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Epinephrine-Induced Vacuole Formation in Parotid Gland Cells and Its Independence of the Secretory Process

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ABSTRACT **Catecholamines cause rapid release of K^+ and formation of vacuoles in acinar gland cells. A high K^+ concentration in the medium bathing parotid gland slices prevents vacuole formation by epinephrine and facilitates the secretion of most of the exportable amylase. N^6-monobutyryl 3′:5′-cyclic AMP does not cause K^+ release and vacuole formation although it efficiently induces amylase secretion. It is suggested that the secretory process is independent of vacuole formation.**

In a previous study (1) it was shown that enzyme secretion induced by catecholamine in the rat parotid gland involves fusion of the secretory granule membrane with the cell membrane that faces the lumen. More recent experiments showed that during the first minutes after intraperitoneal injection of isoprenaline, vacuoles appear in the gland cells. Electron micrographs indicated that the vacuoles are formed from membrane-bound vesicles, in the Golgi area, that take up a large volume of liquid.

The process by which the hormone causes these vacuoles to form and the relation of the process to enzyme secretion was further studied in rat parotid-gland slices.

MATERIALS AND METHODS

Techniques for the study of enzyme secretion in parotid slices were those previously described (2), with the following modifications. The gas mixture (95% O_2–5% CO_2) was continuously bubbled through the medium during the experiments. The Krebs-Ringer bicarbonate medium contained the additional components (mM): β-hydroxylbutyrate, 5; nicotinamide, 10; inosine, 10; adenine, 0.5. When the usual 5 mM K^+ was increased to 50 mM, it replaced an equivalent amount of Na^+. Epinephrine, when added, was 12 μM and N^6-butyryl 3′:5′ cyclic AMP was 1 mM. Secretion of amylase and of K^+ into the medium was expressed as a percentage of the total amount initially present in the slices. Potassium was assayed by atomic absorption. Procedures for phase and electron microscopy were those used in a previous study (1).

RESULTS

Samples of slice tissue after 10 min incubation showed extensive vacuolation only in the system containing the catecholamine (Fig. 1). In all ultrastructural characteristics the vacuoles appearing in the slices were identical with those observed *in vivo*. However, in contrast to the experiments with whole animals, where the vacuoles rapidly disappeared at initial stages of enzyme secretion, the vacuoles in the slices persisted.

We hypothesized that their persistence might be connected with the fact that the slices fail to secrete more than about 50% of their total amylase content (2). A search for medium components that might decrease vacuole formation and increase the extent of secretion was therefore made. We found that when the K^+ concentration in the medium was raised from 5 mM to 50 mM, vacuole formation by epinephrine was almost completely prevented, while amylase secretion often reached 90% of the amount initially present in the slices (Fig. 2). It could therefore be concluded that vacuole formation *per se* was not essential for enzyme secretion.

The findings further indicated that a reduction in the K^+ concentration in the cell might be a cause in vacuole formation when the medium contains the usual 5 mM K^+. Measurements of K^+ release by the slices in such medium indeed showed that epinephrine caused rather rapid and extensive K^+ release while the control slices without hormone showed no loss of this ion during the experiments (Fig. 3). Calcium in the medium was found to be required for the hormone-induced K^+ release and vacuole formation. When incubation was carried out under nitrogen or in the presence of uncouplers of oxidative phosphorylation, K^+ rapidly leaked out of the cells even in the absence of the hormone. However, vacuoles did not form under such conditions whether hormone was present or absent, presumably because ATP is required for this process.

Since cyclic AMP is an intermediate in the induction of enzyme secretion in the parotid gland (2, 3) butyryl cyclic AMP was tested in the medium containing 5 mM K^+. The nucleotide, after a brief lag period (compare refs. 2, 3) caused rapid and extensive enzyme secretion without K^+ release and without vacuole formation (Figs. 2, 3).

DISCUSSION

Previous work (2, 3) showed that butyryl cyclic AMP can be as efficient as the catecholamine in inducing enzyme secretion in parotid slices. The finding that, in the same system, K^+ release and vacuole formation caused by the hormone cannot be reproduced by butyryl cyclic AMP suggests that the cyclic nucleotide may not be an essential intermediate in these latter manifestations of catecholamine action. Accordingly, the following sequence of events might be postulated. Catecholamine binds to receptors in the cell membrane, apparently those which are part of the adenyl cyclase system (4). The binding causes a change in conformation of the membrane components linked to the receptor, which permits leakage of K^+ and entry of various ions from the medium. The resulting changes in the concentration of specific ions in the cell lead to rapid swelling of vesicles in the Golgi area in a process involving ATP. It should be noted that in the perfused rat liver, addition of cyclic AMP did cause release of Ca^{++} and

