells (*4*), we wonder whether the paradoxical behavior here disclosed may not be somehow related to a functional uncoupling of these cells as the result of calcium deprivation (*15*). If so, the present protocol might prove useful for further studies on the significance of intercellular bridging in the physiology and pathology of islet tissue.

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Prostaglandins and the Pulmonary Vasoconstrictor Response to Alveolar Hypoxia

Said and co-workers (*1*) reported a frequent appearance of prostaglandins or prostaglandin-like biologically active substances in the venous effluent from isolated, perfused lungs during hypoxic ventilation. Furthermore, aspirin, which is a potent inhibitor of prostaglandin biosynthesis (*2*), was found to reduce the pulmonary vasoconstrictor response to hypoxic breathing in cats. Several things make this work difficult to assess. Alveolar hypoxia was induced in cat lungs perfused with the extremely low flow of 10 ml/min (*3*). No information was given about pulmonary arterial pressure (PAP), but assuming a normal pressure range, pulmonary vascular resistance (PVR) must have been increased by a factor of 25 or more, indicating grossly abnormal lungs. Furthermore, PAP-rises of as little as 1 mm-Hg were taken as evidence of a true hypoxic response. The average increase in PAP also seemed to be very small (*4*).

The effluent pulmonary perfusate superfused a series of specific smooth muscle assay organs. Whenever alveolar hypoxia elicited pulmonary hypertension, one or more of the assay organs contracted in all but four cases. When used, the rat stomach strip, the rat colon, and the chick rectum contracted in 38, 31, and 19 percent of the cases, respectively. Vane and co-workers recommend the *simultaneous* contraction of rat stomach strip, rat colon, and chick rectum for the bioassay of prostaglandin-like substances (*5*). The incidence of simultaneous contraction of the above muscle organs in the work of Said and co-workers cannot have been higher than 19 percent, which is the lowest percentage given for contraction of one of the assay organs. Other prostaglandin-sensitive tissues such as the guinea pig ileum and guinea pig trachea contracted in 29 and 71 percent of the cases used. As stated by Said and co-workers, some of the organ responses could not be attributed solely to prostaglandins. Addition of aspirin-like drugs to the perfusate might have indicated whether prostaglandins actually were released. An irregular release of prostaglandins from the perfused lungs might well have been an artifact caused by the experimental situation. Prostaglandins are released by various chemical and mechanical stimuli with distortion of cell membranes (*5*). Since the lungs perfused were not normal in the control situation, even slight additional changes might induce prostaglandin-release as a secondary and not as a causal event.

After administration of aspirin to cats a significant reduction of the pulmonary hypoxic response was reported. However, aspirin by itself elevated PVR, whereas the PVR level obtained during hypoxic breathing was identical with that in the control situation. When an aspirin-related reduction in the vasoconstrictor response was claimed, it was based on calculations in percent of the new PVR baseline levels, assuming a linear system. No tests with a pulmonary vasoconstrictor agent were carried out. General and nonspecific depression of vascular smooth muscles has earlier been described for rabbit lungs following the administration of aspirin-like drugs (*6*).

For these several reasons we do not feel convinced that the experiments of Said and co-workers are conclusive as regards a frequent prostaglandin release, and its possible role in the mediation of the pulmonary vascular response to hypoxia. In the notes of their report Said and co-workers also state that another inhibitor of prostaglandin biosynthesis, indomethacin, "sometimes even enhanced" the pulmonary arterial pressor response to hypoxia in cats (*1*).

This last observation is in agreement with recent results obtained in our laboratory (*7*). We used an isolated, ventilated rat lung preparation perfused with blood at constant volume inflow (*8*). Pressor responses elicited by repeated episodes of alveolar hypoxia (*9*) followed a characteristic pattern (*8*). In all experiments PVR was within normal limits for rats. In eight experiments addition to the perfusate (100 μg/ml) of either indomethacin, sodium meclofenamate, or aspirin never gave a reduction in the pressor responses to alveolar hypoxia, but sometimes a moderate increase in these responses was observed. This increase was significant ($P < .04$, Wilcoxon two-sided test). The baseline level of the pulmonary arterial pressure was not in-