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The Interaction of Stimulants on the Function of Isolated Canine Parietal Cells [and Discussion]

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The interaction of stimulants on the function of isolated canine parietal cells

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With isolation, the parietal cell is removed from the effects of the many endogenous substances that may modulate its function in intact mucosa, even in the basal state. The isolated canine parietal cell responds to the major endogenous regulators of secretion: histamine, acetylcholine and gastrin. These agents act on specific receptors as evidenced by (1) the specificity of antagonists (H_2 antagonists, atropine, and dibutyryl cyclic GMP respectively), (2) the binding of radiolabelled ligands, and (3) the existence of separate second messenger systems (cyclic AMP for histamine, calcium influx for acetylcholine, and an unidentified mechanism for gastrin). Potentiating interactions, which occur between histamine and acetylcholine or histamine and gastrin, do not involve extra production of second messenger. When histamine and acetylcholine are given together, the amounts of cyclic AMP generated and of calcium entering the cell are not greater than when each is acting alone. The apparent non-specific effects of inhibitors acting *in vivo*, such as the inhibition of all forms of stimulation by H_2 antagonists, could reflect withdrawal of the potentiating action of the background histamine always present in the mucosa.

Introduction

The regulation of gastric acid secretion has proved very difficult to unravel. This difficulty has largely resulted from the great complexity of the gastric mucosa, with its many cell types and many functions and from the involvement of several pathways in the regulation of acid secretion. The major physiological control of acid secretion is exerted via neurocrine, endocrine and paracrine pathways, with the latter referring to transmitters released from local tissue stores and diffusing to their target across the intercellular space (Grossman 1981). The major chemical transmitters delivered by each of these pathways are respectively acetylcholine, gastrin and histamine. Even this most superficial view of the regulation of acid secretion may be flawed by the complexity of the system.

Vagal pathways

Acid secretion resulting from vagally mediated stimuli, such as sham feeding, is inhibited by atropine, indicating that acetylcholine is the transmitter acting at a muscarinic receptor. However, in man muscarinic agonists are at best only very weak stimulants of acid secretion (Roland *et al.* 1975; Grossman 1979), thus leaving open the question of whether muscarinic agonists only weakly activate the parietal cell or whether such agonists also activate inhibitory pathways. The observation in dogs that activation of vagal pathways by sham feeding inhibits

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the response of a denervated (Heidenhain) pouch to pentagastrin provides evidence for the existence of vagal inhibitory pathways (Sjodin 1975). Vagal inhibitory pathways may even influence parietal cell function in the basal state since section of the trunk of the vagus enhances the response of a denervated pouch to exogenous pentagastrin (Emas & Grossman 1969). Although these vagally released transmitters may be primarily active locally, these last two experiments indicate effects on denervated pouches, thus suggesting delivery of these neurosecretory transmitters via the blood. The specific transmitters involved and the question of whether these inhibitors act directly on the parietal cell or indirectly at other targets are points that remain to be clarified (Grossman 1979, 1981). Thus vagal effects on acid secretion are complex and may involve both inhibitory and stimulatory elements.

Endocrine pathways

Early physiological studies indicated the presence of an antral secretagogue (Edkins 1905; Walsh & Grossman 1976), confirmed in the elegant work of Gregory & Tracy (1964) in which gastrin was isolated and sequenced. Radioimmunoassay of gastrin in blood indicates that the circulating levels produced during a mixed amino acid meal are in the range producing stimulation of acid secretion (Feldman *et al.* 1978). In fact, when the rise in serum heptadecapeptide gastrin produced by such a meal is reproduced by gastrin infusion, the observed acid secretory rates are similar to the meal-stimulated rates. Thus the rise induced in heptadecapeptide gastrin may be the major element mediating the acid secretory response to food. Hormones released from the intestine in response to a meal may also either stimulate or inhibit acid secretion; the physiological importance of these other hormonal influences remains to be established, as do the specific transmitters involved.

