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Ionic currents across pancreatic acinar cell membranes and their role in fluid secretion

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Fluid and enzyme secretion from a number of mammalian exocrine glands is controlled by the action of neurotransmitters and hormones on acinar cell membranes. Sustained stimulation evoking sustained fluid and enzyme secretion also evokes sustained membrane depolarization and increase in conductance. Mouse and rat pancreatic fluid and enzyme secretion, as well as membrane depolarization and conductance increase evoked by sustained stimulation with acetylcholine or cholecystokinin–gastrin peptides, are acutely dependent on extracellular calcium. However, the initial stimulant-evoked conductance increase and secretion appear to be triggered by calcium released from inside the cells. Direct measurement of membrane current during sustained stimulation in voltage-clamp experiments with resolution of the total current into its Na, Cl and K components has allowed calculations of stimulant-evoked Na and Cl uptake into the acinar cells. The NaCl uptake is quantitatively sufficient to account for the stimulant-evoked fluid secretion. The role of the stimulant-evoked transmembrane ionic current appears to be the supply of salt for the fluid secretion. Calcium derived from intracellular sources in the initial phase of secretion, and from the extracellular fluid in the sustained phase, couples fluid and enzyme secretion to hormone–receptor interaction.

1. Introduction

At the Discussion Meeting on 'Active transport of salts and water in living tissues' held at the Royal Society about 10 years ago (Keynes (ed.) 1971) the mechanisms underlying acetylcholine-evoked salivary acinar fluid and electrolyte transport were reviewed. It was shown that acetylcholine (ACh) increased the permeability of the acinar plasma membrane to Na and K resulting in Na influx and K efflux. The Na influx appeared to be an important step in the transepithelial salt and fluid transport. The ouabain-sensitive Na–K pump was shown not to be directly coupled to fluid formation. An ACh-evoked increase in intracellular ionized calcium concentration was mentioned as a possible initiator of fluid secretion (Petersen 1971*b*). The recently acquired detailed picture of the membrane electrical changes evoked by stimulation of salivary acinar cells (Petersen 1980, 1981) and the associated ion fluxes (Putney 1978; Putney *et al.*, this symposium) is in agreement with this hypothesis.

Ten years ago virtually nothing was known about the cellular control of pancreatic acinar secretions. Since then much information, particularly about the role of intracellular calcium

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and cyclic AMP, has become available (Petersen & Iwatsuki 1978; Gardner 1979; Schulz & Stolze 1980; Gardner & Jensen, this symposium; Schulz *et al.*, this symposium; Christophe *et al.*, this symposium). The application of the voltage clamp technique (Hodgkin *et al.* 1952) to pancreatic acini (McCandless *et al.* 1981) has recently permitted calculation of secretagogue-evoked membrane currents (Maruyama & Petersen 1981). This, together with information

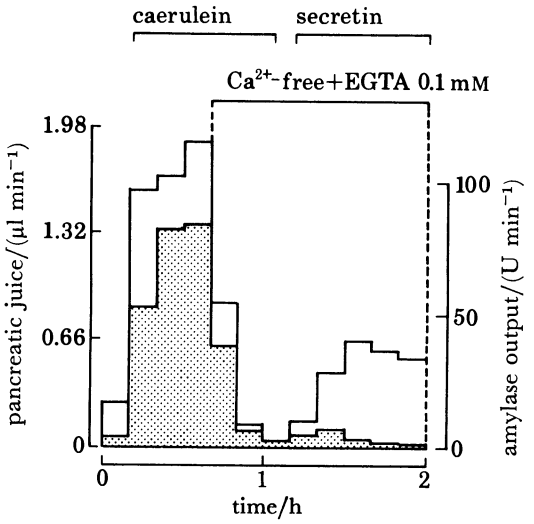

FIGURE 1. Fluid and amylase secretion from isolated perfused rat pancreas. Open columns represent rate of fluid secretion while stippled areas represent amylase output. Caerulein (0.1 nM) and secretin (5.7 nM) were present as indicated. The normal control solution contained 2.6 mM Ca. (From Petersen & Ueda (1976*a*).)

obtained from ion replacement experiments carried out in the isolated perfused rat pancreas (Petersen & Ueda 1977; Ueda & Petersen 1977), enables us now to discuss the control of pancreatic acinar fluid secretion in some detail.

2. TWO TYPES OF FLUID SECRETION

Thaysen (1960), reviewing the field up to 1959, suggested that two kinds of isotonic secretions, both with plasma-like Na and K concentrations, are formed in the pancreas. One product, containing Cl^- as the chief anion and with a high enzyme concentration, comes from the acini. This process is stimulated by the vagal nerve (ACh) but not by secretin. A second product, containing HCO_3^- as the chief anion, but with little enzyme content, emanates from the cuboidal epithelium of the ducts and this process is stimulated by the peptide hormone secretin. Dockray (1972) demonstrated that cholecystokinin–pancreozymin (CCK–Pz) and its analogue caerulein evoked marked fluid and enzyme secretion from the rat pancreas. Sewell & Young (1975) showed that while secretin-evoked pancreatic juice has a high bicarbonate concentration, CCK–Pz stimulates the formation of a juice with a low (plasma-like) bicarbonate concentration.

