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Regulation of pancreatic exocrine secretion *in vitro*: the action of secretagogues

By J. D. Gardner and R. T. Jensen

Digestive Diseases Branch, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205, *U.S.A.*

Pancreatic acinar cells possess two functionally distinct mechanisms by which secretagogues can increase enzyme secretion. One mechanism is mediated by mobilization of cellular calcium and can be activated by any one of four different classes of receptors. The other mechanism is mediated by cyclic AMP and can be activated by either of two different classes of receptors. In addition to stimulating enzyme secretion, a secretagogue can cause potentiation of secretion, desensitization to the subsequent stimulation caused by the same or other secretagogues as well as residual stimulation of enzyme secretion. Although each class of secretagogue receptors can cause the same final effect, stimulation of enzyme secretion, the existence of multiple classes of receptors and the different mechanisms of action endow the acinar cell with a wide range of patterns of response depending on which of the several classes of receptors are activated.

Receptors and mechanisms for stimulating enzyme secretion

Studies of the biochemical basis of action of pancreatic secretagogues have shown that acinar cells possess two functionally distinct mechanisms by which secretagogues can increase enzyme secretion (figure 1) (for reviews, see Gardner 1979; Gardner & Jensen 1980, 1981). One mechanism involves binding of the secretagogue to receptors, mobilization of cellular calcium, and, after a series of undefined steps, stimulation of enzyme secretion. Those secretagogues that cause mobilization of cellular calcium also increase cellular cyclic GMP levels and stimulate the turnover of phosphatidylinositol. Cyclic GMP, however, does not appear to mediate the actions of secretagogues on enzyme secretion (Gardner & Rottman 1980; Gunther & Jamieson 1979), and the role of increased turnover of phosphatidylinositol in the stimulation of pancreatic enzyme secretion is not known (Gardner 1979; Michell 1975). There are four classes of receptor that mediate the actions of those secretagogues that cause mobilization of cellular calcium (figure 1, table 1): one class interacts with muscarinic cholinergic agents, a second class interacts with cholecystokinin (CCK) and structurally related peptides, a third class interacts with bombesin and structurally related peptides, and a fourth class interacts with physalaemin and structurally related peptides. The second mechanism by which secretagogues stimulate pancreatic enzyme secretion involves binding of the secretagogue to receptors, activation of adenylate cyclase, increased cellular cyclic AMP, activation of cyclic AMP-dependent protein kinase and, after a series of undefined steps, stimulation of enzyme secretion (figure 1).† There

† There are marked species differences for the effects on enzyme secretion of agents that increase cyclic AMP in pancreatic acinar cells. For example, vasoactive intestinal peptide and secretin each increase cyclic AMP in pancreas from dog, cat, rat, guinea-pig and mouse, but increase enzyme secretion only in the pancreas from rat or guinea-pig (Robberecht *et al.* 1977).

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are two classes of receptors that mediate the actions of those secretagogues that increase cellular cyclic AMP (figure 1, table 1): one class interacts with secretin, vasoactive intestinal peptide (VIP) and structurally related peptides, and a second class interacts with cholera toxin.

The two mechanisms by which secretagogues can increase enzyme secretion have initial steps that are functionally distinct (Gardner 1979; Gardner & Jensen 1980, 1981). That is, secretagogues that cause mobilization of cellular calcium do not increase cyclic AMP and do

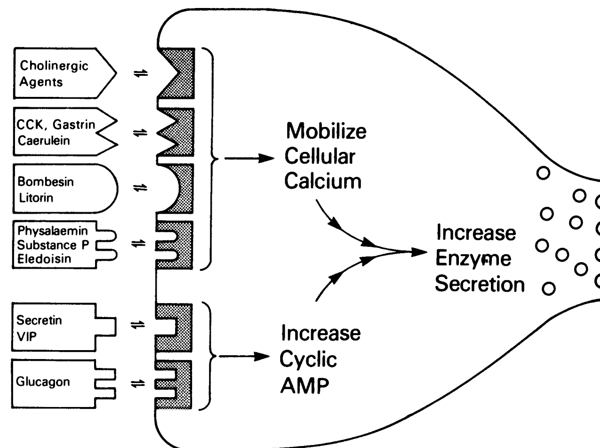

FIGURE 1. Initial steps in the mechanism of action of secretagogues on pancreatic enzyme secretion. There are four classes of receptors for secretagogues that can cause mobilization of cellular calcium and, after a series of undefined steps, stimulation of enzyme secretion. There are two classes of receptors for secretagogues that can cause activation of adenylate cyclase, increased cellular cyclic AMP, activation of cyclic AMP-dependent protein kinase, and, after a series of undefined steps, stimulation of enzyme secretion. The naturally occurring secretagogues that interact with each class of receptors are given in table 1.