Paracrine pathways

The physiological role of histamine in acid secretion was debated until 1972 when Black and coworkers introduced the H_2-receptor antagonists. These agents not only inhibited histamine-stimulated acid secretion, but also acid secretion stimulated by food, gastrin and cholinergic pathways (Black *et al.* 1972; Grossman & Konturek 1974; Gibson *et al.* 1974). These findings not only establish a central role for histamine in the regulation of acid secretion but also underline the interdependence between the effects of the major stimulants of acid secretion, since meal-stimulated acid secretion, which appeared to be largely accounted for by a rise in serum gastrin (Feldman *et al.* 1978), was blocked by histamine H_2 receptor antagonists. Although the finding that the H_2-antagonists block all forms of acid secretion supports the view that histamine is the final common mediator for all other stimulants, the situation was clearly more complex. Interdependence was also demonstrated between cholinergic pathways and gastrin, with anticholinergic agents inhibiting the response to gastrin (Konturek *et al.* 1968; Davison *et al.* 1974; Hirschowitz & Hutchison 1977). In addition, anticholinergic agents inhibited the response to histamine (Code *et al.* 1951; Hirschowitz & Hutchison 1977), with neither of these latter findings consistent with the common mediator hypothesis, which proposed that acetylcholine and gastrin served primarily to stimulate acid secretion by acting to release histamine from local mucosal stores (MacIntosh 1938; Code 1965; Black *et al.* 1972). An alternative theory (Grossman & Konturek 1974) proposed that all three stimulants acted directly at the parietal cell, with interactions occurring at the parietal cell accounting for the interdependence between these three pathways. The possibility of direct receptor interaction

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was initially raised as a possible mechanism for this interdependence (Grossman & Konturek 1974), although other mechanisms now appear more likely.

A basic understanding of the regulation of acid secretion required knowledge of whether histamine, gastrin and acetylcholine were acting directly on the parietal cell or at receptors on other cell types, such as the fundic mucosal histamine-containing cell. Questions such as these are very difficult, if not impossible, to resolve in studies with intact mucosa, a difficulty resulting from acetylcholine and histamine being stored in the mucosa in the vicinity of the parietal cell and exerting influences on parietal cell function even in the basal state. This latter conclusion is based upon the ability of anticholinergic agents and H_2-receptor antagonists to inhibit basal acid secretion (Dotevall *et al.* 1965; Henn *et al.* 1975; Thjodleifsson & Wormsley 1975).

One approach to sorting out the factors regulating parietal cell function is to remove the parietal cell from its mucosal environment, with techniques that preserve functional responses to stimulation. With isolation, the parietal cell can be freed from these background influences, allowing the effects of individual agents, alone or in combination, to be studied. The data obtained from studies with dispersed parietal cells support the view that the parietal cell has receptors for histamine, gastrin, and acetylcholine and that potentiating interactions between stimulants at the parietal cell itself may account for the interdependency observed between stimulants *in vivo*. However, this reductionistic approach can, at best, provide only a partial understanding of the regulation of acid secretion in that the complex integration of regulatory inputs in the whole mucosa obviously cannot be evaluated in studies with dispersed cells.

Experimental approaches

Parietal cells have been dispersed from the fundic mucosa by treatment with enzymes. A variety of specific approaches have been developed (Soll 1981*b*), including rabbit gastric glands prepared with crude collagenase (Berglindh *et al.* 1976), canine parietal cells dispersed with sequential treatment with crude collagenase and EDTA (Soll 1978*a*), and rat (Ecknauer *et al.* 1980) and amphibian (Michaelangeli 1978) parietal cells dispersed with pronase. Several separation procedures have been used to enrich parietal cells, including velocity separation either at unit gravity or in an elutriator rotor and density gradient separations (see Soll (1981*b*) for more detailed review).

