0031-3998/88/2404-0427$02.00/0
PEDIATRIC RESEARCH

Vol. 24, No. 4, 1988
Printed in U.S.A.

The Reserpine-Treated Rat as an Experimental Animal Model for Cystic Fibrosis: Abnormal Cl Transport in Pancreatic Acinar Cells

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ABSTRACT. **Pancreatic acini of control and reserpine-treated rats were incubated with the isotopic tracer ^{36}Cl to compare Cl accumulation in the absence and presence of secretagogues and transport inhibitors. Two phases of Cl accumulation were ascertained in resting control cells: an initial rate (0–5 min) and a steady state level (10–30 min) of accumulation. Both phases were enhanced by acetylcholine (1 μM) and caerulein (10 nM), but not by 10 nM vasointestinal peptide or 10 μM forskolin. Exposure to 1 mM DIDS (4,4′-diisothiocyano-2,2′-stilbene disulfonic acid) inhibited both phases of Cl accumulation, whereas exposure to 1 mM amiloride had a delayed effect on the initial rate and reduced the steady state phase in both resting (unstimulated) or acetylcholine-stimulated cells. Furosemide (1 mM) had no effect on Cl accumulation when added to the cells just before tracer, but reduced it when added 10 min before. Neither the initial phase nor the steady state level of Cl accumulation were enhanced by acetylcholine in acini of reserpine-treated rats and the effect of DIDS on the initial phase was smaller than in control cells. Continued exposure to this inhibitor resulted, furthermore, in a significantly larger steady state Cl content. The inhibitory effects of amiloride and of a 10-min preincubation with furosemide were similar to those observed in control cells. These results suggest that Cl accumulates in rat pancreatic acini by way of DIDS-sensitive mechanisms that are activated by Ca^{2+}-mediated, but not by cAMP-mediated, secretagogues. These mechanisms are altered in acini of reserpine-treated rats. Those responsible for the early (initial) phase are made insensitive to secretagogues but retain some sensitivity to DIDS. Mechanisms responsible for the maintenance of the steady state level are also altered as suggested by the paradoxical effect of DIDS. As this inhibitor blocks Cl conductances and Cl/HCO_3 exchange, either or both of these transport mechanisms may be altered after chronic reserpine. This alteration can explain the reduced in vivo pancreatic fluid secretion observed in the treated animals, as pancreatic fluid secretion is Cl-dependent in the rat. The reserpine-treated rat is an experimental model of cystic fibrosis and a similar defect in Cl transport may also underlie the reduced pancreatic fluid secretion observed in this disease. A generalized defect in Cl transport may be present in exocrine cells of CF patients and of the animal model, involving alterations in the interaction of membrane transport mechanisms with regulatory molecules activated by tissue-specific second messengers. (*Pediatr Res* 24: 427–432, 1988)**

Received October 14, 1987; accepted May 27, 1988.
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Abbreviations

CF, cystic fibrosis
DIDS, 4, 4′-diisothiocyano-2,2′-stilbene disulfonic acid
VIP, vasointestinal peptide

Rats treated for several days with pharmacologic doses of reserpine develop exocrine gland abnormalities resembling those of CF, including structural changes compatible with accumulation of secretory material and alterations in fluid, electrolyte, and protein of glycoprotein secretion (1–10). In the pancreas, there are reductions in the volume of pancreatic juice, in Cl and HCO_3 outputs and in the secretion of amylase, whereas the secretion of proteases is increased (4, 7, 8). A reduced pancreatic fluid secretory response is observed after stimulation with cholinergic agents and peptide hormones, which are thought to act primarily on pancreatic acinar cells in the rat (11–15), and which stimulate secretion via Cl transport mechanisms (12, 14, 15). In view of recent evidence that defective Cl transport is a basic cellular disturbance in two exocrine tissues of cystic fibrosis patients (16–18), we hypothesized that a defect in Cl transport may also be present in pancreatic acinar cells of the reserpine-treated rat. This view is supported by observations that transepithelial Cl transport is abnormal in isolated salivary acinar cells (19) and in isolated, perfused tracheas (20) of this experimental model. To test if Cl transport is likewise altered in pancreatic cells, we compared Cl uptake in acini of control and reserpine-treated rats. The isotopic tracer ^{36}Cl was used to measure Cl accumulation in the absence and in the presence of secretagogues and of transport inhibitors in the two types of acinar cells.

