Blockage of Cell-to-Cell Communication within Pancreatic Acini Is Associated with Increased Basal Release of Amylase

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Abstract. To assess whether junctional coupling is involved in the secretory activity of pancreatic acinar cells, dispersed rat acini were incubated for 30 min in the presence of either heptanol (3.5 mM) or octanol (1.0 mM). Exposure to either alkanol caused a marked uncoupling of the acinar cells which, in control acini, were extensively coupled. Uncoupling was associated with an increased basal release of amylase that was at least twice that of controls. By contrast, carbamylcholine (10^{-5} M)-induced maximal amylase secretion, cytosolic pH, and free Ca^{2+}, as well as the structure of gap junctions joining the acinar cells, were unaffected. Both uncoupling and the alteration of basal secretion were already observed after only 5 min of exposure to heptanol, they both persisted throughout the 30-min exposure to the alkanols, and were reversible after removal of either heptanol or octanol. Since neither of the two uncouplers appeared to alter unspecifically the secretory machinery and the nonjunctional membrane of acinar cells, the data are consistent with the view that junctional coupling participates in the control of the basal secretion of acinar cells.

GAP junctions (11) and coupling (10, 17, 31) have been described for a long time between the acinar cells of the exocrine pancreas, but their function in these cells is still undetermined, as is the case in most other electrically inexcitable adult tissues (13, 20). The observation that dispersed acinar cells do not respond to secretagogues as isolated acini (1, 12, 27, 38) led to suggest that direct interactions between neighboring cells may play a role in the normal functioning of the pancreatic acinus. The additional finding that several secretagogues uncouple the cells of pancreatic acini at concentrations that elicit maximal enzyme secretion (9, 15, 16, 28) further suggested that if junctional coupling is related to the secretory activity of acinar cells (28–30), it is probably not obligatory for their acute maximal secretory response. As yet, however, the possible relationship between acinar cell-to-cell coupling and enzyme secretion has not been investigated. To approach this question, we have studied whether a short period of reversible uncoupling affects amylase secretion from dispersed rat acini.

Among the methods available to induce uncoupling, those that affect cytosolic free Ca^{2+} ($[Ca^{2+}]_i$),[1] cytosolic pH (pH_i), or ATP (20, 34) are not appropriate for this study since these factors are also involved in the control of secretion and, thus, make it impossible to establish whether secretory changes are specifically related to uncoupling. Antibodies directed against gap junctions appear as ideal tools to address this question. Unfortunately, the antibodies so far available are active only upon intracellular application (14, 18, 36) and presently cannot be incorporated into the relatively large number of acinar cells needed to assess secretion. Therefore, we have used heptanol and octanol, two alkanols acting as uncouplers (19) by a still undetermined mechanism that may involve their direct interaction with gap junctions (3, 32, 34, 40).

Here we report that these two alkanols uncouple pancreatic acinar cells and cause a parallel increase in the unstimulated basal release of amylase, while carbamylcholine-stimulated amylase secretion is unaffected. Both coupling and secretion changes are reversible upon removal of the alkanols and occur under conditions that do not cause detectable changes in $[Ca^{2+}]_i$, in cytosolic pH (pH_i), or in the structure and size of acinar cell gap junctions. These data strengthen the hypothesis (22) that junctional communication is involved in the secretory function of gland cells.

Materials and Methods

Preparation of Acini

Acini were isolated as described elsewhere (5) from the pancreas of male Wistar rats weighing ~200 g. Upon isolation, the acini were suspended in a Krebs-Ringer-bicarbonate medium containing 12.5 mM Hepes and 0.1% serum albumin (control KRB)[1] and preincubated for 30 min at 37°C under continuous gassing with 95% O_2/5% CO_2.

Experimental Conditions

Dye injections and measurement of basal amylase secretion were performed after a 30-min incubation of acini in one of the following test media: (*a*) control KRB; (*b*) control KRB containing 3.5 mM 1-heptanol (Sigma Chemical Co., St. Louis, MO); (*c*) control KRB containing 1 mM 1-octanol (Sig-

1. *Abbreviations used in this paper*: $[Ca^{2+}]_i$, cytosolic free Ca^{2+}; control KRB, Krebs-Ringer-bicarbonate medium containing 12.5 mM Hepes and 0.1% serum albumin; pH_i, cytosolic pH.

ma Chemical Co.) dissolved with 0.1% dimethylsulfoxide. At this concentration, dimethylsulfoxide alone had no effect on either acinar coupling or amylase release (not shown).

To assess the reversibility of the alkanol effects, injection and secretion studies were also performed with acini that were first incubated as described above, repeatedly rinsed in control KRB, and finally incubated again for 30 min in this medium.

Amylase release was stimulated by adding carbamylcholine (Sigma Chemical Co.), at concentrations ranging from 10^{-8} to 10^{-4} M, to the above-described media. Some dye injection experiments were also performed in the presence of this secretagogue at the concentration of either 10^{-6} or 10^{-5} M.