With dispersion, parietal cells lose their polar orientation as a component of an epithelium, and thus acid secretion, although detectable (Michaelangeli 1978), is an insensitive index of cell function since acid secreted at the apical surface will be largely neutralized by the concomitant secretion of bicarbonate ions. Indirect functional responses must thus be used. With stimulation, isolated parietal cells undergo a morphologic transformation similar to that observed *in vivo* (Berglindh *et al.* 1976; Soll 1981*b*); tubulovesicles which fill the cytoplasm in the basal state transform into secretory canaliculi. Both oxygen consumption and glucose oxidation provide a good index of the overall degree of cell activation, since the secretion of acid is a highly energy dependent process. The accumulation of weak bases, such as [^{14}C]aminopyrine (AP), provides direct evidence for the secretion of acid by isolated parietal cells (Berglindh *et al.* 1980*a*; Soll 1980*a*). AP, with its pK_a of 5.0, is largely unionized at cytoplasmic pH and freely diffuses across plasma membranes. Once AP has entered an acidic compartment, such as the tubulovesicles and secretory canaliculi of the stimulated parietal cell,

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it becomes ionized and thus locked in by the surrounding plasma membranes. With the aid of fluorescent microscopy, another weak base, acridine orange, has been shown to accumulate within vesicles of stimulated rabbit gastric glands (Berglindh *et al.* 1980*a*). It is important to emphasize that AP accumulation provides an index of the quantity of acid sequestered by parietal cells, rather than of the actual rate of acid secretion.

Receptor specificity

Isolated canine parietal cells appear to possess specific receptors for histamine, acetylcholine and gastrin. Each of these agents stimulates oxygen consumption, AP accumulation and glucose oxidation (Soll 1978*a*, 1980*a*, 1981*b*). In cell separation studies, these secretagogue effects correlate with the parietal cell content of the various fractions from the elutriator rotor and of the unfractionated cells. It is thus unlikely that the action of either cholinergic agents or gastrin is mediated by release of histamine, since mast cells were removed from the parietal cell enriched fractions (Soll *et al.* 1979).

Anticholinergic agents and histamine H_2-receptor antagonists specifically inhibit cholinergic agents and histamine respectively, with the dissociation constants similar to those found for muscarinic and H_2 receptors in other tissues (Soll 1980*a*). It thus appears that the pharmacological properties of these receptors are not grossly altered by the rigours of tissue dispersion and cell separation. Studies with rabbit gastric glands (Berglindh *et al.* 1976; Berglindh 1977*a*) also indicate the presence of these two receptors on the parietal cell.

The direct effects of gastrin on the parietal cell, however, are more controversal. Gastrin does cause a small degree of stimulation of the isolated canine parietal cell (Soll 1978*a*, 1980*a*) and these effects are not blocked by either H_2-antagonists or anticholinergic agents. Gastrin stimulation of AP accumulation is, however, selectively inhibited by dibutyryl cyclic GMP, an agent demonstrated to be a specific receptor antagonist for cholecystokinin (CCK) in pancreatic acinar cells (Jensen *et al.* 1980). The strongest argument for the presence of a specific gastrin receptor on the parietal cells comes from the demonstration of a specific, high affinity receptor for ^{125}I-labelled gastrin on parietal cells (Rutten & Soll 1982). More than 85% of the binding is specific, i.e. displaceable by unlabelled gastrin and reversible. Gastrin and CCK analogues compete for binding of the biologically active label, with a rank order of potency similar to that found for stimulation of AP accumulation. Dibutyryl cyclic GMP was found to inhibit gastrin binding, as it did CCK binding to pancreatic acinar cells. Gastrin thus appears to stimulate the canine parietal cell directly by interacting with a specific receptor that is closely related to the CCK receptor.

The only other species demonstrating a gastrin response *in vitro* is rabbit. Gastrin, in the presence of 3-isobutyl-1-methylxanthine (IMX), but not in the presence of histamine, stimulates AP accumulation by rabbit gastric glands (Berglindh *et al.* 1980*b*). This effect may not be direct since histamine cells are present in the glands and gastrin does release histamine from this preparation (Bergqvist *et al.* 1980). In recent studies, Chew & Hersey (1981) have found gastrin stimulation of AP accumulation by isolated rabbit parietal cells; low concentrations of histamine potentiated this gastrin response. The gastrin response was labile, only being evident within the first 10 min after stimulation and requiring the presence of dithiothreitol.

