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THE EFFECTS OF GASTRIN AND GASTRIN ANALOGUES ON PANCREATIC ACINAR CELL MEMBRANE POTENTIAL AND RESISTANCE

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1 Intracellular recordings of membrane potentials and input resistance have been made from the exocrine acinar cells of mouse and rat pancreas placed in a tissue bath perfused with Krebs-Henseleit solution.

2 The resting acinar cell membrane potential was about −38 mV. The acinar cells were stimulated by cholecystokinin-pancreozymin (CCK-PZ), gastrin and the gastrin-related polypeptides, caerulein and desulphated caerulein. The immediate effect of stimulation with these secretagogues was always a depolarization and a concomitant reduction in input resistance and time constant. Depolarization of the acinar cell membrane by these secretagogues was not abolished in the presence of atropine (1.4 μM).

3 These peptide secretagogues were divided into the gastrin group and the CCK-PZ group according to the time course of the depolarizations and the shape of the dose-response curve. The depolarization evoked by the gastrin group returned quickly to the resting level but that evoked by the CCK-PZ group was long lasting. The time course and the dose-response curve for desulphated caerulein was identical with that of gastrin.

4 It was confirmed electrophysiologically that the activity of gastrin is exerted by the C-terminal tetrapeptide; but the activity of caerulein depends on the C-terminal heptapeptide, especially the presence in the molecule of the sulphated tyrosyl residue at position 7 (numbering from the C-terminus). The equivalent sulphated tyrosyl residue in CCK-PZ is probably necessary for optimal activity of this polypeptide.

5 The dose-response curves obtained by electrophysiological methods indicated that the relative potencies of the peptides on mouse pancreatic acinar cells were caerulein > CCK-PZ > gastrin. Synthetic human gastrin I was found to have a higher potency than either tetra- or pentagastrin.

Introduction

Protein secretion from pancreatic acinar cells is stimulated by the neurotransmitter acetylcholine (ACh) (vagal nerve) and the intestinal hormone cholecystokinin-pancreozymin (CCK-PZ) (Harper, 1967). Dean & Matthews (1972) showed that electrical stimulation of the pancreatic nerve, and CCK-PZ, depolarized the pancreatic acinar cell membrane. ACh and CCK-PZ act on the acinar cell membrane by increasing the membrane conductance (Petersen, 1976). The ACh null (reversal) potential is about −10 mV (Iwatsuki & Petersen, 1976) and since the ACh-evoked depolarization is sensitive to change in extracellular Na and K concentration the mechanism underlying the ACh effect is probably an increase in both Na and K permeability (Nishiyama & Petersen, 1975). It appears that ACh, CCK-PZ and gastrin act in qualitatively the same way (Petersen & Ueda, 1975).

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Gastrin and caerulein (Cn) are peptides with similar structure to the C-terminal pentapeptide of CCK-PZ. CCK-PZ and Cn, having a sulphated tyrosine residue at position 7 from the C-terminus, have a similar spectrum of biological activity (Anastasi, Erspamer & Endean, 1968; Erspamer, 1970). It is now clear that these chemical structures have a close relationship with the biological activity. A study by Tracy & Gregory (1964) of the physiological properties of synthetic peptide derivatives corresponding to various portions of the molecule of gastrin I led to the discovery that of the 17 amino acid residues present, only the C-terminal tetrapeptide amide, try-met-asp-phe-NH_2, was required for display of all the actions of the total molecule. They also showed that this tetrapeptide had a stimulatory effect on amylase secretion by the pancreas. On the other hand, Cn displays a potent stimulatory action on pancreatic secretion similar to that of CCK-PZ. It was reported that this potent activity was caused by the sulphated

tyrosine residue at position 7 from the C-terminus (Bertaccini & Erspamer, 1969).

The present paper concerns the relationship between the biological activity and the primary structure of Cn and gastrin as revealed by electrophysiological studies on mouse and rat pancreas segments superfused *in vitro*.