MATERIALS AND METHODS

Adult male rats of the Sprague Dawley strain were used. Some were given 7 daily intraperitoneal injections of reserpine in a dose of 0.5 mg/kg body weight, as previously described (2–10). The pancreas was removed under pentobarbital anesthesia (6–8 mg/100 g body weight, intraperitoneally) from both untreated controls and drug-treated animals and was rapidly cut into small fragments that were incubated in a dispersion solution of the following composition (in mmol/liter): NaCl, 118; $NaHCO_3$, 25; KCl, 4.7; NaH_2PO_4, 1; $MgCl_2$, 1.1; $CaCl_2$, 1.28; and glucose, 15; pH = 7.4. The medium also contained a basic amino acid mixture (GIBCO, Grand Island, NY), 2 U/mg wet tissue of collagenase (Sigma Chemical Co., St. Louis, MO), 10 U/mg wet tissue of hyaluronidase (Sigma), 0.1 mg/ml of trypsin inhibitor, and 0.2% albumin and was kept at 37°C and continuously bubbled with a 95% O_2–5% CO_2 gas mixture. The fragments were incubated in this solution for 15 min and then resuspended

in fresh dispersion medium containing the enzymes and incubated for an additional 60 min. The fragments were mechanically dispersed by passage through plastic pipettes at 40, 50, and 60 min, filtered through nylon mesh, resuspended in fresh medium and layered over 4% albumin. The resulting suspension was centrifuged, the supernatant was discarded, and the pellet was resuspended in fresh medium and divided into flasks for equilibration in the final incubation medium for 10–15 min.

To measure Cl accumulation, 1 μCi/ml of ^{36}Cl (as the NaCl salt, ICN Chemicals, Irvine, CA, sp. act. 18.1 mCi/g) was added to each flask in the absence or in the presence of test substances and samples of the suspension (200 μl) were subsequently removed in duplicate at timed intervals. The cells were separated from the medium by suction filtration through nucleopore filters (25 mm diameter, 3 μM pore size), and the filters were then washed with 10 ml of ice-cold, isotope-free medium. The radioactivity remaining was measured by liquid scintillation spectrometry in a Beckman 3801 counter. The protein content of appropriate aliquots (0.5 ml) of the various cell suspensions was measured by the Lowry method (21) after sonication of the pellets. Changes in the tracer content of the cells was evaluated in the absence of test substances and in the presence of acetylcholine, caerulein, VIP, and forskolin. The effects of the following transport inhibitors on Cl accumulation were also investigated: amiloride, DIDS, and fuorsemide. The inhibitors were added to the suspensions either at time zero (*i.e.* just before secretagogue and tracer) or 10 min before.

Values for counts in the various samples removed over the course of the experiments, for total counts added, and for the concentration of non-isotopic Cl in the incubation medium (μmol/ml) were used to calculate a "cold" Cl content of the cells at the different time points according to the formula

$$\frac{[Cl]_o}{\text{total cpm}} \times \frac{\text{cpm sample}}{\text{mg/ml protein}} \times 1000 = \text{nmol/mg protein}$$

Mean values for Cl content at the various times after exposure to tracer and to test substances were calculated from the data of similar experiments and compared by Student's t test. Control experiments to measure basal Cl accumulation were performed with each series of experiments involving drugs. The data from several of these control experiments were pooled in the case of similar drug protocols to generate the graphs shown in "Results" for the two types of acinar preparation. Data for experiments with secretagogues or inhibitors refer to the specific experiments in each case.

RESULTS

Accumulation of Cl in absence and presence of secretagogues. A time-dependent accumulation of ^{36}Cl was observed in pancreatic acini of control (untreated) rats not exposed to either secretagogues or transport inhibitors. The values of Cl content calculated as described above from the radiotracer data are shown in Figure 1. The tracer content (and the calculated Cl content) of the cells rose rapidly upon addition of tracer and was 79 ± 5 nmol/mg protein after 1 min of incubation and 102 ± 6 nmol/mg protein after 3 min (Fig. 1). After 5 min of incubation, the calculated Cl content was 114 ± 7 nmol/mg protein (Fig. 1). Between 10 and 30 min, the Cl content varied between 119 ± 8 and 107 ± 7 nmol/mg protein (Fig. 1). The mean of the values between 10 and 30 min of incubation was 111 ± 4 nmol/mg protein and was considered as a steady state content. The data just described show, therefore, two components of Cl (^{36}Cl) accumulation in unstimulated pancreatic acini of control rats: an initial rate of tracer accumulation (*i.e.* between 0 and 4–5 min) and a steady state level (*i.e.* between 10 and 30 min).