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Interdependence between secretagogues

Atropine and cimetidine are specific against stimulation of the function of isolated parietal cells by carbachol and histamine respectively, with these findings thus at odds with the observations *in vivo* that these inhibitors block all forms of acid secretion. This apparent contradiction may reflect the existence of potentiating interactions between secretagogues at the parietal cell itself. Data obtained with studies of both oxygen consumption (Soll 1978*b*) and AP accumulation (Soll 1978*c*) indicate that potentiating interactions occur between histamine and gastrin and between histamine and carbachol but not between carbachol and gastrin, in that the responses to these former two combinations are significantly greater than the sum of the individual responses. A three-way interaction may in addition exist between histamine, carbachol and gastrin (Soll 1978*c*). Interactions between cholinergic agents and histamine (Berglindh 1977*b*) and possibly between gastrin and histamine (Chew & Hersey 1981) have been found in rabbit gastric glands.

In the presence of these potentiating interactions, the actions of cimetidine and atropine display an apparent non-specificity reminiscent of that found *in vivo*. Thus, for example, when gastrin action on isolated parietal cells was enhanced by potentiating interaction with histamine, cimetidine caused an apparent inhibition of the response to gastrin, which presumably reflected withdrawal of histamine's enhancement of gastrin's action (Soll 1978*b*, *c*).

Mechanisms of cell activation

The involvement of cyclic AMP and calcium in activation of parietal cell function by secretagogues has been studied by using dispersed cells.

Cyclic AMP

With intact mucosa, studies of the role of cyclic AMP in parietal cell function have been controversial; work with isolated cells and glands leaves little question that histamine stimulates cyclic AMP production by parietal cells (Major & Scholes 1978; Soll & Wollin 1979; Chew *et al.* 1980). In contrast, neither gastrin nor carbachol enhance cyclic AMP production (Soll & Wollin 1979). In cell separation studies, the major effects of histamine are accounted for by parietal cells (Major & Scholes 1978; Wollin *et al.* 1979). Several findings with canine parietal cells indicate that histamine's enhancement of cyclic AMP production is closely linked to stimulation of parietal cell function (Soll & Wollin 1979). The dose responses for stimulation of cyclic AMP production and stimulation of parietal cell function occur over a similar concentration range of histamine. The action of histamine on cyclic AMP production and on oxygen consumption is enhanced in a parallel fashion by the phosphodiesterase inhibitor isobutyl methyl xanthine. There is a high overall degree of correlation between stimulation of cyclic AMP production by histamine and IMX and stimulation of parietal cell function. With canine parietal cells, low concentrations of histamine and IMX do stimulate AP accumulation, while causing barely detectable changes in cyclic AMP content. This apparent discrepancy probably reflects the sensitivity of AP as an index of parietal cell response (Soll 1980*a*) in contrast to the relatively small rises in cyclic AMP content induced by histamine in canine parietal cells (see below). The cyclic AMP analogue dibutyryl cyclic AMP stimulated both oxygen consumption and AP accumulation. With isolated gastric glands from rabbit, a close

correlation was found between histamine stimulation of cyclic AMP production and stimulation of both AP accumulation and oxygen uptake (Chew *et al.* 1980). Species differences are important in that both the functional and the cyclic AMP responses to histamine are of much greater magnitude in rabbit than in canine parietal cells (Soll 1981*b*); the mechanism accounting for these differences has not been elucidated.

The finding that secretin and prostaglandins, agents that inhibit gastric acid secretion, stimulate cyclic AMP production in fundic mucosa limited the acceptance of any link between AMP generation and stimulation of acid secretion. However, in cell separation studies (Major & Scholes 1978), secretin's effect on cyclic AMP production was found to correlate with the distribution of pepsinogen and not of parietal cells, indicating that secretin may be stimulating cyclic AMP generation only in chief cells. Prostaglandin E_2 (PGE_2), at concentrations above 1 μM, also stimulated cyclic AMP production by isolated mucosal cells. This effect of PGE_2, however, was not correlated with any one cell marker such as pepsinogen or mucous cells staining with periodic acid Schiff. However, PGE_2 stimulation of cyclic AMP production was negatively correlated with the parietal cell content of the fractions ($r = -0.79$, $p < 0.05$), indicating at most a minor stimulatory effect on parietal cell adenylate cyclase.