Methods

The experiments were performed on isolated segments of pancreas from mice and, in a few cases, rats. The segments were placed in a perspex tissue bath (volume 20 ml) which was perfused with a Krebs-Henseleit solution warmed to 37°C and flowing at a rate of about 10 ml/min (Dean & Matthews, 1972; Nishiyama & Petersen, 1974). The solution had the following composition (mM): NaCl 103, KCl 4.7, $CaCl_2$ 2.56, $MgCl_2$ 1.13, $NaHCO_3$ 25, NaH_2PO_4 1.15, D-glucose 2.8, Na pyruvate 4.9, Na fumarate 2.7 and Na glutamate 2.7; it was gassed with 95% O_2, and 5% CO_2. Glass microelectrodes filled with 3 M KCl + 10 mM K-citrate, having a tip resistance of about 20 to 30 MΩ, were used for the measurement of cellular transmembrane potential. Measurements of membrane potential and resistance were made by methods already described in detail by Nishiyama & Petersen (1974). One microelectrode was used for both current injection and recording. Electrode tip resistance compensation was carried out before cell impalement by adjusting the balance output of the electrometer amplifier. The proper balance of the bridge could be verified by looking at the shape of the current pulse induced change in membrane potential.

Stimulation of the tissue was done in the following way. Drugs were added directly to the bath as a single drop (0.01 ml) of a concentrated solution close to the tissue with a 1 ml syringe connected to a small needle ($27\frac{3}{4}$ gauge, diameter 0.4 mm). The doses given are the amounts of drug contained in the single drops.

Drugs were obtained from the following sources: CCK-PZ (mol. wt. 3883) from Boots; 1 mg is equivalent to 3000 Crick-Harper Raper Units; synthetic human gastrin I (mol. wt. 2089) from ICI, BOC pentapeptide (mol. wt. 767.9) from Calbiochem, U.S.A., AOC tetrapeptide (mol. wt. 730.55) from Nihonkayaku Ltd (Tokyo, Japan), caerulein (mol. wt. 1352) and desulphated caerulein (mol. wt. 1272) from Farmitalia, Italy, through Kyowa Hakko Kogyo Ltd, Japan. The amino acid sequences of these peptides are shown in Table 1.

Results

The resting membrane potential

In the mouse pancreas the mean membrane potential was -37.5 ± 0.9 ($n = 370$) and in the rat pancreas the comparable values were -37.5 ± 0.9 ($n = 50$). These values were very similar to results previously described by Dean & Matthews (1972), Matthews & Petersen (1973) and Nishiyama & Petersen (1974) from the same preparation.

The effects of acetylcholine and cholecystokinin-pancreozymin

Previous reports (Matthews & Petersen, 1973; Matthews, Petersen & Williams, 1973) showed that the dose-response curves calculated from continuous exposure of mouse pancreatic acinar cells to either ACh or CCK-PZ had shapes very similar to each other.

We used routinely a single dose of drugs added directly into the tissue bath close to the tissue and the initial experiments were designed to allow comparison between responses obtained using this technique and those previously published.

The effects of acetylcholine The magnitude of maximum depolarization and the time course were approximately similar to those reported by Matthews & Petersen (1973) but the dose-response curve was different in that it had a lesser slope and the initial response was seen at a dose of only 10^{-14} mol. The different experimental results can possibly be

Table 1 Structure of the peptides studied

Peptide	*Structure*
Human gastrin I	Pyr·Gly·Pro·Trp·Leu·Glu·Glu·Glu·Glu·Glu·Ala·Tyr·Gly·Trp·Met·Asp·Phe·NH_2
AOC tetrapeptide	t·Amyloxycarbonyl·Trp·Met·Asp·Phe·NH_2
BOC pentapeptide	t·Butyloxycarbonyl·β·Ala·Trp·Met·Asp·Phe·NH_2
Caerulein	Pyr·Glu·Asp·Tyr·Thr·Gly·Trp·Met·Asp·Phe·NH_2 Ȯ·SO_3H
Desulphated caerulein	Pyr·Glu·Asp·Tyr·Thr·Trp·Met·Asp·Phe·NH_2
CCK-PZ	Lys·Ala·Pro·Ser·Gly·Arg·Val·Ser·Met·Ile·Lys·Asp·Leu·Ser·Leu·Asp· Pro·Ser·His·Arg·Ile·Ser·Asp·Arg·Asp·Tyr·Met·Gly·Trp·Met·Asp·Phe·NH_6 Ȯ·SO_3H

Gastrin II is identical to gastrin I, but the tyrosin residue is sulphated.