Dye Coupling Studies

Aliquots of dispersed acini were plated on 35-mm culture dishes coated with 0.5 mg/ml of poly-L-lysine (150,000–300,000 mol wt) and allowed to rest 10 min at room temperature in a humidified chamber. 2 ml of the appropriate test medium (see above) were then added, and the dishes were kept at 37°C until the end of the experiment. Dishes with attached acini were transferred on the heated (37°C) stage of an inverted ICM405 Zeiss microscope, and a thin layer of mineral oil was placed over the medium to prevent its evaporation. Individual acinar cells were impaled with glass electrodes (with a resistance of 100–150 MΩ when filled with 4 M potassium acetate) that were filled up to the shoulder with a 4% solution of Lucifer Yellow CH (Sigma Chemical Co.) in 1 M lithium chloride, buffered to pH 7.2 with 10 mM Hepes. Just before use, the body of the electrode was filled with 3 M lithium chloride also buffered with Hepes. The microelectrode was connected to an amplifier for passing current pulses and recording voltages as described in reference 2. Upon impalement, 0.1 nA negative square pulses of 900-ms duration and 0.5-Hz frequency were applied to the electrode for 3 min. Cells were used in the study only if a stable resting membrane potential (mean ± SEM was 36.3 ± 0.5 mV, n = 159) was measured from the impalement and up to the end of the injection period. At this time, the electrode was removed, and the injected acinus was photographed at a magnification of 40 using a xenon XBO 75 WZ lamp and filters for fluorescein detection. The same field was then photographed a second time using this system combined with a phase-contrast illumination. Both photographs were taken with a constant exposure time of 90 s on Kodak Ektachrome (400 ASA) color slide film. Injections were restricted to 30 min per dish (usually three acini could be injected and photographed during such a period), and the whole experiment was concluded within 3 h after the acini were isolated.

To quantitatively evaluate the extent of coupling, the photographs taken at the end of each injection were projected and used to trace the profile of the acinus and of the cell(s) containing Lucifer Yellow, at the final magnification of 1,325. The areas of each tracing were then measured using a Tektronix 4953 graphic tablet (Tektronix, Inc. Beaverton, OR) and an IMSAI 8080 microcomputer system (IMSAI Mfg. Co., San Leandro, CA). The absolute values of Lucifer Yellow–stained areas and the percentage of the projected acinus surface they represented were expressed as mean ± SEM and were compared using an unpaired t test. The distribution of coupling extent based on the estimated number of cells containing Lucifer Yellow was also plotted and compared using the χ^2 test. The incidence of dye uncoupling was given in this distribution by the proportion of impalements that did not result in the intercellular transfer of Lucifer Yellow.

Conventional and Freeze-Fracture Electron Microscopy

Aliquots of isolated acini were centrifuged into loose pellets and fixed for 60 min at room temperature in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer, pH 7.4. For conventional electron microscopy, the acini were postfixed in 2% phosphate-buffered osmium tetroxide, stained en bloc with uranyl acetate, dehydrated in graded ethanols, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate. For freeze-fracture electron microscopy, the glutaraldehyde-fixed acini were infiltrated for 60 min in 30% phosphate-buffered glycerol and frozen in Freon 22 that had been cooled with liquid nitrogen. The pellets were fractured and shadowed in a Balzers BAF 301 apparatus (Balzers, High Vacuum Corp., Balzers, Liechtenstein). The replicas were washed in a sodium hypochlorite solution, rinsed in distilled water, and mounted on Formvar–carbon-coated copper grids. Thin sections and replicas were examined in a Philips EM 301 electron microscope.

Freeze-fractured gap junctions were analyzed quantitatively to evaluate possible changes in size and particle arrangement during uncoupling. For size measurements, ~400 randomly selected gap junctions were photographed at a magnification of 14,000 on P- and E-fracture faces of control, heptanol-treated, and octanol-treated acinar cells from three to five experiments. The negatives were projected on a graphic tablet (see above) to measure individual gap junction areas at a final magnification of 70,000. The highly asymmetric distributions of gap junctional areas were compared using the Mann–Whitney and the Kolmogorov–Smirnov nonparametric tests. Average values were expressed as medians and compared using the median test.

To evaluate particle arrangement, 50 selected gap junctional P-faces, each from a different cell, were photographed at a magnification of 33,000 in the control, heptanol-treated, and octanol-treated acini. The negatives were analyzed by optical diffraction using a monochromatic and coherent laser light with a wavelength of 632.8 nm and an optical system giving a diffraction pattern of ~1-cm diam on Ilford FP4 120 film. A square diaphragm of 10-mm side limited the measurement to a gap junctional area containing at least 900 particles. Spacing of the diffraction maxima was measured on 10× enlarged projections of the negatives by one investigator who was not aware of each junction's group. Magnifications of negatives and diffraction patterns were calibrated with a reference grid containing 2,160 lines/mm and