In studies on the isolated vascularly perfused rat pancreas (Petersen & Ueda 1976*a*, 1977; Kanno & Yamamoto 1977; Ueda & Petersen 1977) it was shown that ACh, CCK–Pz, caerulein and secretin each evoked a marked increase in the flow of pancreatic juice when the perfusion fluid contained CO_2–HCO_3^- and calcium (Ca). In the absence of perfusion fluid CO_2–HCO_3^- the secretin-evoked fluid secretion was abolished while the effects of ACh and

caerulein were only slightly reduced. When Ca was removed from the perfusion fluid during sustained caerulein stimulation the flow of pancreatic juice was rapidly reduced to the basal level. Secretin could, however, still evoke a marked fluid secretion in the absence of perfusion fluid Ca (figure 1). The two fluid secretions in the pancreas can therefore be separated by their differing ionic requirements into a Ca-dependent CCK–Pz- or ACh-stimulated neutral fluid and enzyme secretion and a bicarbonate-dependent secretin-stimulated alkaline fluid secretion.

The hypothesis (Thaysen 1960) that the neutral fluid and enzyme secretion is derived from acinar cells while the bicarbonate-rich fluid originates from the duct cells was mainly based on the early experiments of Grossman & Ivy (1946) and Kalser & Grossman (1954) and has received much support from recent studies on electrophysiology, distribution of enzymes, receptor sites, micropuncture and experimental manipulation of gland structure (see reviews in Ribet *et al.* 1980).

In addition to the effects of the classical pancreatic secretagogues, ACh, CCK–Pz and secretin, other stimulants have been studied. The effect of gastrins (Gregory 1974) is exactly the same as that of CCK–Pz and caerulein (Dockray 1973*a*). Following reports showing that dibutyryl cyclic GMP (dbcGMP) is a specific and competitive antagonist of the action of CCK–Pz, gastrin and related peptides on the pancreatic acinar cells (Peikin *et al.* 1979; Philpott & Petersen 1979; Jensen *et al.* 1980; Robberecht *et al.* 1980) it has been demonstrated that fluid and enzyme secretion evoked by pentagastrin or caerulein in the perfused rat pancreas is specifically blocked by dbcGMP (Ueda *et al.* 1980). A very different peptide, bombesin, also evokes pancreatic enzyme and fluid secretion both in the intact rat (Christophe *et al.* 1977) and in an isolated perfused preparation (Ueda *et al.* 1980). The bombesin-evoked fluid secretion would appear to be of the same type as that evoked by ACh and CCK–Pz since it is also relatively independent of the presence of CO_2–HCO_3^- in the perfusion fluid (Ueda *et al.* 1980). The neuropeptide vasoactive intestinal polypeptide (VIP) also elicits fluid secretion from the pancreas (Dockray 1973*b*; Scratcherd *et al.* 1975; Lindkaer Jensen *et al.* 1978). The VIP-evoked secretion is bicarbonate-rich like that evoked by secretin. Recently, Fahrenkrug *et al.* (1979) have presented evidence that VIP is the transmitter in the vagally induced atropine-resistant water and HCO_3 secretion from the porcine pancreas originally described by Hickson (1970). A newly identified and isolated heptacosapeptide PHI (porcine, histidine, isoleucine) (Tatemoto & Mutt 1980) has actions similar to those of VIP on pancreatic secretion (Dimaline & Dockray 1980). Insulin, while not by itself able to evoke pancreatic secretion, potentiates CCK–Pz-evoked fluid and enzyme secretion (Saito *et al.* 1980). Potentiating interactions between secretin and CCK–Pz, both with regard to fluid and enzyme secretion, have also been described (Grossman 1976; Petersen & Ueda 1977; Gardner & Jackson 1977).

3. Ionic requirements for acinar fluid secretion

The neutral fluid secretion evoked by ACh, CCK–Pz, gastrin-like peptides and bombesin requires the presence of Ca, but not CO_2–HCO_3^-, in the perfusion fluid (Petersen & Ueda 1977; Ueda & Petersen 1977). The salivary acinar fluid secretion evoked by ACh is similarly Ca-dependent (Douglas & Poisner 1963; Petersen *et al.* 1967) and bicarbonate-independent (Petersen 1971*a*; Young *et al.* 1980). Figure 2 shows the effect of removing perfusion fluid Na or Cl on fluid and enzyme secretion evoked by sustained caerulein stimulation in the rat pancreas. In both cases fluid secretion is rapidly abolished after ion replacement. Sustained

stimulation with ACh, CCK–Pz or caerulein causes sustained secretion in the perfused rat pancreas, but over a longer period of perfusion (1–2 h) there is a small steady decrease in the secretory rates of both fluid and amylase. In spite of this, readmission of Na to the Na-free perfusion fluid during caerulein stimulation causes a transient increase in both fluid and amylase output while this pattern was not observed after Cl readmission. Ouabain (0.1–1.0 mM) can markedly inhibit caerulein-evoked fluid secretion with much less effect on amylase output (Petersen & Ueda 1977). These pieces of information taken together indicate a particularly important role of Na transport in pancreatic acinar fluid secretion.