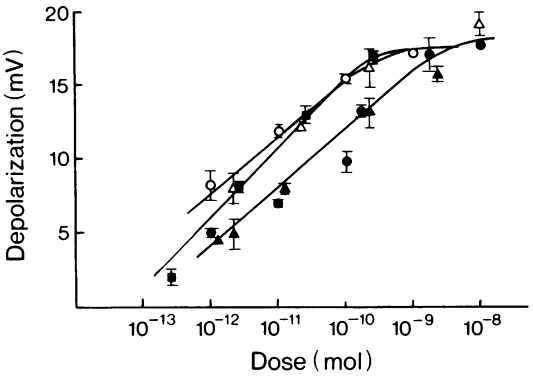

Figure 1 Dose-response curves for the depolarization effect of gastrin analogues on the pancreatic acinar cells of mouse and rat. Mean values ($n=4-13$) are given. Vertical bars show s.e. means. (■) Synthetic human gastrin I; (●) gastrin pentapeptide, mouse; (○) gastrin pentapeptide, rat; (▲) gastrin tetrapeptide, mouse; (△) gastrin tetrapeptide, rat. In this and the following figures, 'Dose (mol)' refers to the amount of agent added to the tissue bath (capacity 20 ml) in a volume of 0.01 ml.

explained by the concentration change caused by dilution at the tissue surface. In all experiments, the effect of ACh was to cause depolarization associated with a marked reduction of input resistance and time constant.

The effects of cholecystokinin-pancreozymin The dose-response curve was very similar to that of Matthews & Petersen (1973). The initial depolarization was seen at a dose of 10^{-15} mol and the magnitude of maximum depolarization was 15 mV at a dose of 10^{-11} mol. The depolarization was long lasting with doses of CCK-PZ over 10^{-12} mol. Signs of electrical uncoupling (Petersen, 1976) were sometimes seen. During the stage of uncoupling it was difficult to know the exact magnitude of depolarization. In a previous report (Nishiyama & Petersen, 1974) it was shown that CCK-PZ stimulation causes depolarization accompanied by a reduction in input resistance and time constant. We confirmed these results in all cases.

The effects of gastrin

Figure 1 shows the dose-response curves for the depolarizing effect of three forms of gastrin. The curve for tetragastrin was indistinguishable from that for pentagastrin. The threshold dose of tetra- and pentagastrin was 10^{-12} mol and maximal depolarization was produced by a dose of 10^{-9} mol of these peptides. The maximum value of depolarization evoked by tetra- and pentagastrin was 18 mV. Depolarization was accompanied by a marked reduction in input resistance (Figure 2). On the other hand the synthetic human gastrin I (SHG I) had a slightly higher potency because an initial depolarization was seen at the dose of 10^{-13} mol and maximal effect was produced by 10^{-10} mol SHG I. The effect of tetra- and pentagastrin was also tested using rat pancreas. The response pattern was the same as in the mice but the dose-response curve was shifted slightly to the left showing that rat pancreatic acinar cells are more sensitive to gastrin than are those of mice.

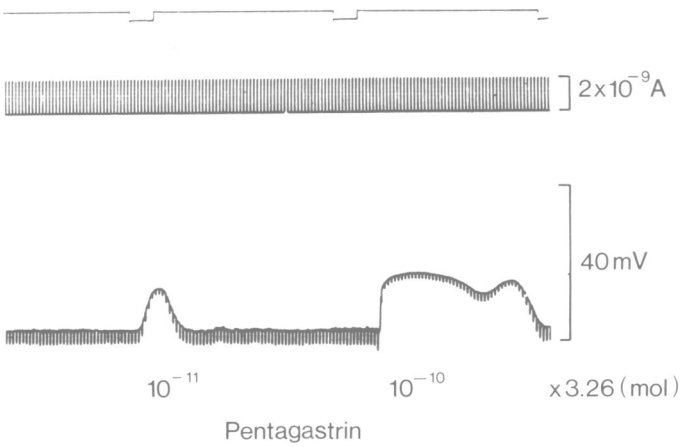

Figure 2 Effect of pentagastrin, added to the tissue bath, on membrane potential and input resistance of rat pancreatic acinar cells. The upper trace shows the time marker, the interval between signals was 1 minute. Rectangular current pulses of 100 ms duration were injected through the recording micro-electrode as indicated by the middle trace.