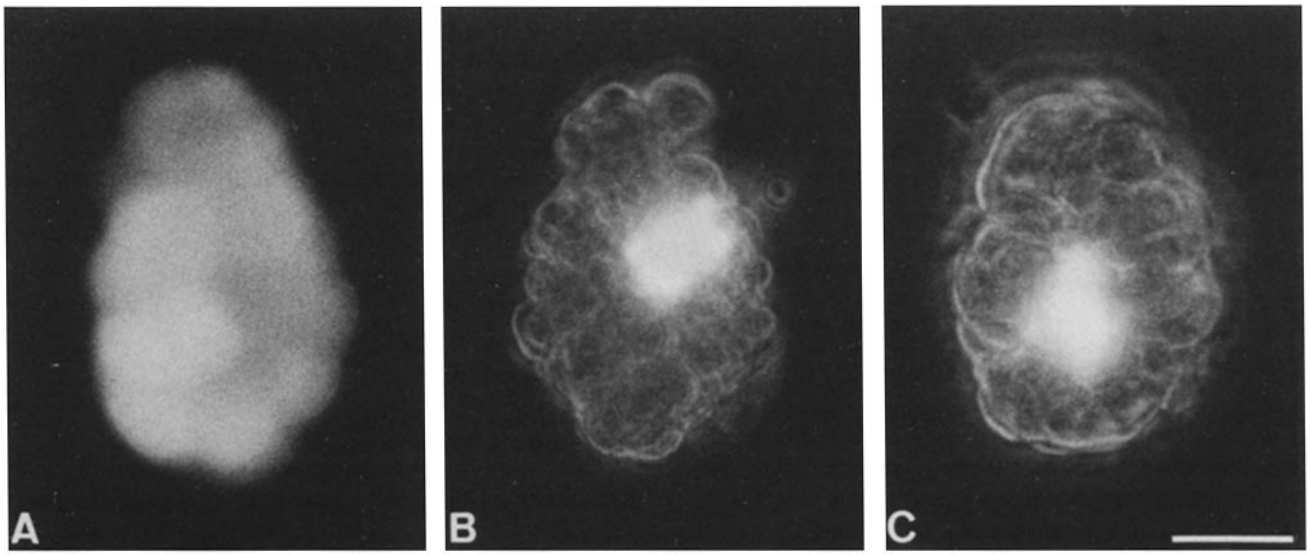

Figure 1. Isolated rat acini injected with Lucifer Yellow. In the control group (*A*), all cells of the acinus were found coupled to each other, as judged by their ability to exchange Lucifer Yellow. By contrast, after incubation with either heptanol (*B*) or octanol (*C*), most acinar cells were found uncoupled from their neighbors, as judged by the lack of diffusion of Lucifer Yellow outside the injected cell. Bar, 20 μm.

a negatively stained catalase crystal (8.75 nm and 6.85 nm periodicity), respectively. The calculated values of particle spacings were expressed as mean ± SEM and compared using an unpaired *t* test.

Secretion Studies

After the preincubation period (30 min), the acini were allowed to sediment and the supernatant was removed and replaced by control KRB. 2-ml aliquots of this acinar suspension (150–200 μg acinar proteins/ml) were placed in glass vials. At the beginning of the incubation (time 0), two 500-μl samples were taken from the acinar suspension, centrifuged 20 s in a Beckman microcentrifuge (Beckman Instruments, Inc., Palo Alto, CA), and the supernatant was analyzed for amylase content, as previously described (4). The pellets were washed twice with cold 0.9% NaCl, sonicated, and then assayed also for amylase content. The total initial amylase content of the acini was then calculated by adding the supernatant and pellet values measured at time 0. At the end of the 30-min test incubation in the presence or absence of carbamylcholine (see above), two 500-μl aliquots were taken from each vial, centrifuged, and amylase was measured in the supernatant as described above. Amylase release was then calculated by subtracting the value measured in the supernatant at time 0 from the value measured in the supernatant at the end of the test incubation and was expressed as a percentage of the total initial content.

To study the reversibility of the effect of uncoupling, acini were first incubated as described above in the presence or absence of either heptanol or octanol. At the end of the 30-min test period, two 500-μl aliquots were taken from each vial, centrifuged, and assayed for amylase measurement, as described above. The remaining acini were centrifuged for 20 s at 80 *g*, washed twice with control KRB, resuspended in 2 ml of this medium, and finally incubated again for 30 min. At the end of this second incubation, two samples from each vial were processed for amylase measurement as described above.

Values of amylase release were expressed as mean ± SEM and compared using an unpaired *t* test.

Measurement of $[Ca^{2+}]_i$

Dispersed acini were suspended in RPMI 1640 culture medium containing 0.5% serum albumin and buffered to pH 7.4 with 25 mM Hepes and were loaded with 50 μM quin2/acetoxymethylester (Sigma Chemical Co.) for 30 min at 37°C (6). After a 20-s centrifugation at 80 *g*, the acini were washed, resuspended in RPMI without albumin, and kept at room temperature until use. Fluorescence measurements and calibrations were performed as previously described (6, 39). Briefly, acini were centrifuged at 80 *g* for 20 s, resuspended in 2 ml of control KRB buffered with 25 mM Hepes and 5 mM $NaHCO_3$ to give a concentration of 6–8 × 10^6 cells/ml, and kept, under continuous stirring at 37°C, in the glass cuvette of a Perkin-Elmer LS3 spectrofluorometer (Perkin-Elmer Corp., Norwalk, CT). After calibration, the $[Ca^{2+}]_i$ was calculated in each experiment according to Eq. 1 of Tsien et al. (35). None of the drugs (heptanol, octanol, and carbamylcholine) added to the acini during these measurements changed the fluorescence of unloaded control acini.

pH_i Measurements

Dispersed acini were loaded with 10 μM 2',7'-bis (carboxyethyl)-5,6-carboxyfluorescein/acetoxymethylester (33) and then processed as described above for the experiments with quin2. The fluorescence signal was calibrated at the end of each trace by lysing the cells with 0.1% Triton X-100 and by measuring the fluorescence of the released dye at various known pH, as described (33). Again, no change in fluorescence was observed upon exposure of unloaded control acini to the different drugs tested.

Results

Dye Coupling

In freshly isolated control acini, microinjection of Lucifer Yellow always resulted in the rapid transfer of the tracer from the injected cell into its neighbors, most often into all cells of the acinus (Figs. 1 *A* and 2). Quantitation showed that Lucifer Yellow labeled an average area of 2,600 μm^2, corresponding to 93–96% of the projected acinus profile (Tables I and II). 2–3 h after the acini were isolated, dye coupling