Figure 1 also shows the results of experiments in which control acini were exposed at time zero to either 1 μM acetylcholine or to 10 nM caerulein. Both secretagogues enhanced the initial rate

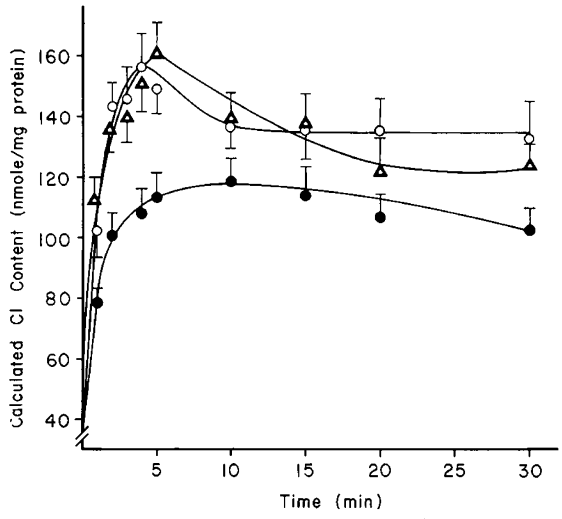

Fig. 1. Time-related changes in Cl content of pancreatic acini of control rats in the absence (*closed circles*) and in the presence of either 1 μM acetylcholine (*open circles*) or 10 nM caerulein (*open triangles*). Acini were exposed to ^{36}Cl and to secretagogue at time zero. Data shown are the means ± SE for each time point from 12 control experiments (six done in parallel with the acetylcholine experiments and six with the caerulein experiments) and of six each for the two secretagogues.

of Cl accumulation (see below) and induced a significantly greater steady state content. In the case of acetylcholine, the Cl content after 1 min of incubation was 102 ± 8 nmol/mg protein and, after 4 min, 157 ± 11 nmol/mg protein. Both values were significantly higher ($p < 0.01$) than the corresponding ones observed in the absence of secretagogue (Fig. 1). The steady state Cl content (*i.e.* between 10 and 30 min) in the presence of acetylcholine (135 ± 2 nmol/mg protein) was also significantly higher ($p = 0.001$) than under basal conditions.

Similar results were obtained with caerulein, although the effect on steady state accumulation was not as sustained as in the case of acetylcholine (Fig. 1). The Cl content in the presence of caerulein was 112 ± 8 nmol/mg protein after 1 min of incubation and 161 ± 10 nmol/mg protein after 5 min (Fig. 1). Both values were significantly higher ($p < 0.05$) than the corresponding basal values in the absence of stimulant. The calculated steady state Cl content in the presence of caerulein was 131 ± 4 nmol/mg protein, which was also significantly higher ($p = 0.01$) than that of unstimulated cells.

Figure 2 shows the results of similar experiments with acini of reserpine-treated rats. The same two components of Cl accumulation were observed in cells not exposed to secretagogues. The Cl content of unstimulated cells after 1 min of incubation was 65 ± 4 nmol/mg protein and, after 5 min, 96 ± 3 nmol/mg protein (Fig. 2). Although the rate of Cl accumulation during the first 5 min of incubation appeared similar to that observed in control acini (see Fig. 4 below), the Cl content at early time points (*i.e.* from 1 to 5 min) was significantly lower than in control acini (compare Figs. 2 and 1). The steady state Cl content in unstimulated acini of reserpine-treated rats was 98 ± 2 nmol/mg protein (Fig. 2). This was also significantly smaller ($p = 0.01$) than the steady state Cl content attained in control acini and shown in Figure 1.

In contrast to control acini, those of reserpine-treated animals did not show an increased Cl accumulation in the presence of secretagogues (Fig. 2). The Cl content in the presence of 1 μM acetylcholine was 78 ± 6 nmol/mg protein after 1 min of incubation and 99 ± 6 nmol/mg protein after 5 min (Fig. 2). The 1-min value was of borderline significance ($p = 0.05$) in relation to the corresponding basal but the 5 min value was not significantly different from the 5 min basal ($p = 0.2$). Similarly, steady state Cl content in the presence of acetylcholine was not

different ($p = 0.2$) from that observed in its absence (101 ± 3 *versus* 98 ± 2 nmol/mg protein, respectively). Exposure to 10 nM caerulein did not significantly enhance Cl accumulation in the acini of reserpine-treated animals (Fig. 2). Cl content values at either the initial period (0–5 min) or the steady state accumulation (105 ± 7 nmol/mg protein) were not significantly different than those observed in the absence of caerulein (Fig. 2).