TABLE 1. CLASSES OF SECRETAGOGUE RECEPTORS ON PANCREATIC ACINAR CELLS AND NATURALLY OCCURRING AGONISTS WHOSE ACTIONS ARE MEDIATED BY THESE RECEPTORS

receptors that cause mobilization of cellular calcium

cholinergic	*CCK*	*bombesin*	*physalaemin*
muscarinic cholinergic agents	CCK	bombesin	physalaemin
	caerulein	litorin	substance P
	gastrin	ranatensin	eledoisin
		alytesin	kassinin

receptors that cause activation of adenylate cyclase

VIP	*cholera toxin*
VIP	cholera toxin
secretin	
PHI	

not alter the increase in cyclic AMP caused by other secretagogues. Similarly, secretagogues that increase cyclic AMP do not alter calcium transport and do not alter the changes in calcium transport caused by other secretagogues.

One might question what purpose is served by having at least six different classes of receptors and two different biochemical mechanisms that can result in the same final effect: stimulation of secretion of enzymes from the pancreatic acinar cell. It could be that pancreatic enzyme secretion is sufficiently important to the animal's overall metabolism and survival that the redundancy of stimulatory processes reflects an evolutionary fail-safe mechanism such that

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pancreatic enzyme secretion does not depend exclusively on the presence of only one intact regulatory pathway. Recent studies, however, indicate that the multiple receptors and the different cellular mechanisms of action endow the acinar cell with a wide range of different patterns of response depending on which of the several classes of receptors is activated.

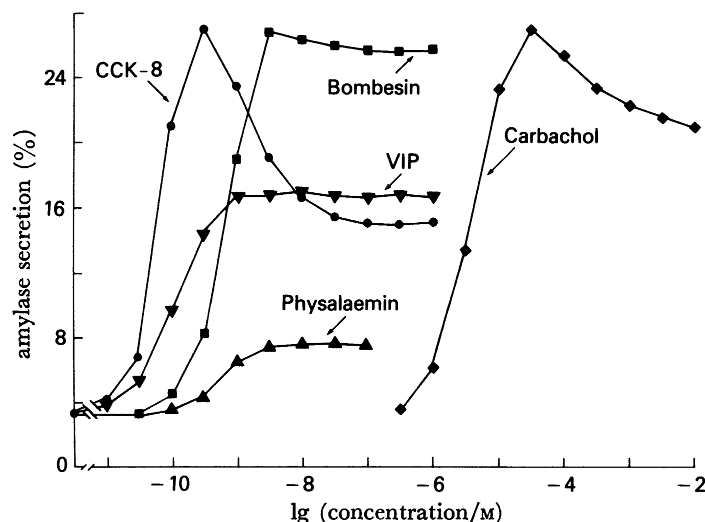

FIGURE 2. Dose–response curves for the secretagogue-induced increase in amylase secretion by dispersed acini from guinea-pig pancreas. Acini were incubated with the secretagogues specified for 30 min at 37 °C and amylase secretion is expressed as percentage release. CCK-8 is the abbreviation for the C-terminal octapeptide of CCK, and this fragment has the same efficacy as, and is more potent than, native CCK. Results are from Gardner & Rottman (1979), Gardner *et al.* (1979), Jensen & Gardner (1979), Jensen *et al.* (1978, 1980), Peikin *et al.* (1978) and Uhlemann *et al.* (1979).

PATTERNS OF STIMULATION OF ENZYME SECRETION

For those secretagogues that stimulate enzyme secretion by mobilizing cellular calcium, there is a different stoichiometric pattern of action depending on the class of receptors with which the secretagogue interacts (figure 2). For those secretagogues that interact with CCK receptors, as the concentration of the secretagogue increases, enzyme secretion increases, becomes maximal and then decreases. The dose–response curves for the action of cholinergic agents on enzyme secretion, like those for CCK-related peptides, also show submaximal stimulation with supramaximal secretagogue concentrations; however, with cholinergic agents the magnitude of the decrease in the dose–response curve is not as great as it is with CCK-related peptides (figure 2). Those secretagogues that interact with bombesin receptors also show a small but statistically significant decrease in enzyme secretion with supramaximal secretagogue concentrations, but the magnitude of this decrease is substantially less than that which occurs with cholinergic agents or with CCK-related peptides (figure 2). With a maximally effective concentration of a secretagogue that interacts with bombesin receptors, the increase in enzyme secretion is the same as that caused by a maximally effective concentration of a secretagogue that interacts with cholinergic receptors or with CCK receptors (figure 2). None of the secretagogues that interact with physalaemin receptors show submaximal stimulation of enzyme secretion with supramaximal concentrations of the secretagogue (figure 2). In terms of the maximal increase in enzyme secretion caused by CCK-related peptides, bombesin-related

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peptides or cholinergic agents, those secretagogues that interact with physalaemin receptors are substantially less efficacious (figure 2).