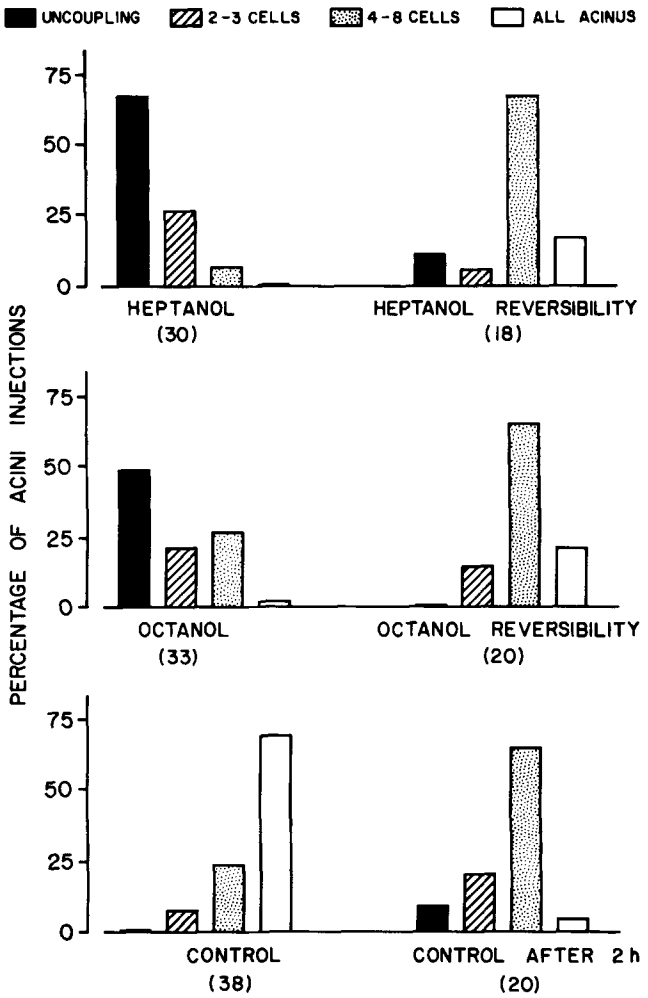

Figure 2. Extent of dye coupling in control, heptanol-treated, and octanol-treated acini. Extensive coupling was always observed in control acini at the beginning of the experiment (*lower left*). 2 h after isolation, acinar cells were still frequently coupled although to a lesser extent (*lower right*). Heptanol markedly increased the proportion of uncoupled cells and decreased the spatial diffusion of Lucifer Yellow among the remaining coupled cells (*upper left*). A similar pattern was observed upon exposure to octanol (*middle right*). After removal of heptanol or octanol (*upper right* and *middle right*), acinar cells were found coupled as in control acini (*lower right*).

was still very frequent between acinar cells, but fewer acini showed coupling of all their cells (Fig. 2). As a result, the average diffusion of Lucifer Yellow was limited to an area corresponding to 54–58% of the acinus profile (Tables I and II).

After a 30-min exposure to heptanol, Lucifer Yellow often remained within the injected cell (Figs. 1 *B* and 2). As seen in Table I, uncoupling was then observed in 67% of the cases, as compared with 0% in the controls. When a larger diffusion of Lucifer Yellow was seen, it was usually restricted to a few cells, and the tracer was never exchanged between all cells of an acinus (Fig. 2). Quantitation revealed that the absolute and relative areas labeled by Lucifer Yellow within an acinus were decreased ($P < 0.001$) 3.3- and 5.3-fold, respectively, as compared with control values (Table I). Similar experiments performed after either a 5- or 10-min

Table I. Effects of Heptanol on Dye Coupling within Isolated Rat Pancreatic Acini

Group	Acini injected	Uncoupled cells	Extent of dye diffusion*	
			μm²	% of acinus area
		%		
Control	19	0	2,597.4 ± 181.7	96.3 ± 5.6
Heptanol	30	66.7	774.1 ± 78.6‡	18.5 ± 2.5‡
Heptanol reversibility	18	11.1	2,076.8 ± 223.3§	66.8 ± 7.5§‖
Control after 2 h	8	25.0	1,915.6 ± 517.2	58.1 ± 15.0¶

* Values are mean ± SEM.
‡ $P < 0.001$, heptanol vs. control.
§ $P < 0.001$, heptanol reversibility vs. heptanol.
‖ $P < 0.005$, heptanol reversibility vs. control.
¶ $P < 0.05$, control after 2 h vs. control.

Table II. Effects of Octanol on Dye Coupling within Isolated Rat Pancreatic Acini

Group	Acini injected	Uncoupled cells	Extent of dye diffusion*	
			μm²	% acinus
		%		
Control	19	0	2,567.7 ± 252.9	93.1 ± 6.2
Octanol	33	48.5	1,111.3 ± 132.1‡	33.5 ± 4.8‡
Octanol reversibility	20	0	2,107.5 ± 191.9§	62.3 ± 6.3§‖
Control after 2 h	12	0	2,126.3 ± 331.5	53.7 ± 6.9¶

* Values are mean ± SEM.
‡ $P < 0.001$, octanol vs. control.
§ $P < 0.001$, octanol reversibility vs. octanol.
‖ $P < 0.001$, octanol reversibility vs. control.
¶ $P < 0.001$, control after 2 h vs. control.

Table III. Time Course of Heptanol Effect on Basal Amylase Release and Acinar Cell Coupling

Group	Amylase release*	Uncoupling	Coupling of all acinus	No. of injections
Control				
5 min	1.0 ± 0.2 (5)	0	18	25
10 min	1.4 ± 0.3 (4)	0	6	7
30 min	2.8 ± 0.3 (5)	0‖	26‖	38‖
Heptanol				
5 min	3.4 ± 0.2‡ (5)	4	0	5
10 min	3.7 ± 0.3‡ (4)	6	0	9
30 min	7.7 ± 1.4‡ (5)	20§	0§	30§

* Values are mean ± SEM; values in parentheses are numbers of experiments.
‡ $P < 0.001$, heptanol vs. control.
§ Data from Table I.
‖ Data from Tables I and II.

exposure to heptanol already revealed uncoupling of acinar cells (Table III). At each of these time points, the distribution of coupling extent was significantly ($P < 0.001$) different in the heptanol-treated and in the control acini. The uncoupling effect quantitated after a 30-min incubation was reversible upon removal of heptanol, rinsing, and incubation of the acini for 30 min in control medium. Under these conditions, the distribution (Fig. 2) as well as the absolute and relative areas of dye diffusion (Table I) were similar to those of control acini processed in parallel (control after 2 h group).