Effects of DIDS and amiloride. Acini from control and reserpine-treated rats were exposed to either 1 mM DIDS or 1 mM amiloride at time zero (*i.e.* immediately before tracer) and tracer accumulation was monitored as indicated. The results are shown in Figure 3. In control cells, DIDS inhibited both the initial phase and the steady state accumulation of Cl. The calculated Cl content was significantly smaller after 1 min (42 ± 2 nmol/mg protein, $p = 0.001$) and 5 min (85 ± 8 nmol/mg protein, $p = 0.01$) of incubation than in the absence of the inhibitor. Steady state Cl content was also significantly smaller ($p = 0.001$) than in cells not exposed to DIDS (86 ± 2 nmol/mg protein, Fig. 3). The effect of amiloride on control acini was not clear cut in the first min of incubation, when the Cl content (76 ± 3 nmol/mg protein) was similar ($p = 0.2$) to that of cells not exposed to this inhibitor (Fig. 3). At 5 min, however, the Cl content in the presence of amiloride (87 ± 4 nmol/mg protein) was significantly lower ($p = 0.01$) than in unexposed cells and steady state Cl content was 85 ± 2 nmol/mg protein, a value significantly lower ($p = 0.001$() than in cells not exposed to this inhibitor (Fig. 3).

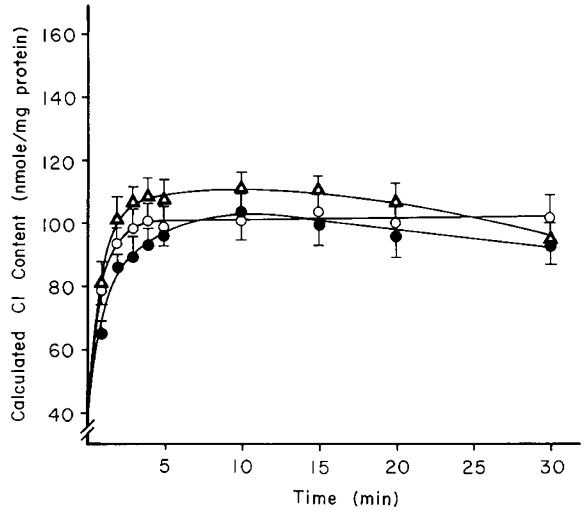

Fig. 2. Time-related changes in Cl content of pancreatic acini of reserpine-treated rats incubated in the absence (*closed circles*) and in the presence of either 1 μM acetylcholine (*open circles*) or 10 nM caerulein (*open triangles*). Protocol was similar to that of Figure 1. Data are the means ± SE from eight control experiments (no secretagogue) and four each for the two secretagogues indicated.

DIDS also significantly reduced the initial phase of Cl accumulation in acini of reserpine-treated rats (Fig. 3). Cl content in the presence of this inhibitor was 44 ± 3 nmol/mg protein after 2 min of incubation ($p = 0.001$ when compared to basal) (Fig. 3). After this initial reduction in Cl content, however, the Cl content increased significantly in the acini of reserpine-treated rats exposed to DIDS, so that Cl content was 137 ± 9 nmol/mg protein at 10 min and reached approximately 180 nmol/mg protein between 20 and 30 min of incubation. This was significantly higher ($p = 0.001$) than the steady state Cl in cells not exposed to DIDS (Fig. 3). As in control acini, the effect of amiloride was not clear cut in the first min of incubation, when the Cl content (68 ± 7 nmol/mg protein) was not significantly different ($p = 0.2$) from that observed in the absence of this inhibitor (65 ± 4 nmol/mg protein, Fig. 3). Cl content at subsequent time points was, however, significantly smaller in the presence of amiloride and steady state Cl content was 75 ± 1 nmol/mg protein, a value significantly smaller ($p = 0.001$) than in the absence of amiloride (Fig. 3).

The data shown in Figures 1–3 for the initial phase of Cl accumulation were further analyzed by calculating rates of Cl accumulation between 1 and 5 min of incubation from the mean values shown in the figures. The reciprocals (1/rate) of the rates at the various time points were then plotted against time and the results are shown in Figure 4. It can be seen that, in control acini, the rate of Cl accumulation is enhanced by acetylcholine (as suggested by the reduced 1/rate relationship), whereas DIDS reduces it (as indicated by the increased reciprocal of the rate). As indicated by the data in Figure 3, the effect of amiloride, however, is more clear in the latter part of the accumulation period (Fig. 4). In acini isolated from reserpine-treated rats, the