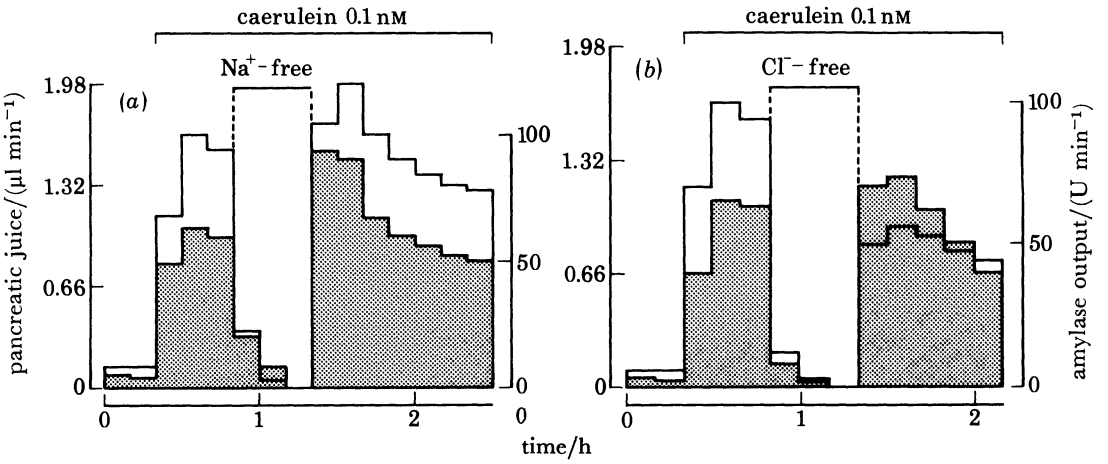

FIGURE 2. The effect of (*a*) Na or (*b*) Cl removal from the perfusion fluid on amylase and fluid secretion from isolated perfused rat pancreas. Na was replaced by Tris and Cl by sulphate. (Part (*a*) from Petersen & Ueda (1977).)

In the perfused cat submandibular gland it has been shown that 50% replacement of perfusion fluid Na by for example Li causes a dramatic decrease in the ACh-evoked flow of saliva, whereas a 50% replacement of Cl by for example sulphate has virtually no effect on stimulated fluid secretion (Petersen & Poulsen 1969). Ouabain (0.1 mM) inhibits ACh-evoked salivary secretion (Petersen & Poulsen 1967), but it is possible to use a dose of ouabain (10 µM) that abolishes active Na–K transport across the basal acinar cell membrane without affecting markedly the rate of ACh-evoked secretion during a 1 min stimulation period (Petersen 1971*a*). From micropuncture studies of electrolyte concentration in rat salivary primary fluids (samples obtained from intercalated ducts) it appears that there is a good linear correlation between the Cl concentration in the primary secretion and a plasma ultrafiltrate (Young & Martin 1971; Young & van Lennep 1979; Young *et al.* 1980). All these results taken together demonstrate the particularly important role of Na transport also in the process of salivary acinar fluid secretion.

4. Electrochemical gradients across the acinar plasma membrane

While there is much information on membrane potential measurements in exocrine acini from many glands and species (Petersen 1980, 1981) there are comparatively few data on intracellular ionic activities in mammalian acini. Table 1 summarizes results from recent studies on the mouse pancreas (Poulsen & Oakley 1979; Graf, Maruyama & Petersen, unpublished

1981). It is clear that neither Na nor K is in equilibrium across the plasma membrane. While Cl is close to being in equilibrium there is apparently a difference between the Nernst potential for Cl and the resting membrane potential. The difference would be physiologically significant if it could be assumed that the Cl-sensitive microelectrode was behaving ideally. Our recent studies were carried out with two separate microelectrodes inserted into the same functional acinar unit, one electrode monitoring the membrane potential, the other being an ion-sensitive (liquid ion exchanger) microelectrode. When the Cl-sensitive electrode penetrated the membrane after an ordinary microelectrode had been previously inserted there was always a small change in the potential measured with the Cl electrode, indicating that Cl was not in equilibrium. It cannot be vigorously excluded, however, that the difference in potential measured with the

TABLE 1. INTRACELLULAR ION ACTIVITIES IN ACINI FROM ISOLATED SUPERFUSED MOUSE PANCREATIC FRAGMENTS

(The K values of Poulsen & Oakley (1979) were obtained by using double-barrelled microelectrodes, one barrel filled with liquid ion exchanger, the other serving as a potential monitor, while in our own measurements on K, Na and Cl we used two separate micropipettes inserted into two electrically coupled neighbouring acinar cells, one electrode filled with 3 M KCl or 3 M K acetate, the other with the appropriate liquid ion exchanger. The extracellular ion activities in these experiments (assuming activity coefficients of 0.75) were: $a_{K,o} = 3.5$ mM, $a_{Cl,o} = 86.3$ mM, and $a_{Na,o} = 108.8$. Mean values ± s.e. (number of observations) are listed. The Nernst potentials (E_K, E_{Cl} and E_{Na}) are also shown.)

$a_{K,i}$	93.0 ± 3.0 mM ($n = 12$)	(Poulsen & Oakley 1979)
E_K	−88 mV	
$a_{K,i}$	102.0 ± 2.7 mM ($n = 15$)	
E_K	−90 mV	
$a_{Cl,i}$	34.3 ± 1.3 mM ($n = 36$)	
E_{Cl}	−25 mV	
$a_{Na,i}$	6.6 ± 0.4 mM ($n = 44$)	
E_{Na}	+75 mV	

Cl electrode and the ordinary microelectrode is also influenced by factors other than the intracellular Cl activity ($a_{Cl,i}$). The pancreatic acinar plasma membrane appears to be very permeable to Cl. Thus there are marked transient potential changes after removal and readmission of extracellular Cl (Nishiyama & Petersen 1975*a*; Iwatsuki & Petersen 1977*a*, *b*; Petersen & Philpott 1980) and the specific membrane resistance is doubled after extracellular Cl removal (McCandless *et al.* 1981). Since the steady state membrane potential measured in Cl-free solution is relatively close to that obtained during control conditions (Nishiyama & Petersen 1975*a*) it can still not be excluded that, despite the small apparent difference between the Cl Nernst potential and the membrane potential (table 1), Cl is in equilibrium.