Analogous, although smaller effects on coupling were seen after a 30-min exposure of acini to octanol (Figs. 1 *C* and 2). In this condition, uncoupling was observed in 48% of the cases, and the area of dye diffusion was decreased ($P < 0.001$) 2.3–2.8-fold as compared with controls (Table II). Removal of octanol was accompanied by restoration of a dye coupling extent similar to that of controls (Table II).

In addition to these experiments, which were all performed in control KRB, i.e., in the absence of carbamylcholine, a few acini were also microinjected after exposure to

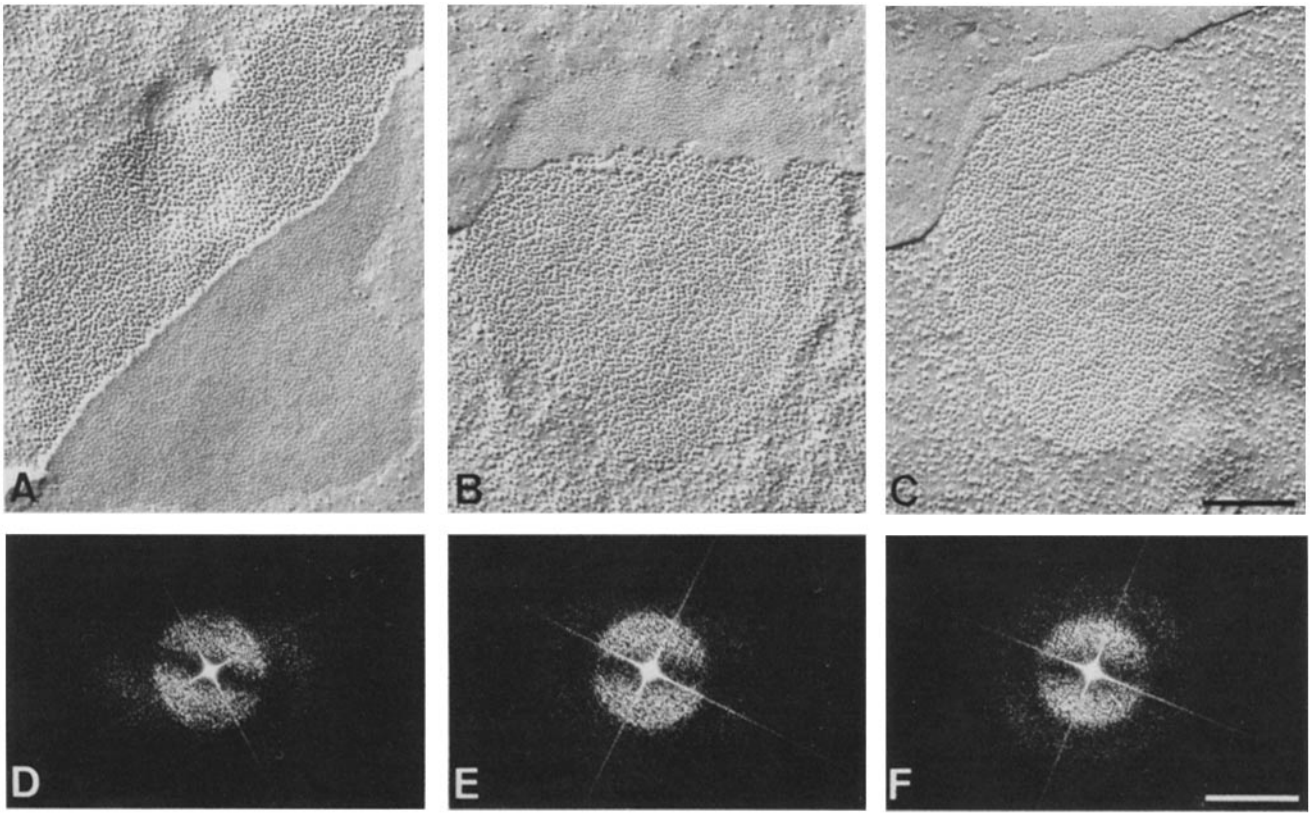

Figure 3. (*A–C*) Freeze-fracture replicas of gap junctions in control (*A*), heptanol-treated (*B*), and octanol-treated dispersed acini (*C*). The qualitative comparison of the three figures shows a similar appearance of the junctions between coupled (*A*) and between alkanol-uncoupled acinar cells (*B* and *C*). Bar, 200 nm. (*D–F*) Optical diffraction patterns of the gap junctions shown in *A–C*. Virtually all diffraction patterns obtained showed a circular shape, indicating a random arrangement of particles in the gap junctions of both control (*D*) and alkanol-treated acinar cells (*E* and *F*). The radius of these circular profiles appeared similar in the three groups studied (see also Table IV), indicating no major differences in the average spacing of particles within the gap junctions of coupled and uncoupled acinar cells. Bar, 8 nm.

maximally stimulatory concentrations (10^{-6}–10^{-5} M) of this secretagogue. As expected (9, 15, 16, 28), carbamylcholine rapidly depolarized and uncoupled at least partially the acinar cells.