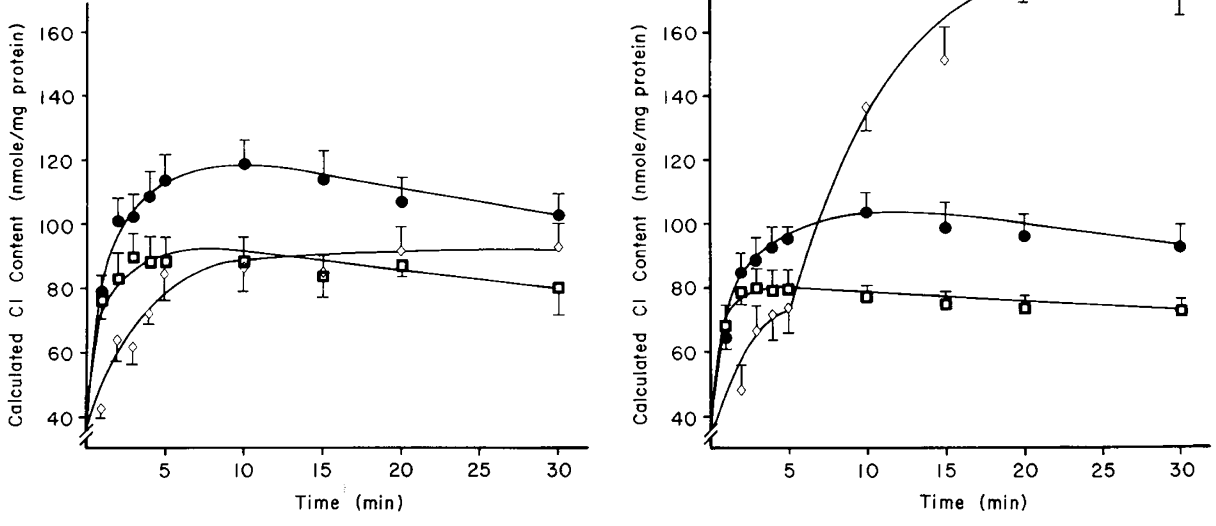

Fig. 3. *Left panel*, time-related changes in Cl content of pancreatic acini from control rats incubated in the absence (*closed circles*) and in the presence of either 1 mM DIDS (*open diamonds*) or 1 mM amiloride (*open squares*). Cells were exposed to ^{36}Cl and to test substances at time zero. Data are means ± SE from 10 control experiments and five each for the two inhibitors. *Right panel*, time-related changes in acini of reserpine-treated rats incubated in the absence (*closed circles*) and in the presence of either 1 mM DIDS (*open diamonds*) or 1 mM amiloride (*open squares*). Data are the mean ± SE of eight control experiments and four each for the two inhibitors.