In contrast to those secretagogues that mobilize cellular calcium, all secretagogues that act by increasing cellular cyclic AMP show the same stoichiometric pattern of action on enzyme secretion (figure 2). That is, the dose–response curves for secretin and VIP have the same monophasic configuration as does that for cholera toxin and none of these secretagogues show submaximal stimulation of enzyme secretion with supramaximal concentrations. Moreover, those secretagogues that increase cellular cyclic AMP are equally efficacious in stimulating enzyme secretion, and the magnitude of the stimulation caused by a maximally effective concentration of a secretagogue such as VIP is approximately half of that caused by a maximally effective concentration of CCK (figure 2).

Table 2. Potentiation of enzyme secretion caused by VIP in combination with CCK

secretagogue	amylase secretion (%)
none	2.1 ± 0.4
VIP (1 nM)	9.2 ± 0.6
CCK (1 nM)	13.0 ± 1.0
VIP + CCK	27.4 ± 2.4 (20.1)

Dispersed acini from guinea-pig pancreas were incubated with the secretagogues indicated for 15 min at 37 °C. Values for amylase secretion represent the percentage of the amylase activity present in the acini at the beginning of the incubation that was released into the medium during the incubation. Results given are means ± 1 s.d. from four separate experiments. The number in parenthesis is the calculated additive value.

In contrast to the multiple stoichiometric patterns of action of secretagogues on enzyme secretion, there are only two distinct kinetic patterns of action, and these kinetic patterns correlate with the cellular mechanism of action of the secretagogue, not with the class of receptors with which it interacts (Gardner & Jensen 1981; Peikin *et al.* 1978). With VIP or secretin the rate of enzyme secretion remains constant for at least the first 2 h of incubation (Peikin *et al.* 1978). Although cholera toxin does not alter enzyme secretion during the first hour of incubation, once stimulation begins the rate of enzyme secretion remains constant for the duration of the incubation (Gardner & Rottman 1979). In contrast to secretagogues that increase cellular cyclic AMP, with secretagogues that mobilize cellular calcium, the rate of enzyme secretion during the initial minutes of incubation is greater than it is later (Gardner & Jensen 1981; Peikin *et al.* 1978).

Potentiation of enzyme secretion

Although the initial steps in the two different biochemical mechanisms by which secretagogues can stimulate enzyme secretion are functionally distinct, these two mechanisms do interact at some currently undefined step. One consequence of this interaction is that there is 'potentiation' of enzyme secretion (Gardner & Jensen 1981; Peikin *et al.* 1978). For example, as illustrated in table 2, the increase in enzyme secretion produced by the combination of a secretagogue that causes mobilization of cellular calcium (e.g. CCK) and a secretagogue that increases cellular cyclic AMP (e.g. VIP) is significantly greater than the sum of the increase caused by each secretagogue acting alone. Thus, when one of the two cellular mechanisms is activated by a particular secretagogue, there will be amplification of the response produced by a second secretagogue whose action is mediated by the other mechanism. Furthermore, some of the nerves

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that innervate secretory tissues, including pancreas, contain VIP, a secretagogue that increases cyclic AMP, and acetylcholine, a secretagogue that mobilizes cellular calcium. Stimulation of these nerves could cause release of both secretagogues, activation of both cellular mechanisms and, as a consequence, a potentiated enzyme secretory response.

Desensitization of enzyme secretion

In addition to causing stimulation of enzyme secretion, some pancreatic secretagogues can cause desensitization of enzyme secretion. That is, first incubating pancreatic acini with a particular secretagogue can reduce the subsequent secretory response to that same secretagogue measured during a second incubation (table 3).