Ultrastructure

At both light and electron microscopic levels, the organization of cells within dispersed acini was similar to that seen in the native pancreas and was not affected by exposure to either heptanol or octanol (not shown). Membrane structure, as revealed by freeze-fracture, appeared also similar in control, heptanol-treated, and octanol-treated acini. In particular, no obvious difference in the size of gap junctions or in the arrangement and spacing of their constitutive particles was apparent between the three groups studied (Fig. 3, *A–C*). These observations were confirmed by a quantitative analysis that showed that the areas of individual gap junctions and the average spacing of the gap junction particles were similar between coupled and alkanol-uncoupled acinar cells (Table IV). The optical diffraction patterns (Fig. 3, *D–F*), which were used for the latter measurements, also revealed that in virtually all the gap junctions studied, particles were randomly arranged. Only five out of the 162 gap junctions studied gave diffraction patterns with distinct spots, indicating a hexagonal packing of the particles (not shown). Three of these junctions were found in control acini and two were seen in octanol-treated acini. These numbers represent 5.1 and 3.8%, respectively, of the junctions studied in these groups.

Amylase Secretion

At the end of the 30-min exposure to either heptanol or octanol, which was used for most experiments, the basal release of amylase was increased ($P < 0.001$) twofold over con-

Table IV. Area and Particle Spacing of Acinar Cell Gap Junctions

	Gap junctional area (μm^2)			Particle spacing (nm)		
Group	*n*	Range	Median	*n*	Range	Mean ± SEM
Control	468	0.005–12.11	0.21	59	7.07–8.66	8.02 ± 0.04
Heptanol	438	0.010–10.15	0.19	50	7.20–8.69	7.99 ± 0.05
Octanol	417	0.008–12.98	0.21	53	7.13–8.50	7.91 ± 0.04

n, no. of gap junctions analyzed.

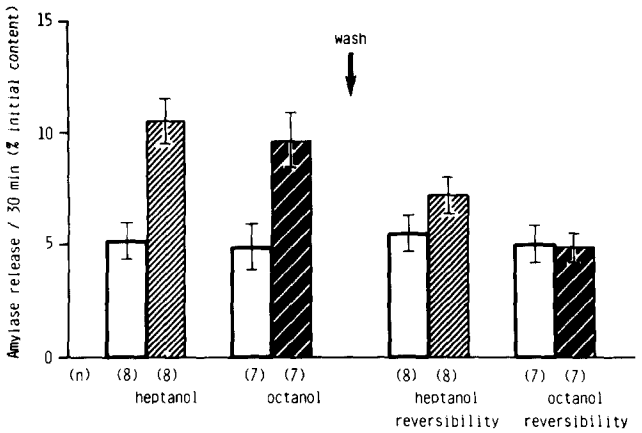

Figure 4. Effect of alkanols on basal amylase release of dispersed rat pancreatic acini and reversibility of this effect. As seen in the left half of the figure, a 30-min incubation of the acini in the presence of either heptanol or octanol caused a marked increase ($P < 0.001$ for heptanol; $P < 0.01$ for octanol) in their basal amylase secretion as compared with controls (open bars). The right half of the figure shows that after removal of the alkanols, washing and incubation for 30 min in control KRB, the basal secretion of the treated acini decreased significantly ($P < 0.02$ for heptanol; $P < 0.005$ for octanol). Thus, at the end of this second incubation, the basal secretion of both heptanol- and octanol-pretreated acini was similar to that of controls. Values are mean ± SEM. The number of experiments is indicated in parentheses.

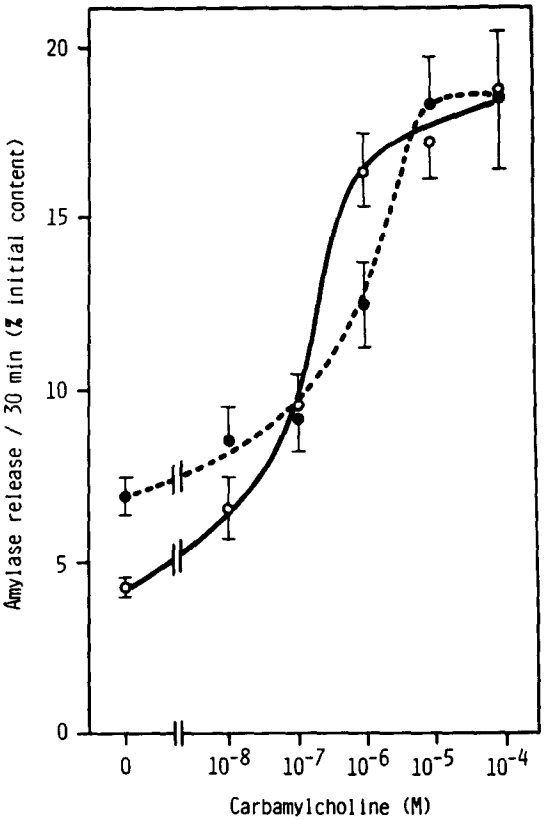

Figure 5. Effect of heptanol on basal and carbamylcholine-stimulated amylase release of dispersed rat pancreatic acini. Heptanol-treated acini (--●--) showed an increased basal release as compared with controls (—○—). In contrast, amylase release in response to maximal stimulatory concentrations of carbamylcholine was similar in the two groups. Values are mean ± SEM of eight independent experiments.

trol values (Fig. 4). Parallel experiments showed that, as for uncoupling, this change was already established ($P < 0.001$) after 5 min of incubation. Thus, after 5 and 10 min of exposure to heptanol, it represented 44 and 48% of the value observed at the end of the 30-min incubation, respectively (Table III). Furthermore, it can be calculated from these data that the amylase release per minute was 2.7 times higher in the presence than in the absence of heptanol from the beginning (first 5 min) to the end (10–30 min) of the experiment. As shown in Fig. 4, the change in basal amylase secretion was completely (octanol) or almost completely (heptanol) reversible upon removal of either alkanol, repeated washing, and a 30-min incubation of the acini in control medium. Under these conditions, the basal release of amylase from acini pretreated with either heptanol or octanol was significantly reduced and similar to the basal release of control acini (Fig. 4).