Prostaglandins, although they do not have a major effect in stimulating adenylate cyclase in parietal cells, do directly inhibit parietal cell function (Soll 1980*b*). This inhibitory effect has been demonstrated for prostaglandins of the E_2 and I_2 groups and has been shown to be selective for histamine. The stimulation of parietal cell function by carbachol and gastrin when studied as single agents is not inhibited. However, when carbachol or gastrin action has been enhanced by interaction with histamine, but not dibutyryl cyclic AMP, the effects are inhibited by prostaglandins, with the residual response similar to that found with either gastrin or carbachol alone. Histamine stimulation of cyclic AMP is also inhibited by prostaglandins (Major & Scholes 1978; Soll 1980*b*) over the same nanomolar concentration range of prostaglandins in which inhibition of histamine-stimulated aminopyrine accumulation is found. The view that prostaglandin inhibition of histamine-stimulated function is related to inhibition of histamine-stimulated cyclic AMP formation and not stimulation of cyclic AMP formation by the prostaglandins themselves is underlined by the effects of the 16-phenoxy analogue of prostacyclin, which was found to potently inhibit histamine-stimulated cell function and cyclic AMP formation but not to stimulate cyclic AMP formation by itself (Soll & Whittle 1981). These data suggest that the antisecretory effects of prostaglandins result from specific interference with histamine activation of parietal cell function. The apparent lack of specificity of prostaglandins *in vivo* may result from a mechanism similar to that proposed for H_2 blockers, i.e. impairment of potentiating interactions with endogenous histamine.

Extracellular calcium and cholinergic stimulation

In many cell types, increases in cytosol calcium appear to mediate cell activation by chemical transmitters. Such increases in cytosol calcium can be achieved by either enhanced influx of extracellular calcium or mobilization of intracellular calcium. The studies available at present indicate that cholinergic stimulation of parietal cell function is coupled to enhanced influx of extracellular calcium. The experiments supporting this conclusion include demonstration of a sharp dependence of cholinergic stimulation on the concentration of extracellular calcium (Berglindh *et al.* 1980*b*; Soll 1981*a*). Furthermore, lanthanum, which blocks calcium fluxes across plasma membranes, also caused marked impairment of cholinergic action (Soll 1981*a*).

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Lastly, carbachol stimulation is associated with enhanced $^{45}Ca^{2+}$ influx into parietal cells, which in turn is closely correlated with cholinergic stimulation of oxygen consumption and AP accumulation (Soll 1981*a*). In contrast to these findings, histamine stimulation of parietal cell function was only modestly impaired by removal of extracellular calcium and was not blocked by lanthanum nor associated with enhanced influx of $^{45}Ca^{2+}$. Gastrin action showed an intermediate dependence upon the concentration of extracellular calcium, and treatment with lanthanum caused modest impairment of gastrin responsiveness. However, stimulation by gastrin was not found to be associated with enhanced calcium influx, and although gastrin action may be linked to mobilization of intracellular calcium, studies available with cells preloaded with $^{45}Ca^{2+}$ have failed to support this possibility (Soll 1981*a*). Further studies will be necessary to elucidate the secondary mechanisms involved in gastrin's action on isolated parietal cells.

Mechanisms underlying potentiation

Only negative data are available regarding the mechanisms underlying the potentiating interactions found between secretagogues. Potentiation does not appear to occur at the receptors themselves or at the initial steps of cell activation. Histamine-induced increases in cellular cyclic AMP content are the same when cells treated with histamine alone are compared with cells treated with histamine plus gastrin or carbachol (Soll & Wollin 1979). Since these studies were done in the presence of a phosphodiesterase inhibitor, they do not exclude the possibility of modulation of phosphodiesterase activity by gastrin or carbachol. However, potentiation of oxygen consumption is evident in the presence of phosphodiesterase inhibitors so that other mechanisms in addition to inhibition of phosphodiesterase activity appear likely. Furthermore, the rate of $^{45}Ca^{2+}$ uptake induced by cholinergic agents is not altered by the addition of either histamine or histamine plus a phosphodiesterase inhibitor (Soll 1981*a*). With parietal cells, as with many other cell types (Rasmussen & Waisman 1981), potentiation appears to involve the convergence of a cyclic AMP dependent pathway and a calcium dependent pathway, although the mechanisms accounting for this interaction remain to be elucidated.