Table 3. Bombesin-induced desensitization of amylase secretion from dispersed acini from guinea-pig pancreas

first incubation	second incubation	amylase secretion (%)
none	none	2.6
none	10 mM bombesin	23.9
10 mM bombesin	10 mM bombesin	9.3

Acini were first incubated for 120 min at 37 °C with or without 10 mM bombesin, washed, and reincubated for 30 min at 37 °C with or without bombesin. Amylase secretion was measured during the second incubation and is expressed as percentage release.

First incubating pancreatic acini with bombesin causes desensitization of pancreatic enzyme secretion measured during a subsequent incubation (table 3) (Lee *et al.* 1980). Bombesin-induced desensitization does not depend on protein synthesis, is temperature-dependent and reversible (Lee *et al.* 1980). Bombesin-induced desensitization reduces the subsequent response only to those secretagogues that interact with bombesin receptors (i.e. bombesin, litorin, ranatensin and alytesin). Bombesin does not cause desensitization to the effects of other secretagogues that cause mobilization of cellular calcium (i.e. A23187 or secretagogues that interact with receptors for CCK, cholinergic agents or physalaemin), and does not cause desensitization to secretagogues that increase cellular cyclic AMP (i.e. VIP, secretin or 8-bromo cyclic AMP). Litorin, ranatensin and alytesin, other secretagogues that interact with bombesin receptors, induce desensitization with the same characteristics as that induced by bombesin. Although the mechanism by which bombesin causes desensitization is not known, this phenomenon appears to reflect changes that occur at the receptor (Jensen *et al.* 1978; Lee *et al.* 1980). Effects of bombesin that occur at steps after receptor occupation cannot account for the selectivity of bombesin-induced desensitization, and the close correlation between the dose–response curve for bombesin-stimulated enzyme secretion and that for bombesin-induced desensitization favour an action at the receptor for bombesin and related peptides (Jensen *et al.* 1978; Lee *et al.* 1980).

First incubating pancreatic acinar cells with CCK also reduces the subsequent enzyme secretory response to CCK by as much as 75% (Abdelmoumene & Gardner 1980). CCK-induced desensitization, like that induced by bombesin, does not depend on protein synthesis, is temperature-dependent and reversible, and the rate of onset as well as the rate of reversal depend on the concentration of CCK (Abdelmoumene & Gardner 1980). CCK-induced desensitization develops somewhat more rapidly than that induced by bombesin in that the former is maximal

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after 1 h of incubation, whereas the latter requires 2 h of incubation for maximal induction. As shown in table 4, CCK-induced desensitization is not restricted to secretagogues that interact with the CCK-receptor but also reduces the subsequent response to all secretagogues that increase enzyme secretion by causing mobilization of cellular calcium (i.e. CCK, cholinergic agents, bombesin and physalaemin). Cholinergic agents such as carbamylcholine can also induce desensitization of pancreatic enzyme secretion and, as occurs with acinar cells that have been first incubated with CCK, carbamylcholine-induced desensitization reduces the subsequent response to all secretagogues that act by causing mobilization of cellular calcium (Abdelmoumene & Gardner 1980). The desensitization induced by CCK or by a cholinergic agent is selective,

Table 4. CCK-induced desensitization of amylase secretion from dispersed acini from guinea-pig pancreas

first incubation	second incubation	stimulation of amylase secretion (%)
10 nM CCK	0.1 nM CCK	30
10 nM CCK	30 μM carbachol	27
10 nM CCK	10 nM bombesin	34
10 nM CCK	10 nM physalaemin	25
10 nM CCK	10 nM VIP	100
10 nM CCK	1 μM secretin	100

Acini were first incubated for 75 min at 37 °C with or without CCK, then for 10 min with 3 mM dibutyryl cyclic GMP, washed, and reincubated for 30 min at 37 °C with the secretagogues indicated. Dibutyryl cyclic GMP was added to abolish CCK-induced residual stimulation of enzyme secretion (for example, see figure 6). Stimulation of amylase secretion was calculated as the percentage of the stimulation obtained with acini that were first incubated without CCK.

however, because first incubating acinar cells with either of these secretagogues does not reduce the subsequent stimulation of enzyme secretion caused by secretagogues such as VIP, secretin or 8-bromo cyclic AMP, agents which act by increasing or mimicking cellular cyclic AMP (table 4) (Abdelmoumene & Gardner 1980). Although CCK-induced desensitization, like stimulation of enzyme secretion, is initiated by occupation of cell surface receptors for CCK, this desensitization, unlike stimulation, persists after CCK dissociates from its receptors (Abdelmoumene & Gardner 1980; Jensen *et al.* 1980). The mechanism by which CCK and carbachol cause desensitization is not known; however, this action appears to reflect some post-receptor change, because CCK as well as cholinergic agents desensitize acinar cells to all secretagogues that cause mobilization of cellular calcium (table 4) (Abdelmoumene & Gardner 1980).