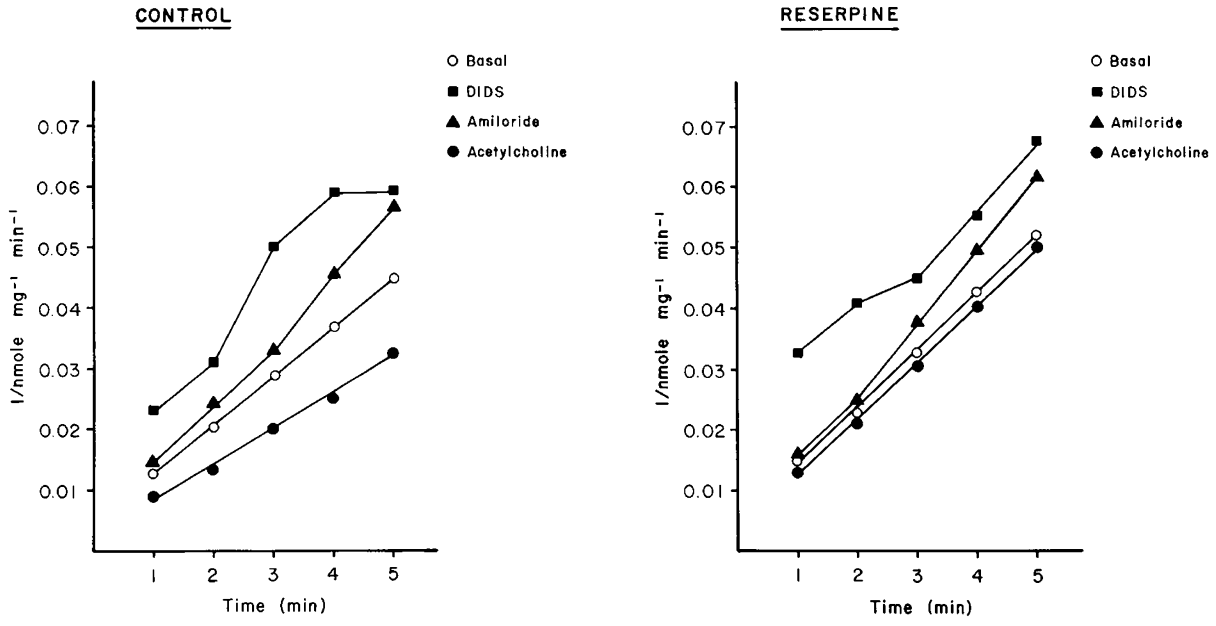

Fig. 4. *Left panel*, reciprocals of the rates of Cl accumulation (1/rate) in control acini in the absence (*open circles*) and presence of the test substances indicated. Data shown are derived from those shown in Figure 1 and the *left panel* of Figure 3. *Right panel*, reciprocals of the rates of Cl accumulation in acini of resperine-treated rats under the same conditions shown in the *left panel* for control acini. Data shown are derived from those shown in Figure 2 and the *right panel* of Figure 3.

basal rate of early Cl accumulation was similar to that of control cells (Fig. 4). In contrast to the latter, however, acetylcholine did not enhance the initial rate of Cl accumulation in cells of reserpine-treated animals (Fig. 4). However, DIDS inhibited the initial rate of Cl accumulation in these cells (Fig. 4), although the effect was somewhat different from that in control acini and became smaller with time (Fig. 4). In view of the effect of this inhibitor on steady state Cl in the cells of the treated animals (Fig. 3), the results shown in Figure 4 suggest complexities in these cells relative to either the Cl transport systems responsible for Cl accumulation or their interaction with DIDS.

Effects of furosemide. Furosemide is a loop diuretic that inhibits coupled cation/anion transport systems in epithelial cells; there is evidence that such a transport system is present in pancreatic cells (31). We therefore investigated the effect of 1 mM furosemide on Cl accumulation in the acinar preparations. In control acini, furosemide had essentially no effect when added to the cell suspensions at time zero, but reduced Cl accumulation significantly when added 10 min before addition of tracer (Fig. 5). Both the initial rate and the steady state accumulation were reduced in cells preincubated for this period of time with furosemide. The Cl content of the acini was 52 ± 8 nmol/mg protein after 1 min of incubation in the presence of the inhibitor and 74 ± 7 nmol/mg protein after 5 min (Fig. 5). The steady state Cl accumulation was 74 ± 6 nmol/mg protein when furosemide was present in the medium for 10 min before tracer (Fig. 5). All these values are significantly smaller from the corresponding ones in the absence of furosemide (Fig. 5).

Furosemide also inhibited Cl accumulation in unstimulated acini of reserpine-treated rats. The effect was also evident, as in control acini, in both phases of Cl accumulation. The Cl content at 1 and 5 min was, respectively, 28 and 46 nmol/mg protein and the steady state content was 44 ± 3 nmol/mg protein. These were significantly reduced in relation to the corresponding values in the absence of inhibitor.

Effect of transport inhibitors on acetylcholine-stimulated acini. As shown in Table 1, DIDS and amiloride inhibited Cl accumulation in control acini exposed to 1 μM acetylcholine. The inhibitory effect was evident in both the initial phase and the steady state phase of Cl accumulation (Table 1). Furosemide also inhibited Cl accumulation in these control cells when added 10

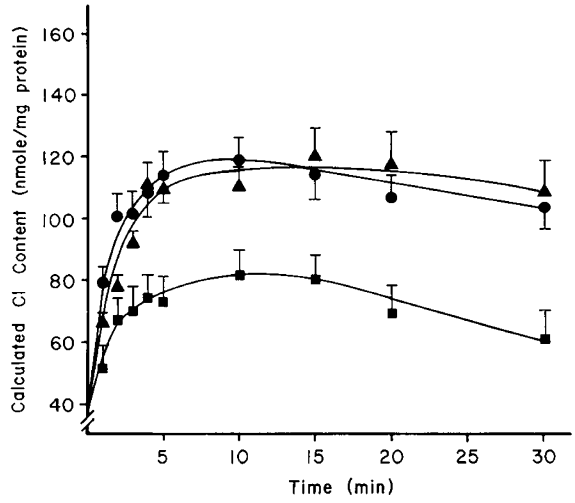

Fig. 5. Time-related changes in Cl content of control acini incubated in the absence (*closed circles*) and in the presence of 1 mM furosemide, added to the suspensions either at time zero (*triangles*) or 10 min before tracer (*squares*). Data from six control experiments and from four experiments each for the two furosemide protocols.