In the salivary glands a major reduction in acinar intracellular K activity occurs following stimulation with secretagogues (Poulsen & Oakley 1979). This is not surprising in view of the classical findings of Burgen (1956) showing that nerve stimulation causes marked release of K from salivary glands into both saliva and blood. Later studies have shown that ACh and other secretagogues increase membrane Na and K permeability (Petersen 1970*a*, *b*, 1971*a*, *b*). In the pancreatic acinar cells there is no measurable ACh-evoked change in $a_{K,i}$ (Poulsen & Oakley 1979). Our recent unpublished data indicate the absence of any marked rapid stimulant-evoked change in $a_{Cl,i}$ and $a_{Na,i}$.

5. Secretagogue-evoked ionic currents across the acinar membrane

Previous studies on stimulant-evoked membrane potential and resistance changes in exocrine acinar cells have been extensively reviewed elsewhere (Petersen 1980, 1981). Here only some recent direct measurements of membrane current in voltage clamped pancreatic acini will be mentioned. Figure 3 shows ACh-evoked membrane potential and inward current responses in the same acinar unit. Similar responses are seen after stimulation with peptides belonging to the cholecystokinin–gastrin and bombesin groups. Most of the stimulant-evoked inward current is carried by Na since the inward current response is essentially abolished after removal of external Na (McCandless *et al.* 1981). The reversal potential for the secretagogue-evoked current response is about −15 mV during exposure to control conditions, whereas during exposure to Cl-free solutions (steady state) this value is changed to about +10 mV (McCandless

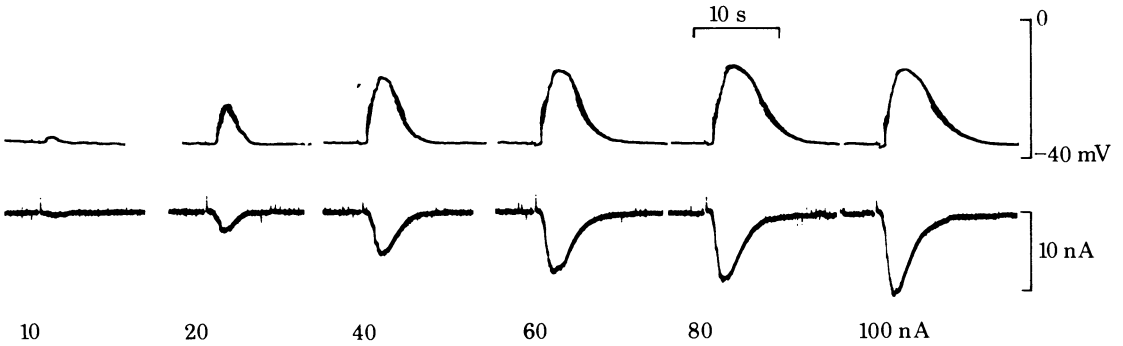

Figure 3. Mouse pancreatic fragment superfused *in vitro*. Dose–response relations for ACh-evoked depolarization and inward current in the same acinar unit. Upper trace shows the membrane potential and the effects of extracellular ionophoretic ACh applications (ejecting currents from 10 to 100 nA, as indicated, for 0.5 s; retaining current 20 nA) on the potential. Below are shown the effects of exactly the same doses of ACh on membrane current under voltage-clamp still in the same unit. A two-microelectrode voltage-clamp circuit with the electrodes in neighbouring electrically coupled cells was used (McCandless *et al.* 1981). The holding potential was the same as the spontaneous resting potential, i.e. −37 mV. (A. Nishiyama & O. H. Petersen, unpublished experiment.)

et al. 1981). Figure 4 summarizes the available data on reversal potentials, including information about the action of neutral amino acids, and compares these values with the resting membrane potential and the Na, K and Cl Nernst potentials. For the initial action of secretagogues (responses to short-lasting pulses of stimulants), it has been calculated that at the reversal potential for the action of the secretagogues (−15 mV) the stoichiometry of transport through the secretagogue-controlled channels is 3 Na^+ taken up accompanied by 2 Cl^- while 1 K^+ exits (Iwatsuki & Petersen 1977*b*).

In order to relate secretagogue-evoked membrane currents to secretion it is useful to work with sustained stimulation. Figure 5 shows the result of a voltage-clamp experiment in which the effect of exposing the acinar unit to a low concentration of the CCK–Pz analogue caerulein is investigated. It is seen that caerulein evokes an inward current and that the inward current response is sustained for the entire period of stimulation. Cessation of stimulation with simultaneous addition of the specific CCK–Pz antagonist dbcGMP (Peikin *et al.* 1979; Philpott & Petersen 1979) caused an immediate return to the resting situation. A series of short-lasting (100 ms) voltage jumps of varying magnitude were applied before and during the period of

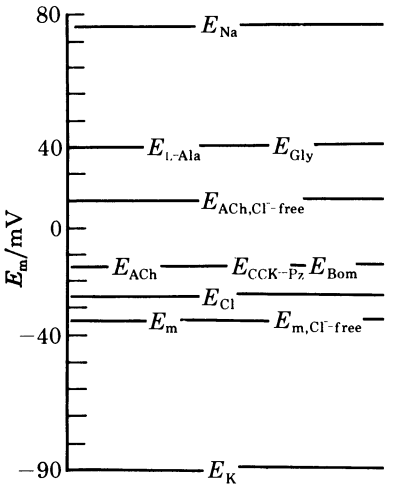

FIGURE 4. Values for membrane potential (E_m), reversal potentials for the action of ACh, CCK–Pz peptides, bombesin peptides, L-alanine and glycine (E_{ACh}, $E_{CCK\text{-}Pz}$, E_{Bom}, $E_{L\text{-}Ala}$, E_{Gly}) (short pulses applied by microiontophoresis) and Nernst potential for Na, K and Cl (E_{Na}, E_K, E_{Cl}) based on measurements with ion-sensitive microelectrodes (see also table 1).