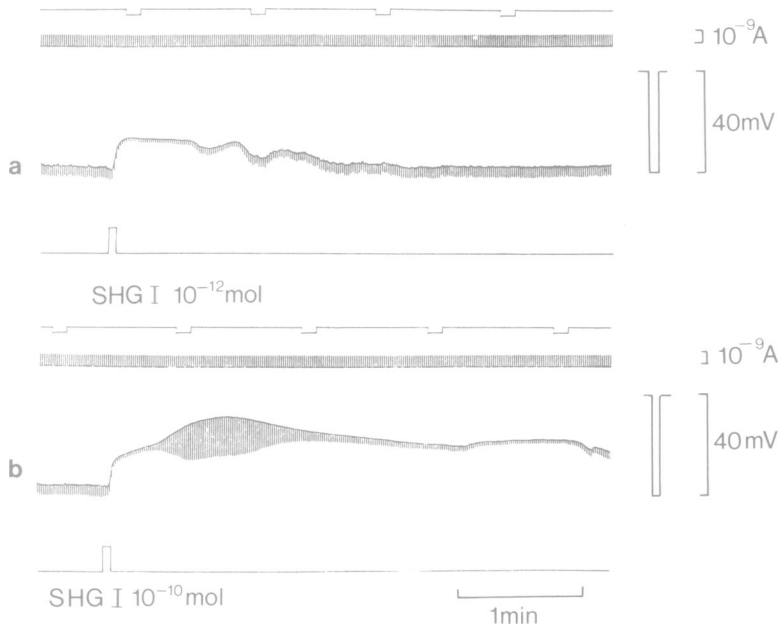

Figure 3 The effect of synthetic human gastrin I (SHGI) on membrane potential and input resistance of rat pancreatic acinar cells. The upper record (a) shows the result of the addition of a small dose of SHGI to the tissue bath and the lower record (b) shows the effects of a larger dose of SHGI. In each record the top trace is time marker (1 min intervals), the second trace indicates the injection of rectangular current pulses (100 ms duration) through the micro-electrode, the third trace is the potential recording and the lowest trace is an event marker showing the addition of drug to the bath.

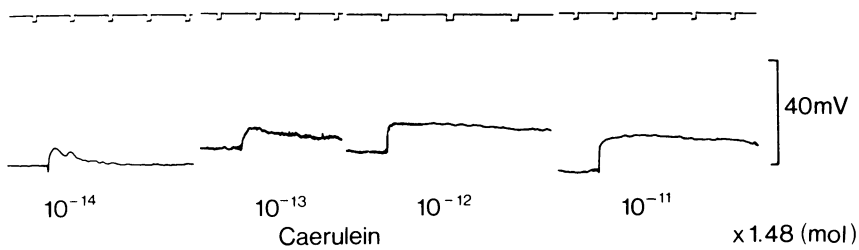

Figure 4 Dose-dependent depolarization of mouse pancreatic acinar cells produced by caerulein. The upper trace shows the time marker (interval between signals is 1 minute).

The decrease in resistance accompanying the initial depolarization was associated with a reduction of the membrane time constant in both mouse and rat pancreatic acinar cells. In some cases the initial depolarization was followed by a period of marked increase in input resistance (Figure 3b). The time constant during the delayed depolarization was shorter than in the resting state in spite of the increased resistance. Petersen & Ueda (1976) and Iwatsuki & Petersen (1976) have shown that pancreatic acinar cells are electrically coupled. The phenomenon observed here of an increase in resistance without an accompanying increase in time constant is indicative of acinar cell uncoupling during the gastrin-induced depolarization. Depolarization of the acinar cell membrane evoked by gastrin was not abolished in the presence of atropine (1.4 μM).