Table 1. *Effect of transport inhibitors on Cl accumulation in acetylcholine-stimulated acini*

Inhibitor	Type of cell	*n*	Cl accumulation (% of values with acetylcholine alone) 1 min	5 min	10–30 min
DIDS	Control	4	42	58	67
	Reserpine	4	54	78	157
Amiloride	Control	4	83	78	68
	Reserpine	4	86	78	73
Furosemide	Control	3	32	33	31
	Reserpine	3	40	49	43

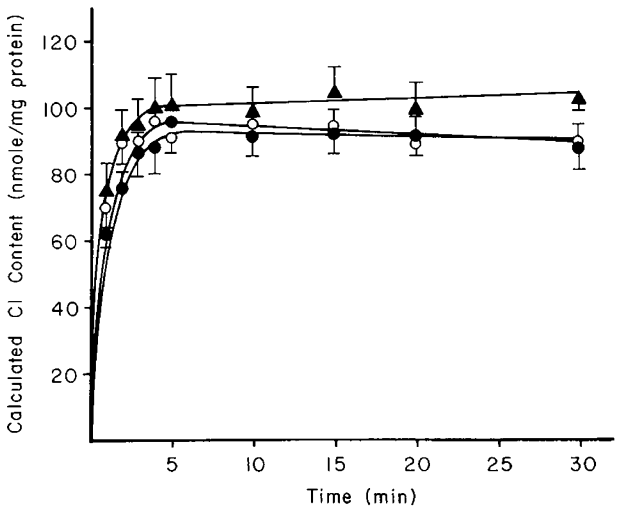

Fig. 6. Time-related changes in Cl content of control acini incubated in the absence (*closed circles*) and in the presence of either 10 mM vasointestinal peptide (*open circles*) or 10 μM forskolin (*closed triangles*). Data are from six control experiments and four each for the two test substances.

min before tracer (Table 1). As in unstimulated cells (see Fig. 3), the effect of DIDS on Cl accumulation by acetylcholine-stimulated acini of reserpine-treated rats was biphasic (Table 1). An initial inhibition of Cl accumulation was followed by a significant increase in Cl content, which was 157% of the corresponding value in cells not exposed to DIDS between 10 and 30 min of incubation (Table 1). The effect on the initial phase of accumulation was less than in control acini (Table 1). The inhibitory effect of furosemide on Cl accumulation was also significant in acini of reserpine-treated rats (Table 1), when the cells were exposed to this inhibitor for 10 min before secretagogue and tracer.

Effects of VIP and forskolin. We evaluated the effects of two other secretagogues on Cl accumulation in control acini. VIP acts in pancreatic epithelium through cAMP, whereas acetylcholine and caerulein use Ca^{2+} as their intracellular mediator (11, 14). Forskolin is a disterpene derivative that activates adenylate cyclase and cAMP formation (22).

As shown in Figure 6, neither VIP nor forskolin modified Cl accumulation in control acini. The initial and steady state phases of accumulation were essentially similar to those of cells not exposed to these substances.

DISCUSSION

The ion transport systems operating in pancreatic acinar cells have not been fully elucidated (14), but studies with isolated, perfused tissues (13) and with isolated cells (23) suggest that, in the rat, they include a pair of functionally linked antiports in the basolateral cell membrane that exchange Na for H and Cl for HCO_3. The former is sensitive to amiloride and the latter to stilbene sulfonic acid derivatives such as DIDS (24, 25). The results of our experiments with control acini can be interpreted as lending support to this model, as these inhibitors reduced Cl accumulation in both resting and acetylcholine-stimulated cells. Stilbene derivatives also inhibit, however, Cl conductances in several epithelial and non-epithelial cells (26–29) and acetylcholine activates a Cl conductance pathway of fairly high permeability in pancreatic acinar cells (30). In general, the anion exchanger is sensitive to lower doses of these inhibitors (*i.e.* in the μM range), whereas inhibition of Cl conductance pathways and other ion transport systems require higher stilbene concentrations (29). Although the inhibitory effect of DIDS on Cl accumulation in control pancreatic acinar cells could result from blockade of the proposed Cl/HCO_3 antiport, it could represent equally well effects on a Cl conductance pathway of the high concentrations of DIDS used. Amiloride also blocks cholinergic receptors in the pancreas (31) and its inhibition of Cl accumulation in control acini could be due to this effect, rather than to blockade of the proposed Na/H exchange.