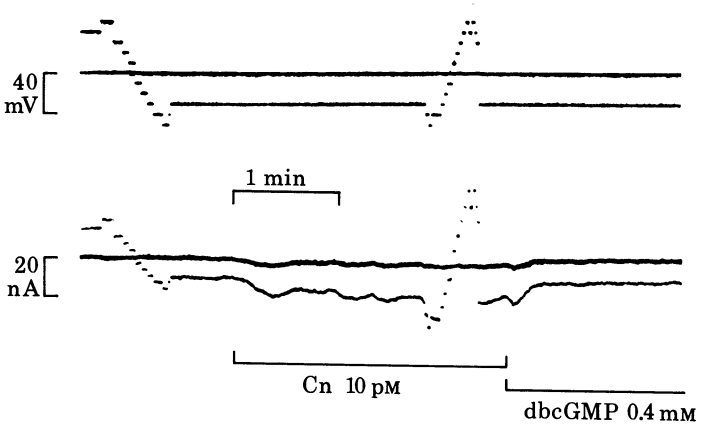

FIGURE 5. Effect of caerulein (Cn) (10 pM) on membrane current in voltage-clamped mouse pancreatic acinus. The holding potential was −34 mV. Hyperpolarizing voltage steps of 30 mV (100 ms duration) were repetitively applied. Complete voltage–current relations were obtained before and during Cn stimulation. Dibutyryl cyclic guanosine 3′,5′-monophosphate (dbcGMP) (0.4 mM), a specific antagonist of CCK–Pz, Cn and gastrin actions (Philpott & Petersen 1979), was present after the period of caerulein stimulation. Downward deflexion in current trace represents inward current and in voltage trace increased internal negativity. (From Maruyama & Petersen (1981).)

stimulation. For the same size voltage jump the associated current change was much larger during stimulation than during rest, indicative of the secretagogue-evoked increase in membrane conductance. Subtraction of the resting currents from the currents obtained in the presence of caerulein enabled us to plot the caerulein-evoked membrane current as a function of the membrane potential. A linear relation was obtained showing reversal of the caerulein-evoked current at about −20 mV. While the magnitude of the caerulein-evoked current was dependent on the secretagogue concentration, the reversal potential was constant at about −20 mV. Similar results were obtained with ACh and bombesin (Maruyama & Petersen, in preparation).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A detailed ionic analysis of the currents flowing during sustained stimulation has not yet been carried out, but since the reversal potential for the secretagogue-evoked currents is of nearly the same magnitude during sustained stimulation as in the initial phase of stimulation (short pulse experiments) it is reasonable for the time being to assume that the ionic events are essentially the same. Assuming that the current is carried only by Na, Cl and K (Iwatsuki & Petersen 1977*b*) we can, from our knowledge of the values of the reversal potential for the secretagogue-evoked current during control and Cl-free conditions (McCandless *et al.* 1981) and the recently obtained values for intracellular ion activities (table 1), in principle decompose the total secretagogue-evoked current into its Na, K and Cl components by using the Goldman–Hodgkin–Katz equations (Hodgkin & Katz 1949). The previously calculated relative permeability of the secretagogue-controlled pathways (Iwatsuki & Petersen 1977*b*) must now be corrected since the intracellular ion activities, particularly for Na, recently measured directly (table 1), differ substantially from the intracellular activities estimated by Williams (1975) and used in previous calculations. By using the values for the reversal potentials measured by Iwatsuki & Petersen (1977*b*) (now verified in the recent voltage-clamp experiments of McCandless *et al.* (1981)) and the ion activities from table 1, the relative permeabilities of the secretagogue-controlled pathways for Na, K and Cl, calculated in the same way as in the paper of Iwatsuki & Petersen (1977*b*), are $P_{Cl}:P_{Na}:P_K = 5:1.2:1$. The following example shows the calculation of ACh-evoked Na uptake across the acinar membrane at one particular concentration of the stimulant.

1 μM ACh depolarized the acinar cell membrane from −35 to −28 mV. When the potential was clamped at −28 mV, ACh (1 μM) evoked an inward current of 3.5 nA.

From $P_{Na}:P_{Cl}:P_K = 1.2:5:1$ and constant field equation we obtain

$$I_{Na} = 4\,\text{nA}.$$

Assuming that specific membrane capacitance is 1 μF cm^{-2} (see Petersen 1980) we calculate from the average input capacitance in mouse pancreatic acinar units of 2.5 nF that one acinar unit contains 2.5×10^5 μm^2 plasma membrane. We obtain

$$I_{Na} = 1.5\,\mu\text{A cm}^{-2}.$$

According to the morphometric data of Bolender (1974) obtained from guinea-pig pancreas, 1 g pancreas contains 3770 cm^2 acinar plasma membrane. Therefore

$$I_{Na} = 6\,\text{mA g}^{-1}.$$

We divide the current by the Faraday constant and obtain the Na flux through the ACh-controlled pathway

$$J_{Na} = 4\,\mu\text{mol min}^{-1}\,\text{g}^{-1}.$$

In this example the 1 μM ACh concentration was chosen because this is the concentration that causes maximal amylase secretion in isolated fragments of mouse pancreas. In the absence of direct information on fluid secretion in isolated mouse pancreatic fragments (impossible to measure) we assume that at 1 μM ACh, fluid secretion is also maximal. In the mouse pancreas *in vivo* (Mangos *et al.* 1973) maximal juice flow induced by cholinergic stimulation (pilocarpine administered intraperitoneally) is about 10 μl g^{-1}. The micropuncture data of Mangos *et al.* (1973) show that [Na] in the acinar secretion is 145 mM. Therefore maximal Na secretion evoked by cholinergic stimulation is about 1.5 μmol min^{-1} g^{-1}. In the rat pancreas *in vivo* similar

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

maximal juice flow rates are obtained in response to stimulation with CCK–Pz or caerulein (5 µl min^{-1} g^{-1}) (Sewell & Young 1975). In our voltage-clamp experiments, inward current responses quantitatively similar to those obtained with 1 µM ACh were obtained by using 100 pM caerulein stimulation.