Figs. 5 and 6 show that the amylase release of the control acini, which were used in the dye injection experiments, was already significantly increased by 10^{-8} M carbamylcholine and reached maximal levels (4–5-fold stimulation) in the presence of carbamylcholine concentrations $>10^{-6}$ M. The dose-dependent stimulation induced by carbamylcholine was still observed in the presence of either heptanol (Fig. 5) or octanol (Fig. 6). Neither of the two alkanols affected the maximal secretory level of amylase in the batches of acini in which increased basal secretion was observed (Figs. 5 and 6).

$[Ca^{2+}]_i$ and pH_i

Measurements of intracellular quin2 fluorescence confirmed (6, 25, 26) that $[Ca^{2+}]_i$ rapidly increased in dispersed acini exposed to 10^{-5} M carbamylcholine (Fig. 7 *A*). By contrast, addition of either heptanol (Fig. 7 *B*) or octanol (Fig. 7 *C*) at the concentrations used in the coupling and the secretion studies did not change $[Ca^{2+}]_i$. In addition, neither alkanol affected the kinetics and magnitude of the subsequent calcium response of acini exposed to carbamylcholine (Fig. 7, *B* and *C*).

Similarly, heptanol (Fig. 8 *B*) and octanol (Fig. 8 *C*) had no effects on pH_i and did not prevent the subsequent acidification and alkalinization of the cells by lactate and NH_4Cl, respectively (Fig. 8, *B* and *C*).

Discussion

We have shown that, as in mouse pancreatic fragments (10, 17), virtually all cells of freshly isolated rat acini were dye coupled. Heptanol and octanol rapidly caused most acinar cells to become uncoupled from their neighbors and markedly reduced the extent of communication between the few cells that remained coupled. Under the nonstimulatory conditions that were used throughout the study, this uncoupling was reversible after removal of the alkanols and a short incubation of the acini in control medium. Separate experiments performed with the alkanols in the presence of 10^{-6}–10^{-5} M carbamylcholine also showed uncoupling of the acinar cells. However, this effect could not be unequivocally interpreted

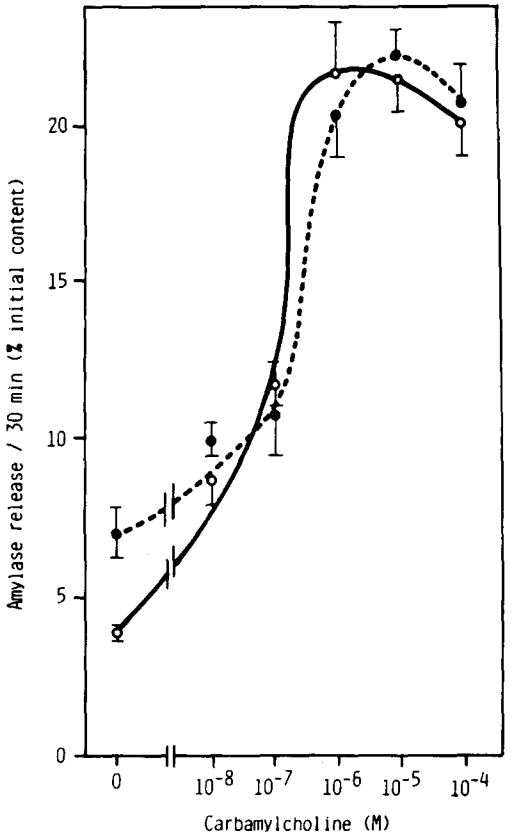

Figure 6. Effect of octanol on basal and carbamylcholine-stimulated amylase release from dispersed rat pancreatic acini. Octanol-treated acini (--●--) showed an increased basal release as compared with controls (—○—). In contrast, amylase release in response to maximal stimulatory concentrations of carbamylcholine was similar in the two groups. Values are mean ± SEM of six independent experiments.

since at these concentrations, which elicit maximal amylase secretion, carbamylcholine alone could uncouple the cells of dispersed acini, as previously reported for several other pancreatic secretagogues (9, 15, 16, 28).

That the two alkanols tested did not perturb intercellular communication by damaging the cells was shown by the normal ultrastructure of the acini, their preserved maximal secretory response to carbamylcholine, and the rapid recovery of normal coupling and basal secretion after the acini were returned to control medium. Since uncoupling did not either result from the disappearance of gap junctions, it is likely that heptanol and octanol decreased the permeability of existing junctional channels. Cytosolic free calcium and cytosolic pH are considered the most likely mediators of junctional permeability in most tissues (13, 20, 34), including the pancreatic acinar cells (15, 16, 29). However, fluorescence measurements from acini loaded with either quin2 or 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein revealed no effect of the alkanols on either one of these two factors, indicating that they were probably not involved in the uncoupling mechanism. Whether this implies that the alkanols acted directly on the junctional membranes, as suggested by previous studies on intact systems (3, 32, 34) as well as on in vitro reconstituted junctional channels (40), remains to be ascertained. Our experiments did not reveal obvious effects of the