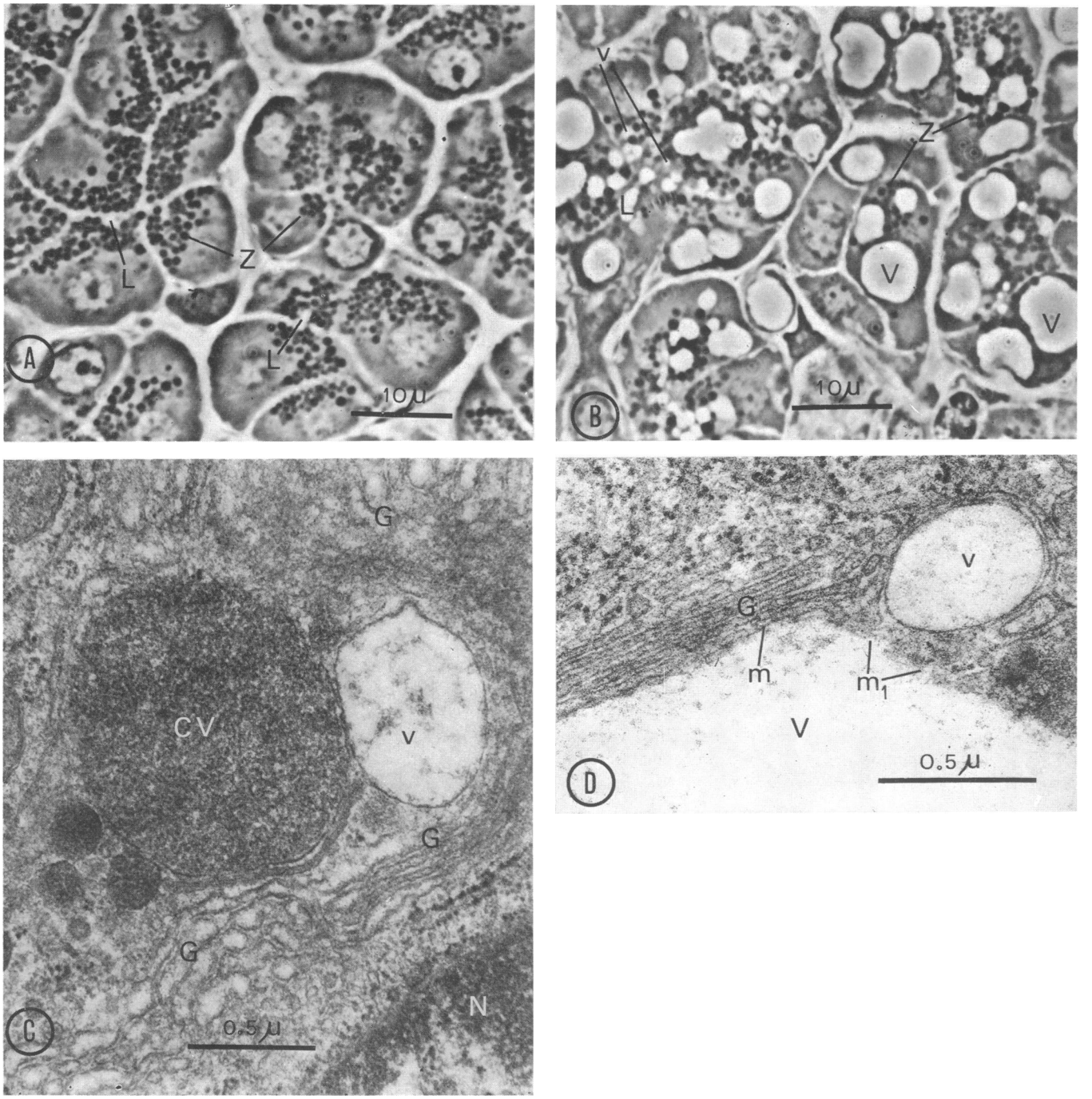

FIG. 1. Epinephrine-induced formation of vacuoles within the acinar cells of the rat parotid gland. (*A*) Phase-contrast micrograph of a section of a slice incubated in the absence of epinephrine. Normal appearance of the acini loaded with zymogen granules (*Z*) and small lumens (*L*). (*B*) As in Fig. 1*A*, but after incubation with epinephrine. Extensive vacuolation within the acinar cells; size of vacuoles varies from 0.5 μm (*v*) to about 6–10 μm (*V*). (*C*) Electron micrograph of tissue incubated with epinephrine, showing a small vacuole (*v*) appearing within the Golgi area (*G*) adjacent to a developing zymogen granule (condensing vacuole, *CV*). Vacuole is enveloped by a smooth membrane and appears to develop from a Golgi vesicle. *N*, nucleus. (*D*) As in Fig. 1*C;* An electron micrograph showing a small vacuole (*v*) and only a part of an extensively swollen vacuole (*V*), both originating within the Golgi area (*G*). Because of the swelling, the smooth membrane (*m*) enclosing the large vacuole (*V*) is ruptured in several places (m_1).