From these calculations based on the actually measured secretagogue-evoked acinar membrane currents, one may conclude that the Na influx through the secretagogue-controlled pathways is sufficient to account quantitatively for the stimulated acinar Na secretion. It is considerably more difficult precisely to quantify the Cl fluxes through the secretagogue-controlled pathways. In contrast to Na, the equilibrium potential for Cl (E_{Cl}) is close to the reversal potential for the secretagogue-evoked currents (E_{ACh}, E_{CCK-Pz}, E_{Bom}) and very close to the membrane potential actually measured in the presence of the normally used secretagogue concentrations. A slight deviation of the intracellular Cl activity from that estimated by using the Cl-sensitive electrodes would have marked effects on the calculated Cl fluxes. It therefore seems unwise to attempt a precise quantification of the Cl current through the secretagogue-controlled pathways. As seen in the example calculation above, the inward current through the secretagogue-controlled pathway is roughly equivalent to the Na current (this was so throughout the dose range investigated) which means that the Cl and K currents are relatively small. However, under conditions *in vivo*, when the acinar membrane is not under voltage clamp, the Na uptake must be accompanied by Cl, or K must move in the opposite direction. At a membrane potential equal to the reversal potential for the action of the secretagogues where there is no net current flowing through the secretagogue-controlled pathways, most of the Na influx is accompanied by Cl (Iwatsuki & Petersen 1977*b*). In contrast to what has been found in the salivary acinar cells, secretagogue action does not reduce intracellular K activity (Poulsen & Oakley 1979) while causing uptake of both radioactive Na and Cl (Putney *et al.* 1980; Putney & Van de Walle 1980). It would therefore appear that under conditions *in vivo* the secretagogue-evoked Na uptake is mainly accompanied by Cl and it therefore does seem likely that the Cl uptake is quantitatively sufficient to account for the acinar Cl secretion.

It may be relevant to discuss briefly the important question of the site of the secretagogue-evoked membrane permeability change. In an isolated superfused tissue fragment preparation the luminal fluid compartment would in principle be expected to be in electrical contact with the bath fluid and it should therefore not be possible to distinguish between changes occurring at the luminal or basolateral plasma membrane. However, electrophysiological studies have been carried out on the rat pancreas *in vivo* (Petersen & Ueda 1975) in a situation where in principle only the basolateral membrane parameters are measured. Results *in vitro* and *in vivo* were exactly the same, indicating that also *in vitro* the electrical parameters measured are dominated by the characteristics of the basolateral membrane. Since there is no information about the conductance of the tight junctional paracellular pathway in pancreatic acini (see also Petersen 1980) it cannot be excluded that a paracellular shunt exists and that luminal membrane characteristics influence recordings of the basolateral membrane properties. While it can now be excluded that the exocytosis process itself contributes to the secretagogue-evoked membrane permeability changes measured (Petersen 1980; Iwatsuki & Petersen 1981; Pearson *et al.* 1981), it is possible that specific electrogenic luminal membrane ion transport pathways are activated. However, the luminal membrane area, as estimated by the morphometric work of Bolender (1974), is only a small fraction of the total plasma membrane area (about 5%). If the electrical properties measured with intracellular electrodes were dominated by the properties of the luminal membrane the specific conductance of that membrane, both during

rest and stimulation, would need to be orders of magnitude higher than that of the basolateral membrane. There is good evidence available showing that transport events confined to the basolateral membrane influence the electrical properties as measured with intracellular microelectrodes. Activation of the ouabain-sensitive Na–K pump, exclusively located to the basolateral membrane in all exocrine gland cells so far investigated (see Petersen 1980), has been demonstrated to cause marked membrane hyperpolarization (Petersen 1973). Activation of Na-amino acid co-transport systems responsible for pancreatic acinar amino acid uptake from the blood (Schulz & Ullrich 1979) causes marked membrane depolarization and resistance reduction (Iwatsuki & Petersen 1980) and in voltage-clamp experiments inward current (Petersen & Singh 1981). There is so far no evidence that specific luminal transport events influence the intracellular acinar electrical recordings obtained, but it can nevertheless not be excluded that part of the currents and conductance changes measured occur at the luminal membrane.