Unlike secretagogues that mobilize cellular calcium, those secretagogues that increase cyclic AMP do not cause desensitization of pancreatic enzyme secretion *in vitro* (Lee *et al.* 1980; Jensen *et al.* 1980). For example, first incubating acinar cells with VIP does not alter the subsequent response to VIP, secretin or 8-bromo cyclic AMP and actually potentiates the subsequent stimulation caused by those secretagogues that mobilize cellular calcium (Lee *et al.* 1980; Jensen *et al.* 1980).

Residual stimultion of enzyme secretion

Another type of pancreatic secretory response that can be evoked by a secretagogue is what we have termed 'residual stimulation' of enzyme secretion (Collins *et al.* 1981 *a*). That is, when

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pancreatic acini are first incubated with a secretagogue, washed to remove the free secretagogue and then reincubated in fresh, secretagogue-free incubation solution, there is a significant stimulation of enzyme secretion measured during the second incubation (figure 3). This type of response is not unique to acinar cells because hormone-induced residual stimulation of target cell function has also been observed with hormones acting on other target tissues. For example, in thyroid tissue incubated with TSH for 2 min and then washed, TSH-stimulated glucose oxidation persists for at least 2 h after removal of TSH from the incubation medium (Pastan

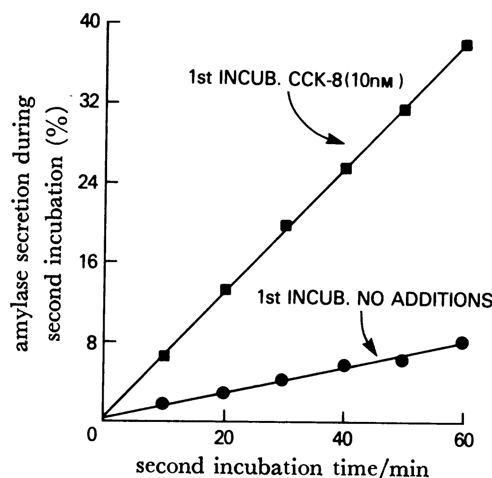

FIGURE 3. CCK-induced residual stimulation of enzyme secretion from dispersed acini from guinea-pig pancreas. Acini were first incubated for 10 min at 4 °C with or without CCK-8, washed, and reincubated for up to 60 min at 37 °C. Amylase secretion is expressed as the percentage of the amylase activity in the acini at the beginning of the second incubation that was released into the extracellular medium during the second incubation.

et al. 1966). In some instances, particularly with hormones that increase cellular cyclic AMP, hormone-induced residual stimulation is caused by a persistent increase in one of the cellular mediators of the hormone's action (e.g. cyclic AMP), and it is this persistently activated intermediate step that actually causes the residual stimulation. This type of mechanism appears to account for the residual stimulation of pancreatic enzyme secretion caused by secretagogues such as VIP and secretin (Lee *et al.* 1980). In other instances, hormone-induced residual stimulation reflects persistent occupation of receptors after the free hormone is removed from the incubation medium. This type of mechanism appears to account for the residual stimulation of pancreatic enzyme secretion caused by CCK and bombesin (Collins *et al.* 1981*a*; Lee *et al.* 1980).

In pancreatic acinar cells, CCK-induced residual stimulation of amylase release is reversible and specific for CCK (Collins *et al.* 1981*a*, *b*). Although induction *per se* is temperature-independent, the induction process does occur more rapidly at 37 °C (maximal by 1 min) than at 4 °C (maximal by 10 min). Once induced, residual stimulation persists for 60–75 min at 37 °C and for more than 90 min at 4 °C. The reversal of CCK-induced residual stimulation, which occurs spontaneously after 75 min of incubation at 37 °C, does not reflect the development of non-specific impairment of cell responsiveness nor the depletion of the amount of enzyme available for secretion (Collins *et al.* 1981*a*, *b*). Because desensitization also occurs in acinar cells that have been first incubated with CCK, this desensitization contributes to the spontaneous reversal of residual stimulation that occurs at 37 °C. Although the spontaneous

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rate of reversal of CCK-induced residual stimulation is slow, this residual stimulation can be reversed immediately by adding dibutyryl cyclic GMP, a specific CCK-receptor antagonist (figure 4) (Collins *et al.* 1981*b*). Thus, CCK-induced residual stimulation of enzyme secretion appears to be caused by persistent occupation of receptors by CCK (Collins *et al.* 1981*a*, *b*; Jensen *et al.* 1980). Adding an antagonist, such as dibutyryl cyclic GMP, is believed to cause displacement of CCK from its receptors and thereby abolish residual stimulation (Collins *et al.* 1981*b*). Carbachol, whose mechanism of action on pancreatic enzyme secretion is the same as that of CCK, does not cause residual stimulation of enzyme secretion, and bombesin causes residual stimulation that is only 20–30% of that caused by CCK (Collins *et al.* 1981*a*; Lee *et al.* 1980).