The effect of caerulein and desulphated caerulein

Caerulein (Cn) produced a dose-dependent depolarization of the acinar cells (Figure 4). The initial

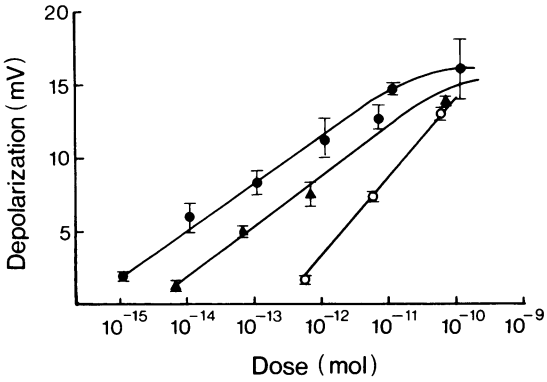

Figure 5 Dose-response curves for the depolarizing effect of cholecystokinin-pancreozymin (▲), caerulein (●) and desulphated caerulein (○) on mouse pancreatic acinar cells. Each point is the mean of between four and fifteen measurements; vertical bars show s.e. means.

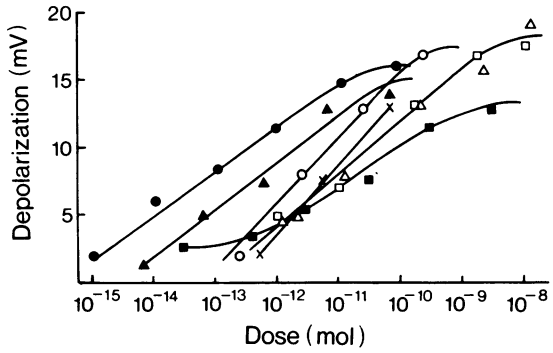

Figure 7 Dose-response curves for depolarizing effects of secretagogues studied on mouse pancreatic acinar cells: (■) ACh; (▲) cholecystokinin-pancreozymin; (●) caerulein; (×) desulphated caerulein; (□) gastrin tetrapeptide; (△) gastrin pentapeptide; (○) synthetic human gastrin I. Each point shows mean value ($n = 4-18$).

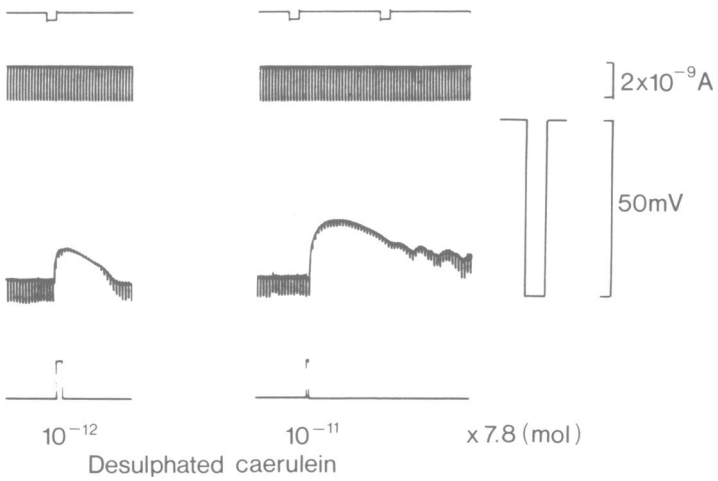

Figure 6 The effect of desulphated caerulein on the membrane potential and resistance measurement of mouse pancreatic acinar cells. The traces are arranged as in Figure 3.

depolarization appeared at a dose of 10^{-15} mol and the maximum depolarization was 16 ± 0.2 mV at a dose of 10^{-10} mol. Caerulein caused depolarization of both mouse and rat pancreatic acinar cell mebrane and the dose-response curve had a shape more like that for CCK-PZ than that for ACh (Figures 5 and 7). Doses of Cn in excess of 10^{-12} were followed by long-lasting depolarization which had not returned to the resting potential 1 h later. Depolarization caused by 10^{-12} mol Cn returned to the level of the resting potential after about 20 minutes. In many cases the initial depolarization was followed by a further depolarization which, however, was accompanied by marked increase in input resistance without a corresponding increase in time constant, indicating electrical uncoupling of acinar cells. The presence of atropine (1.4 μM) in the Krebs-Henseleit solution reduced the uncoupling produced by Cn. Ueda (1974) has reported that intravenous administration of atropine reduces the Cn-induced amylase secretion by the pancreas *in vivo*. The relationship between the effect of atropine in decreasing both electrical

uncoupling and amylase secretion induced by Cn is not clear.