Our results suggest that there are two distinct phases of Cl accumulation in rat pancreatic acini. The initial rate is stimulated by acetylcholine and by caerulein and is inhibited by DIDS. A steady state level of accumulation is also increased by secretagogues and is reduced to approximately the same extent by DIDS and by amiloride. The effect of amiloride on the initial rate of Cl accumulation is delayed but can be ascertained after 2–3 min of incubation (see Fig. 3). The steady state level attained is likely to be related to the initial rate of accumulation and the effects of the various drugs on such level could result, therefore, from their effects on this initial rate and the resulting changes in cell Cl concentrations, cell volume, or both.

Cl accumulation is altered in pancreatic acini of reserpine-treated rats, as indicated by the failure of acetylcholine to increase Cl accumulation and by the large and sustained accumulation of Cl in the continued presence of DIDS. In contrast to control acini, no significant increase in the initial rate of Cl accumulation was seen with acetylcholine. This suggests that reserpine administration blocks the activation by the secretagogue of the transport mechanism responsible for early Cl accumulation. However, both basal and acetylcholine-stimulated early accumulation of Cl were inhibited by DIDS in the cells of the treated animals, although to a lesser extent than in control acini. Thus, the transport mechanisms in question retain a good portion of their DIDS sensitivity but lose their acetylcholine sensitivity after reserpine administration. This suggests complex changes in the transport mechanism or mechanisms responsible for early Cl accumulation after chronic reserpine. As DIDS can inhibit both Cl conductances and Cl/HCO_3 exchange (see above), either or both of these mechanisms may be affected in reserpine-treated rats. Analysis of dose-related responses to DIDS will help clarify the issue further.

The sustained increase in Cl accumulation in the continued presence of DIDS observed in the acini of reserpine-treated animals suggests an alteration in Cl transport mechanisms responsible for the maintenance of steady state Cl content. This could result from disturbances in Cl exit (efflux) or from a reversal in the direction of transport by a DIDS-sensitive Cl/HCO_3 exchange. The mechanisms of Cl efflux in pancreatic acini are not clear, but there is evidence that they may involve a furosemide-sensitive Na, K, 2Cl cotransport system oriented in the opposite direction to that of salivary acini and other secretory epithelial (32). The effects of preincubation with furosemide were similar in pancreatic acini of control and reserpine-treated rats. This argues against a disturbance in Cl exit in the latter cells, at least by way of the cotransport mechanism. A reversal in the direction of Cl/HCO_3 exchange (moving Cl out of the cell) could occur, however, if there were changes after reserpine administration in intracellular HCO_3, Cl, or H (pH) concentrations or in metabolic functions critical for anion transport. Clearly, additional studies are necessary to explore these questions.

Cl accumulation was enhanced in control acini by secretagogues that use Ca^{2+} as their intracellular mediator, but not by those that act through cAMP (14). As previous evidence indicated that Cl accumulation in pancreatic acini is dependent on the presence of Ca^{2+} in the incubation medium (33), it is possible that the alterations in Cl accumulation in acini of reserpine-treated rats is the result of a disturbance in the mediator role of Ca^{2+}. This requires further study, but the alteration in Cl accumulation observed in this study can explain the reduced fluid secretion observed *in vivo* in reserpine-treated rats (4, 7, 8), as Cl is required for secretagogue-induced fluid secretion in the rat (12). Transcellular Cl transport is thought to be important also in human pancreatic acini (34) and alterations in pancreatic fluid

and anion secretion are prominent in CF (35). It is possible, therefore that these alterations are related to abnormal Cl transport in pancreatic acini of affected individuals, perhaps similar to those observed in this study on an experimental animal model of this disease.

The Cl transport defect in sweat glands (36) and airways (17, 18) of CF patients seems to be related, however, to a disturbance in a cAMP-mediated pathway regulating Cl transport. As Cl transport appears to be regulated by Ca^{2+}, rather than by cAMP, in rat salivary (37) and pancreatic acinar cells (33), a question arises about the relevance of the findings of this and the previous study on salivary cells of the reserpinized rat model. Ion channels and conductances are variously regulated by Ca^{2+} or by cAMP in exocrine and nonexocrine tissues (38) and one can speculate that the disturbance of Cl transport in exocrine glands of CF patients and of the reserpine rat model may be due to a regulatory malfunction by either of these intracellular messengers in accordance with the tissue involved. This may involve the interaction between membrane transport proteins and regulatory molecules activated by either Ca^{2+} or cAMP. Studies on the regulation of Cl transport in human salivary and pancreatic cells are clearly necessary to establish the validity of this speculation.

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