Overview

Isolation of parietal cells allows testing of the hypothesis that secretagogues modulate acid secretion by directly interacting with receptors on the parietal cell itself. Several lines of evidence obtained with isolated cells indicate that canine and probably rabbit parietal cells have receptors for histamine, gastrin and acetylcholine and that potentiating interactions are a major mechanism underlying the interdependence between secretagogues *in vivo*. This approach, however, is reductionistic and does not consider integration of the neural, hormonal and paracrine inputs that occur *in vivo*. Release of each of the transmitters may be modulated by a variety of inputs serving to integrate the paracrine, neurocrine and endocrine elements regulating parietal cell function by mechanisms in addition to potentiation at the parietal cell itself. There are data available indicating that gastrin release is modulated by neurosecretory input, with bombesin being a major candidate for the stimulatory transmitter, and by paracrine input, with somatostatin being the major inhibitory transmitter involved (Saffouri *et al.* 1980). There are few data available to establish the major factors modulating release of histamine or acetylcholine. In the rat, histamine is contained in endocrine-like cells and gastrin stimulates histamine release (Aures *et al.* 1968; Synder & Epps 1968). In contrast, in dog and man, species

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in which fundic mucosal histamine is contained in mast-like cells (Soll *et al.* 1979), gastrin has not been found to release histamine or enhance its formation. It is likely that release of acetylcholine is modulated by local factors; this possibility has not been directly studied. Knowledge regarding the modulation of release of all of the transmitters involved is essential to a full understanding of the regulation of acid secretion.

Additional effects that occur over a longer time scale may also be of major importance. Such 'trophic' effects may include phenomena such as neural input and gastrin modulating parietal cell mass or gastric mucosal histamine stores. The finding of direct effects of secretagogues on the parietal cell do not establish that these direct effects fully account for the actions *in vivo* of the compound in question. A challenge for future work will be to gain insight into relating the characteristics *in vitro* of isolated mucosal cells to the regulation of acid secretion *in vivo*.

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Discussion

J. S. Davison (*Department of Medical Physiology, University of Calgary, Alberta, Canada*). Our experience with dibutyryl cyclic GMP (dbcGMP) is somewhat different from Dr Soll's (*J. Physiol. Lond.* **312**, 62P (1981)). In our isolated, whole mouse stomach preparation we have been unable to inhibit the stimulation of gastric acid secretion by pentagastrin with dbcGMP. Our results were identical whether we attempted to reverse stimulation by subsequent addition of

dbcGMP or to block a response by prior addition of dbcGMP. However, we were able to block the inhibition of secretion by CCK–Pz or autoinhibition by gastrin itself, though not inhibition mediated by glucagon. Our current interpretation of these results is that there are two receptors, a high-affinity receptor, not blocked by dbcGMP, which mediates secretion, and a low-affinity receptor, blocked by dbcGMP, which mediates inhibition of secretion.

Does Dr Soll think it possible that the differences in our results might reflect a difference between receptor properties of the isolated parietal cell and receptor properties in the intact gastric epithelium? For example, as Dr Soll suggested in his presentation, could we be dealing in the intact mucosa with a heterogeneous population of gastrin receptors on cells other than just the parietal cell?

A. H. Soll. One is left with few data when considering gastrin receptors on cells other than the parietal cell itself, particularly if these additional receptors are assumed to be inhibitory. Assuming that dbcGMP interacts with the gastrin receptor on the parietal cell, the failure to see inhibition may reflect the competitive nature of dbcGMP inhibition of gastrin, with the concentrations of dbcGMP delivered to the receptor too low to block the gastrin present. Inhibition might be only found against low concentrations of gastrin, concentrations that might not produce much stimulation in the isolated mouse stomach. Since CCK inhibition of gastrin action and autoinhibition by gastrin itself generally are only produced at high concentrations, these effects might be expected to be inhibited by a concentration of dbcGMP insufficient to block stimulation by gastrin alone. I favour the explanation that these latter effects involve interaction with the gastrin receptor on the parietal cell, reflecting either a low-potency effect of the hormone, or interaction with a low-affinity subpopulation of gastrin receptors or a low-affinity configuration of a gastrin receptor capable of negative cooperativity. Although, as Dr Davison suggests, CCK or high concentrations of gastrin could interact with inhibitory receptors on other cell types, I see little reason to choose this hypothesis. Alternatively, dbcGMP itself may independently stimulate acid secretion, an effect that counterbalances inhibition of gastrin action. Species differences in gastrin receptors may also be important.