Slices were incubated for 10 min in the medium containing 5 mM KCl. In the presence of epinephrine, about 26% of the amylase initially in the slices was secreted.

K^+ (5). Further experiments will therefore be necessary to decide whether cyclic AMP is really not involved in K^+ release and vacuole formation caused by catecholamines in the parotid gland.

The present findings also appear to shed some light on a number of seemingly independent phenomena reported in the literature. Since the work of D'Silva (6) it has been known that epinephrine injection causes a transient rise in blood

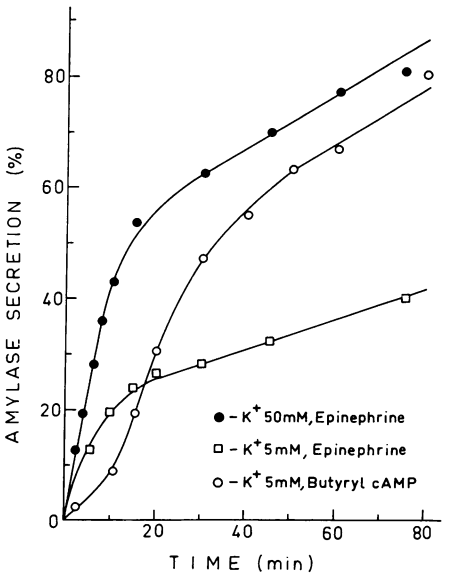

FIG. 2. Dependence of epinephrine-induced secretion of amylase on the K^+ concentration in the medium.

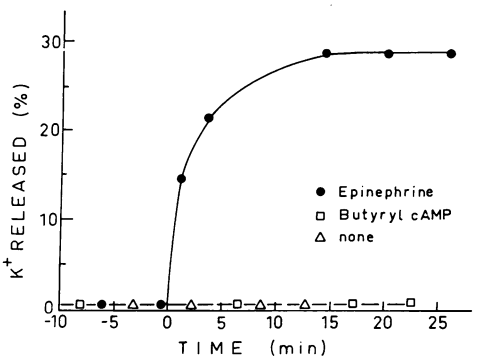

FIG. 3. Epinephrine-induced release of K^+ by parotid gland slices. Samples of medium were periodically removed for K^+ determination by atomic absorption. At time zero epinephrine was added to one system while a second system received butyryl cyclic AMP. The amount of K^+ initially in the slices (80 μeq/g wet weight) was defined as 100%. The rates of amylase secretion were essentially the same as those shown in Fig. 2.

K^+, due to release of this ion by the liver (7). An increase in blood K^+ can also be obtained simply by placing the animal under hypoxic conditions (7). Even the latter hyperkalemia can be prevented by dibenamine—a catecholamine-blocking agent (7). Trowell independently showed that hypoxic conditions caused vacuolation in the liver (8). The present work suggests that these events in the liver are like those in the parotid gland. The animals that are in a state of alarm because of the hypoxic conditions presumably secrete epinephrine into the bloodstream, thus causing the observed increase in blood K^+. The loss of K^+ from the liver in turn brings about vacuolation by the process discussed above for the parotid gland. There also seems to be little doubt that this process is responsible for the vacuolation produced in the submandibular gland by feeding animals after a period of starvation (9).

Tapp (9–11) reported on vacuolation produced *in vitro* in submandibular, parotid, and pancreas preparations. These findings are similar to the present results in that vacuolation occurred in the Golgi area (10) and required the presence of Ca^{++} in the medium. However, in the experiments of Tapp, no hormone was added to any of the systems and the formation of vacuoles required an extended incubation period in unoxygenated media (9–11). Although these particular incubation conditions might have been responsible for ion movements leading to vacuolation, the possibility that the process was due to the action of hormone released from endogenous stores within the tissue (compare, ref. 12) has apparently not been excluded.

The loss of K^+ caused by catecholamines seems to have additional consequences in the cell. In an attempt to restore ion balance the ($Na^+ + K^+$)-stimulated ATPase (EC 3.6.1.3) would become maximally activated by the change in ion concentration. This would lead to a large expenditure of ATP and an increase in ADP, which is required for respiration in tightly-coupled mitochondria (compare ref. 13). Thus at least part of the increased oxygen uptake usually caused by epinephrine (2, 14, 15) might be due to the observed K^+ release.

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