6. Mechanism underlying secretagogue-evoked membrane permeability change

This is a topic that has recently been reviewed in considerable detail (Petersen 1980, 1981; Petersen *et al.* 1981). The main conclusion is that the similar nature of the membrane responses obtained after activation of three different receptors (ACh, CCK–Pz and bombesin) (Petersen & Philpott 1979) is due to a common intracellular messenger, Ca. The arguments in favour of the Ca hypothesis (Iwatsuki & Petersen 1977*c*; Petersen & Iwatsuki 1978) are based on three lines of evidence: (1) while the membrane electrical responses to short pulses of extracellular secretagogue stimulation are essentially independent of the presence of extracellular Ca (Nishiyama & Petersen 1975*a*), intracellular (but not extracellular) micropipette application of Ca (but not Mg or K) can mimic the secretagogue effects (Iwatsuki & Petersen 1977*c*) and the reversal potential from the membrane response to intracellular Ca application is similar to that obtained for secretagogue stimulation (Petersen & Iwatsuki 1978); (2) intracellular EGTA application by micropipettes inhibits the secretagogue-evoked (but not amino acid-evoked) membrane responses, in such a way that responses to small doses are abolished while the inhibitory action of intracellular EGTA can be overcome at higher secretagogue doses (Laugier & Petersen 1980*b*); (3) during sustained secretagogue-evoked membrane depolarization and conductance increase, removal of extracellular Ca abruptly abolishes the response, which is rapidly restored by Ca readmission during the continued secretagogue exposure. In contrast, removal of extracellular Ca during sustained amino acid-evoked depolarization only causes further depolarization (Laugier & Petersen 1980*a*, *b*). On the basis of these experimental data and in accordance with a suggestion made earlier (Petersen & Ueda 1976*b*), the Ca messenger hypothesis was formulated in some detail (Petersen & Iwatsuki 1978; Petersen *et al.* 1981) stating that the initial secretagogue action was to release intracellular Ca (most likely from the plasma membrane), the resulting enhanced $[Ca^{2+}]_i$ would trigger enzyme secretion and open channels in the plasma membrane permeable to Na, Cl and K, giving rise to NaCl uptake. In the sustained phase of stimulation the membrane Ca permeability would be enhanced and Ca influx from the extracellular space would occur, maintaining enzyme secretion and the open state of the ionic pathways. Intracellular Ca would therefore couple the two important functions of the acinar cells, enzyme and fluid secretion, to hormone–receptor interaction (figure 6).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

7. Mechanisms underlying acinar fluid secretion

It has been described above that stimulants such as ACh, CCK–Pz and bombesin evoke acinar fluid secretion and also evoke a membrane conductance increase. The inward Na current resulting from the conductance increase has been quantified and shown to be of sufficient magnitude to account for the stimulant-evoked acinar Na secretion. This, however, does not prove that the secretagogue-evoked membrane conductance increase is causally related to fluid secretion. Indeed all the evidence is circumstantial. Clearly, exactly the same receptor mechanisms are involved in the secretagogue-evoked acinar fluid secretion and the secretagogue-evoked membrane conductance increase (Petersen & Ueda 1977; Petersen & Philpott 1979;

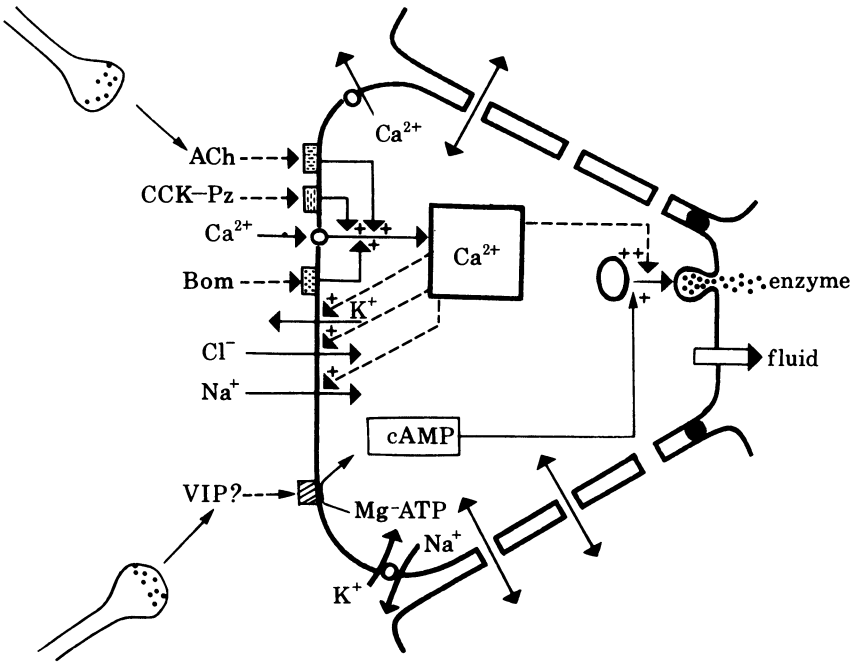

Figure 6. Simplified diagram showing some of the steps involved in nervous and hormonal control of acinar fluid and enzyme secretion.

Philpott & Petersen 1979; Ueda *et al.* 1980). The Ca-dependence is also, at least qualitatively, the same. Thus sustained fluid secretion evoked by activating the CCK–Pz receptor is acutely dependent on extracellular Ca (Ueda & Petersen 1977) in a way similar to the sustained depolarization and conductance increase (Laugier & Petersen 1980*a*). There is also, however, an initial entirely transient Ca-independent fluid secretion after stimulation (Kanno & Yamamoto 1977); this would seem to correspond to the initial transient depolarization and conductance increase after stimulation observed during exposure to Ca-free solution (Laugier & Petersen 1980*a*). From the electrophysiological data it has been concluded that secretagogues evoke electrogenic Na and Cl uptake (Iwatsuki & Petersen 1977*a*, *b*). In fragments of isolated rat pancreas, ACh and caerulein cause an increase in ^{22}Na uptake (Case *et al.* 1978; Bobinsky & Kelly 1979). Secretagogue-evoked uptake of ^{22}Na and ^{36}Cl into isolated rat pancreatic acinar cells is mediated by the same receptor mechanisms as those involved in the acinar fluid secretion

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

and the membrane conductance changes. This stimulant-evoked uptake of both Na and Cl is Ca-dependent (Putney *et al.* 1980; Putney & Van de Walle 1980). Finally, an internal Ca control of Na permeability in isolated pancreatic membrane vesicles that is qualitatively entirely in accord with the electrophysiological data has been demonstrated (Schulz & Heil 1979).