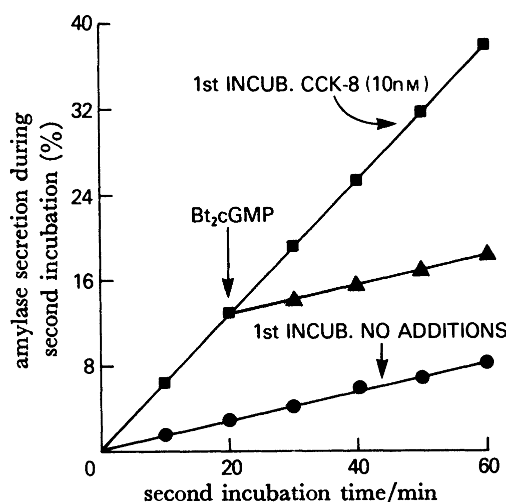

FIGURE 4. Ability of dibutyryl cyclic GMP to reverse the residual stimulation caused by CCK-8. The experimental conditions were the same as those for the experiments illustrated in figure 3 except that dibutyryl cyclic GMP (Bt_2cGMP) was added after 20 min of the second incubation.

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Discussion

J. S. Davison (*Department of Medical Physiology, University of Calgary, Canada*). I wonder if Dr Gardner's definition of potentiation is adequate. The demonstration that a combination of two agonists will produce a greater response than the sum of the responses to each agonist acting singly might not distinguish between additive and superadditive effects if only a single dose of each agent is used. A single agent can satisfy this criterion for potentiation on certain parts of the dose–response curve: i.e. it can apparently potentiate itself. In defence of the simple definition of potentiation, Magee *et al.* (*Gastroenterology* **55**, 648–650 (1968)) present dose–response curves to illustrate the difference between additive and potentiated responses. However, as pointed out by Grossman (*Gastroenterology* **56**, 815–816 (1969)), these figures show clearly that on parts of the curve doubling the dose will more than double the response, hence not satisfying the simple definition. For this reason he proposed alternative criteria for potentiation (for details see reference above). Do Dr Gardner's data satisfy any of these other criteria?

J. D. Gardner. Dr Davison's comment raises three questions. The answer to the first question is that the demonstration that a combination of the two agonists produce a greater response than the sum of the response to each agonist acting alone is *by definition* a 'superadditive' response. The statement concerning 'autopotentiation' is correct, but it is important to realize that this is an unusual phenomenon. If the effect of an agonist is mediated by a single class of non-interacting receptors, the dose–response curve can be described by a rectangular hyperbola ($y = ax/(b+x)$), and it is clear on mathematical grounds alone that the two doses in combination will *never* give a response that is greater than the sum of the response to each dose alone. Those rare agents that do show what Dr Davison refers to as 'autopotentiation' are usually characterized as showing 'positive cooperativity', which is an uncommon phenomenon. Finally, most of the confusion concerning the adequacy of definitions of potentiation seems to me to have arisen because of the failure to realize that the definition is purely operational. As we have pointed out previously (*Gastroenterology* **74**, 348 (1978)), we use the definition of potentiation given in this paper because of its predictive ability. That is, for two agents that show potentiation by our criterion, the two agents (1) at some point have a final common mediator or effector, (2) have steps before this common mechanism that are functionally distinct and (3) act on the mediator or effector in such a way to cause

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multiplication. There are two other definitions of potentiation: (1) the response to two agents in combination exceeds the larger of the responses to twice the dose of each agent alone and (2) the response to the combined agents exceeds the observed maximal response obtainable by either agent alone. The first definition is too restrictive because when one agent alone causes a response that is much greater than that caused by a second agent, potentiation would be said not to have occurred even though the response to the combination was significantly greater than the sum of the individual responses. The second definition is too broad because actions of two agents that occur via independent mechanisms and which are additive would be labelled 'potentiation' by this definition.

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