Desulphated caerulein (d-Cn) which lacks a sulphate group on the tyrosine residue at position 7 evoked a dose-dependent depolarization of the pancreatic acinar cell membrane. The dose-response curve (Figure 5) had a shape rather similar to that for gastrin. Depolarization was always associated with a marked reduction in input resistance. Figure 6 shows the effect of d-Cn on membrane potential and resistance. The response is surprisingly similar to that induced by gastrin. This finding suggests that sulphated tyrosine residue at the position 7 is necessary for the activity characteristic of Cn and that desulphated caerulein acts as gastrin tetrapeptide. Depolarizations of the acinar cell membrane evoked by Cn and d-Cn were not abolished in the presence of atropine. Figure 5 summarizes the dose-response curves of Cn, d-Cn and CCK-PZ.

Discussion

The essential finding in this study is that two stimulants of pancreatic protein secretion, caerulein and desulphated caerulein, reduced considerably the acinar cell membrane potential and input resistance in both mouse and rat, and that the effects of caerulein were similar to the effects of CCK-PZ. On the other hand the effects of desulphated caerulein were very similar to the effects of gastrin on acinar cell potential and resistance. We may therefore conclude that these agonists also act on acinar cells by increasing the permeability to Na and K.

In 1964 Tracy & Gregory described the isolation from hog antral mucosa of two very similar polypeptide hormones (gastrin I and II), both of which evoked a remarkable range of physiological responses from alimentary tract structures. Anderson, Barton, Gregory, Hardy, Kenner, Macleod, Preston & Sheppard (1964) succeeded in synthesizing gastrin in the same year. Tracy & Gregory (1964) studied the actions of gastrin I and II and discovered that they stimulated both exocrine pancreatic and gastric acid secretion in dogs. They discovered that of the 17 amino acid residues present, only the C-terminal tetrapeptide amide, try-met-asp-phe-NH_2, was required for the display of all the actions of the total molecule and that the activity of the tetrapeptide was almost entirely abolished by removal of the C-terminal amide group. Lengthening the peptide chain in the direction of the N-terminus increased the potency but did not alter the general pattern of biological activity. The extended study by Morley, Tracy & Gregory (1965), of the properties of a large series of analogues of the C-terminal tetra- and pentapeptide amides showed that the most important feature of the molecules was the aspartic carboxylic terminal amide group. In the anaesthetized cat pentagastrin is less potent than gastrin II in evoking pancreatic amylase secretion (Beswick, 1968). From the results in Figure 1 it can be seen that gastrin I has a higher potency with regard to acinar cell membrane depolarization than either tetragastrin or pentagastrin.

The polypeptide hormones gastrin I and II have the same effect on protein and fluid secretion from the rat pancreas as CCK-PZ (Dockray, 1973). Electrophysiologically the time course of depolarization evoked by gastrin (gastrin I and tetra- and pentagastrin) was very similar to that of ACh and the effect of gastrin was fully reversible in a relatively short time. However the depolarization evoked by CCK-PZ is sustained for more than 30 minutes. The effect of gastrin, as well as of CCK-PZ, was not blocked by atropine in a concentration sufficient to abolish ACh-induced depolarization. A previous report (Matthews & Petersen, 1973) and our results suggest the existence of two distinct membrane receptors for these agonists. It is indicated, however, that the affinity for the membrane receptor of CCK-PZ is greater than that of gastrin.