So far the Ca-modulated Na and Cl transports across the acinar membrane, characterized electrophysiologically as well as in radioactive tracer experiments, remain the only membrane transport events studied that are controlled by the same receptor-mechanisms that control the Ca-dependent acinar fluid secretion. It would therefore be unreasonable for the time being to propose a model for secretagogue-evoked acinar fluid secretion that did not focus on these secretagogue-controlled membrane transport processes. The model shown in figure 6 is an attempt to explain in part the receptor-mediated control of fluid secretion. No details are given in respect of transport events at the luminal membrane because there is as yet no evidence that the electrophysiological parameters measured can be influenced by specific luminal transport events. However, it cannot be excluded that part of the conductance changes measured occur at the luminal membrane.

There has recently been a great deal of interest in Na-coupled Cl transport by epithelial tissues (see, for example, Frizzell *et al.* 1979). In the pancreatic acini it is clear that secretagogue-evoked Cl uptake (figure 6) would be expected to be Na-dependent. The Na inward current is responsible for the depolarization and the depolarization provides a driving force for Cl. The increase in Cl conductance would tend to speed up Cl influx if a driving force exists. Based on a model proposed by Eveloff *et al.* (1978) for NaCl transport across rectal gland cells it has previously been tentatively proposed that Cl may be transported through the cells, the uptake at the basolateral membrane occurring by the mechanism depicted in figure 6, the transport mechanism across the luminal membrane remaining unclear, while the Na taken up is pumped back by the Na pump with Na entering the lumen via a possible paracellular shunt (Petersen & Iwatsuki 1979). At the moment, however, there is no evidence that activation of the Na–K pump occurs following receptor activation of pancreatic acinar cells (Nishiyama & Petersen 1975*b*). In contrast, in salivary acinar cells, where secretagogues evoke a change in membrane Na and K permeability resulting in Na–K exchange (Petersen 1971*b*), the initial receptor-controlled conductance increase is followed by a ouabain-sensitive hyperpolarization not associated with a conductance increase (Roberts *et al.* 1978). There is also no direct evidence available from pancreatic acini with regard to a possible paracellular shunt (see Petersen 1980), although indirect evidence indicates that at least the cat and rabbit pancreatic epithelia are leaky (Dewhurst *et al.* 1978; Jansen *et al.* 1979); the localization of this leak is at present unknown. The model presented in figure 6 is therefore deliberately vague on a number of important points, but focuses attention on transport events and mechanisms that have been demonstrated.

8. Dissociation between stimulant-evoked secretion and electrical changes

In acini that are not voltage-clamped, stimulation with secretagogues such as ACh, CCK–Pz and bombesin evokes membrane depolarization. The depolarization is unlikely by itself to be of importance for triggering enzyme secretion since various neutral amino acids can evoke even larger depolarizations without stimulating amylase secretion (Laugier & Petersen 1981). It has been reported, however, that depolarization of guinea-pig pancreatic acinar cells by increasing

extracellular K concentration ($[K]_o$) to 75 mM evokes atropine-resistant and Ca-dependent amylase secretion. Scheele & Haymowitz (1980) concluded from these observations that depolarization opened membrane Ca channels resulting in Ca influx stimulating exocytosis, a hypothesis in obvious disagreement with the results from our work as summarized in figure 6. Recently we presented evidence for the existence of a non-cholinergic nervous pathway controlling enzyme secretion in the guinea-pig pancreas. Excitation of intrinsic nerves in isolated superfused pancreatic fragments by electrical field stimulation evokes membrane depolarization, amylase secretion and ^{45}Ca efflux. The effects on membrane potential and Ca efflux were abolished by atropine and were therefore of cholinergic origin, while the effect on amylase secretion was only slightly reduced by atropine, i.e. nerve stimulation in the presence of a high atropine concentration (10 μM) still evoked an immediate and marked increase in amylase output which was rapidly reversible upon cessation of stimulation. The nerve stimulation-evoked increase in amylase output could be blocked by 1 μM tetrodotoxin. Pearson *et al.* (1981 *a*) concluded from these experimental results that in addition to nerves releasing ACh there were nerves releasing another transmitter also able to control amylase secretion, but working through a different intracellular coupling mechanism. Since VIP immunoreactive nerves have been demonstrated in pancreas (Larsson *et al.* 1978; Sundler *et al.* 1978) and since VIP does not cause membrane electrical changes (Petersen 1981) or changes in Ca fluxes (Gardner 1979; Gardner & Jensen, this symposium) it seems probable that the unknown transmitter is VIP or a similar peptide. We have recently shown that nerve stimulation (field stimulation) or exposure to a solution with a high $[K]_o$ (75 mM) (both in the presence of atropine) or VIP at 0.1 μM cause the same biphasic increase in tissue cyclic AMP level accompanied by smaller biphasic changes in cyclic GMP concentration (Pearson *et al.* 1981 *b*). Figure 6 includes this newly discovered nervous control mechanism for acinar enzyme secretion. One important conclusion is that cellular control mechanisms for exocytosis can be dissociated from control of membrane conductance. Can control of fluid secretion also be divorced from control of membrane conductance? So far this has not been demonstrated. As described in §2, VIP does indeed evoke fluid secretion. However, this is of the bicarbonate-dependent type and existing evidence suggests that this process occurs in duct cells. It is likely, therefore, that excitation of the non-cholinergic secretomotor fibres evokes enzyme secretion from the acinar cells and bicarbonate-rich fluid secretion from the duct cells.

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