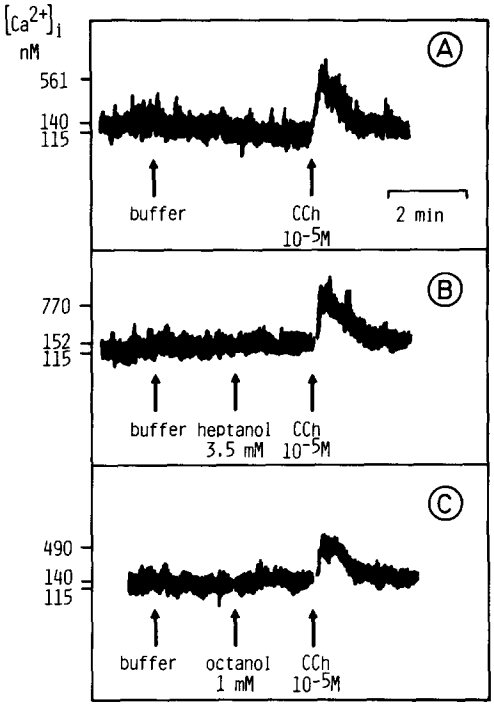

Figure 7. Effect of heptanol and octanol on $[Ca^{2+}]_i$ of dispersed rat pancreatic acini. $[Ca^{2+}]_i$ was not affected by either heptanol (*B*) or octanol (*C*). Neither of these two alkanols prevented the subsequent increase of $[Ca^{2+}]_i$ upon carbamylcholine stimulation (*A–C*). All traces are from the same experiment, which is representative of three independent experiments.

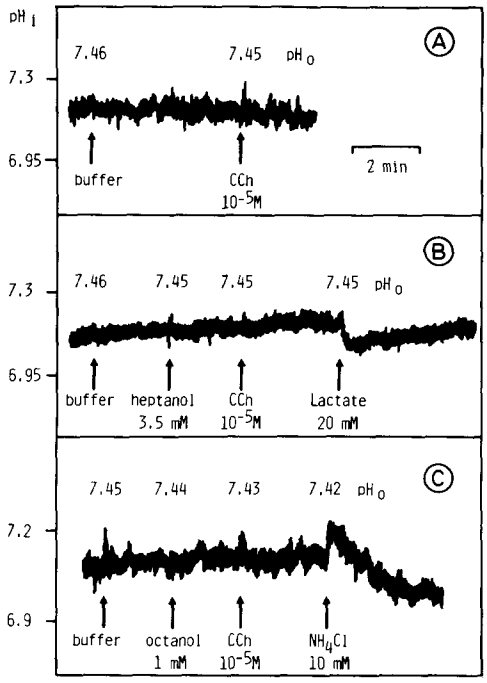

Figure 8. Effect of heptanol and octanol on pH_i of dispersed rat pancreatic acini. Heptanol (*B*) and octanol (*c*) did not affect pH_i. Neither of these two alkanols prevented the subsequent changes of pH_i induced by exposure to lactate (*B*) or NH_4Cl (*C*). The extracellular pH (pH_o) is indicated on top of each trace. All traces are from the same experiment, which is representative of three independent experiments.

alkanols on the permeability of the nonjunctional membrane to the injected tracers or on the appearance of gap junctions that were formed by similarly spaced and randomly arranged particles between both coupled and uncoupled acinar cells. Thus, in the controversial issue on the structure of gap junctions during uncoupling (3, 7, 21, 24), the present data confirm (21) that the freeze-fracture ultrastructure of acinar cell gap junctions is apparently unchanged after a short period of fully reversible uncoupling.

During the 30-min exposure to either heptanol and octanol, which was used for most experiments, the uncoupled acinar cells showed a marked increase in their basal secretion of amylase. This change was already detected, with uncoupling, after 5 min of heptanol exposure and, as uncoupling, it persisted throughout the incubation with the alkanols and was reversible upon their removal. The return to the control level of unstimulated secretion and of cell-to-cell communication was slightly less complete in the acini exposed to heptanol than in those exposed to octanol. This difference may be related to the variable efficiency of the two alkanols, since heptanol appeared more effective than octanol on both coupling and secretion of acinar cells. As expected (9, 15, 16, 28), maximally stimulating doses of carbamylcholine caused uncoupling of the acinar cells. Addition of the alkanols did not modify this effect and did not alter the maximal secretion of the stimulated acini, further supporting the view that the secretory machinery of acinar cells (23) was not aspecifically perturbed by either heptanol or octanol.

Taken together, the results indicate that the changes in cell-to-cell communication induced by the alkanols were paralleled by specific secretory changes and suggest that these two events may be causally related. However, since some of the factors that are thought to control junctional communication (13, 20, 34) are also likely to play critical roles in the regulation of secretion (28–31, 37), an obvious alternative possibility is that the secretion and the coupling changes were independent events, regulated simultaneously by a common messenger sensitive to alkanols. This possibility was not supported by the observation that pH_i and $[Ca^{2+}]_i$ as well as the maximally stimulated release of amylase were unaffected by the alkanols. Furthermore, there is evidence that the n-alkanols can directly interact with target membrane proteins (32), including those of reconstituted permeable junctions (40), and it is established that the effect of heptanol and octanol on native junctional channels is not mediated by unspecific membrane changes (19, 32, 34). Thus, it is conceivable that these two alkanols acted directly on the junctions of the acinar cells to decrease their permeability and block intercellular communication. Whether this effect is alone responsible for the perturbed basal secretion of uncoupled acinar cells awaits the results of future studies using other uncouplers. Antibodies directed against the main gap junctional peptide(s) (14, 18, 36) and reacting with pancreatic acinar cells (8) appear among the most promising tools for such studies. However, the difficult technical problem of their introduction into relatively large numbers of small secretory cells will have to be solved before these highly specific probes could be used to test this issue. At this stage, our data are consistent with the view that blockage of junctional cell-to-cell communication within pancreatic acini may be responsible for the elevated basal enzyme secretion that was observed under such conditions, thereby providing evidence for a specific regulatory role of coupling in the secretory activity of adult acinar cells.

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