The decapeptide caerulein, obtained for the first time in a pure form from methanol extracts of the skin of *Hyla caerulea* displays a potent, and relatively long lasting, hypotensive action in the dog, potently stimulates some extravascular muscles and possesses a remarkable action on several external secretions of the digestive tract including exocrine pancreatic secretion in the dog (Erspamer, Roseghini, Endean & Anastasi, 1966). The juice produced by caerulein is rich in enzymes, like that produced by CCK-PZ, and 1 μg Cn in equiactive to 30 to 40 μg human gastrin I, and 10 to 20 μg pure CCK-PZ (Erspamer, Bertaccini, De Caro, Endean & Impicciatore, 1967).

The amino acid sequences described in Table 1 show the striking resemblance in structure existing between caerulein, CCK-PZ and the C-terminal pentapeptide of gastrin I. Initial depolarization induced by caerulein is seen following relatively smaller doses than that of CCK-PZ but the magnitude of the maximum depolarization and the time course of the effects produced by these two peptides is very similar. On a molar basis, caerulein is 10 to 30 times as active as gastrin I and 3 to 6 times as active as CCK-PZ on pancreatic volume flow and amylase output in the dog (Bertaccini *et al.*, 1969; Stening & Grossman, 1969). In the present study (Figure 7) caerulein is about 7 times as potent as CCK-PZ and synthetic human gastrin I about one-tenth as potent as CCK-PZ. Accurate relative potencies between CCK-PZ and gastrin I could not be estimated because the two dose-response curves were not parallel. Adding the N-terminus of gastrin to tetrapeptide amide increased its potency. In contrast, the addition of amino acids to the N-terminus of the octapeptide caerulein to form CCK-PZ resulted in a decrease in potency.

Whereas the activity of gastrin depends largely on the C-terminal tetrapeptides, the activity of caerulein and CCK-PZ depends on the C-terminal heptapeptide, an especially necessary prerequisite for activity being the presence of O-sulphated tyrosyl residue at position 7 from the C-terminus. A shift of this residue towards the C-terminus, as in gastrin, produced a reduction in biological activity. Desulphated caerulein was about 20 times less active on the pancreatic secretion and 6 to 20 times less active on gastric secretion, and 160 times less active on gallbladder contraction in the dog (Johnson, Stening & Grossman, 1969; Anastasi, Bernardi, Bertaccini, Bosisio, De Castiglione, Erspamer, Gaffredo & Impicciatore, 1968). The drastic decrease in potency due to desulphation of caerulein is seen in Figure 5. The initial depolarization evoked by caerulein occurs at a dose of 10^{-15} mol and that evoked by desulphated caerulein occurs at a dose of more than 10^{-13} mol. The dose-response curve of desulphated caerulein has a shape rather similar to that of gastrin (Figure 7). Furthermore, the time course of depolarization evoked by desulphated caerulein was surprisingly similar to that of gastrin (Figure 6), never long-lasting even if the dose was increased. The frequency of electrical uncoupling by these two peptides is nearly the same. It was concluded from the above electrophysiological results that the biological activity of desulphated caerulein was dependent on only the C-terminal tetrapeptide amide, and desulphated caerulein acted as gastrin tetrapeptide on pancreatic acinar cells. It was confirmed that the presence of a sulphated tyrosyl residue at position 7 from the C-terminus is a necessary prerequisite for the manifestation of the CCK-PZ-like actions of caerulein and the equivalent residue in CCK-PZ is possibly also required for the activity of these polypeptides.

In our experiments gastrin, caerulein and desulphated caerulein, as well as ACh and CCK-PZ, all depolarized the pancreatic acinar cells of mouse and rat concomitant with reduced membrane input resistance. The depolarizing effect of ACh, but not that of other stimulants, was abolished by atropine, suggesting the existence of two distinct receptors for these stimulants. Furthermore, we can divide peptide agonists into two groups: the CCK-PZ group and the gastrin group. The first group requires the presence of a sulphated tyrosyl residue at position 7 from the C-terminus; the second group requires the presence of tetrapeptide amide. The CCK-PZ group has a stronger affinity for the receptor than the gastrin analogues. It was confirmed by the dose-response curve of these agonists on pancreatic acinar cells of mouse and rat that the biological activity was in the following order of potency: Cn > CCK-PZ > gastrin.

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