T. Berglindh (*Department of Physiology, University of Uppsala, Sweden*). I am very happy that we seem to agree that histamine is the key secretagogue, without which no acid secretion will take place under *physiological* conditions. And I think we agree that histamine, to fulfil its role, perhaps only has to be present at background concentrations, and thus set the stage for the other secretagogues. Like Dr Soll, with the isolated gastric dog cells, we have over the past few years been able to obtain a gastrin response in the isolated rabbit gastric glands, but only in the presence of phosphodiesterase inhibitor like IMX. This is in agreement with Dr Soll's finding that IMX is necessary to see potentiation between histamine and gastrin. To explain all the data we must probably, as Dr Soll has described, assign three different receptors to the parietal cell (i.e. histamine, acetylcholine and gastrin). Since, at least in the rabbit glands, the gastrin receptor seems to be of a passive type, additional effects of gastrin must be sought. In that context it is of interest that both gastrin and acetylcholine have been shown to release histamine from isolated gastric glands in a dose-dependent manner (Bergqvist *et al.* 1980). These results agree very well with the findings that the gastrin and acetylcholine responses seen in the presence of IMX can be blocked by low concentrations of cimetidine in the absence of exogenously added histamine.

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A. H. Soll. I should like to make a couple of points pertaining to the effects of gastrin, histamine and IMX on parietal cell function. Ackerman has recently reported that gastrin stimulates acid secretion may occur *in vivo*. Our studies with canine parietal cells and Chew's recent ineffective. Furthermore, this gastrin response is not blocked by H_2 antagonists. Thus, although there is no doubt that histamine is of great importance to the regulation of acid secretion, Ackerman's study suggests that without histamine action some gastrin stimulation of acid secretion my occur *in vivo*. Our studies with canine parietal cells and Chew's recent demonstration of a cimetidine-resistant gastrin stimulation of rabbit gastric glands lead to the conclusion that gastrin has a direct action on parietal cells independent of histamine. The mechanism of cell activation remains to be elucidated; I would be reluctant to call it 'passive'. Whether gastrin stimulation of acid secretion requires actions on other cell types remains to be established. In rat, and possibly rabbit, species in which histamine is stored in endocrine-like cells, gastrin stimulates histamine formation and release, although the data available do not establish that this effect actually mediates gastrin stimulation of acid secretion. Establishing this latter point is of major importance. In the dog, a species in which fundic mucosal histamine stores are in mast-like cells, there is no indication that gastrin releases histamine. In our studies we have removed histamine-containing cells from the parietal cell-enriched fractions, thus leaving little chance that gastrin action reflects indirect effects on mast-like or endocrine-like cells.

IMX is not necessary for histamine–gastrin interactions on canine parietal cell function. We have found histamine–gastrin interactions without IMX present, using both the accumulation of aminopyrine and the stimulation of glucose oxidation as indices of response. In the studies of oxygen consumption, we were unable to detect interactions in the absence of IMX, but I suspect that this result was a matter of the sensitivity of the system. The actions of IMX alone on canine parietal cells are blocked by cimetidine (Soll 1980*a*), an effect that was interpreted as reflecting a partial dependency of IMX action on residual endogenous histamine. Thus in the studies with canine parietal cells, the inhibition of IMX plus gastrin by cimetidine may not reflect more than inhibition of the enhancement of gastrin action by residual endogenous histamine. In contrast, in the rabbit gastric glands, gastrin may either cause a pulse release of histamine from endogenous stores or enhance the effect of residual endogenous histamine available to the parietal cell. I see no way of choosing among these possibilities with the present data.

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