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STIMULATION OF PANCREATIC GROWTH BY CHOLECYSTOKININ IS MEDIATED BY HIGH AFFINITY RECEPTORS ON RAT PANCREATIC ACINAR CELLS

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Pancreatic acinar cells possess both high and low affinity receptors for cholecystokinin. The cholecystokinin analog caerulein, which exerts a trophic effect on the rat pancreas, acts as an agonist at both types of receptors. In contrast, the synthetic analog CCK-JMV-180, which also acts as an agonist at high affinity receptors, opposes the action of caerulein on the low affinity receptors. We report that infusion of either caerulein or CCK-JMV-180 into rats increases [^{3}H]-thymidine incorporation into pancreatic DNA and causes the pancreatic weight as well as content of DNA, RNA, and protein to increase. CCK-JMV-180 also stimulates in-vitro incorporation of [^{3}H]-thymidine into DNA of cultured rat acini. The finding that both caerulein and CCK-JMV-180 exert the same trophic effect on pancreatic acinar cells indicates that this effect is mediated via high affinity acinar cell cholecystokinin receptors. © 1993 Academic Press, Inc.

Cholecystokinin (CCK) and its analog caerulein are known to have a trophic effect on the exocrine pancreas (1-3). Previous studies have indicated that this effect is mediated via CCK receptors on acinar cells and that, when administered exogenously, CCK or caerulein increase pancreatic weight, DNA content, and incorporation of thymidine into DNA (4,5). The acinar cells of the pancreas possess both high and low affinity CCK receptors (6-8). The former are believed to mediate CCK-stimulated digestive enzyme secretion, while the latter are believed to mediate the inhibition of enzyme secretion which occurs when acinar cells are exposed to high concentrations of CCK (9,10). It is not known whether the trophic effect of CCK on the pancreas is mediated by the high or low affinity CCK receptors.

CCK-JMV-180 is a recently developed synthetic analog of CCK which, like caerulein and CCK, binds to high affinity CCK receptors and stimulates digestive enzyme secretion from acinar cells (11,12). CCK-JMV-180 also interacts with low affinity acinar cell CCK receptors but, in contrast to CCK and caerulein, this interaction with low affinity receptors does not result in the inhibition of secretion (13). Rather, CCK-JMV-180 acting via low affinity receptors, can prevent CCK- and caerulein-induced high dose inhibition of secretion (14). These observations

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indicate that CCK, caerulein and CCK-JMV-180 act as agonists at high affinity CCK-receptors but that, at low affinity receptors where CCK-JMV-180 has an effect opposing that of either caerulein or CCK, caerulein and CCK are agonists while CCK-JMV-180 is an antagonist.

We reasoned that the receptor state that mediates CCK-stimulated pancreatic growth could be identified by comparing the trophic response elicited by caerulein to the effects following CCK-JMV-180 administration. Thus, if both CCK analogs were found to stimulate pancreatic growth, it could be concluded that this response is mediated via high affinity CCK receptors. Conversely, if low affinity CCK receptors mediate the trophic response, CCK-JMV-180 would be found to (a) not stimulate growth and to (b) antagonize the trophic response elicited by caerulein. Our findings indicate that the trophic effect of CCK on the pancreas is mediated via high affinity CCK receptors.

Materials and Methods

All studies were performed using male Wistar rats (200-225 g) purchased from Charles River Breeding Laboratories (Wilmington, MA). Caerulein was purchased from Peninsula Laboratories (Belmont, CA) and CCK-JMV-180 from Research Plus (Bayonne, NJ). [^{3}H] thymidine (24.5 mCi/mmol) was obtained from Amersham Corp (Arlington Heights, IL). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MI).

In-vivo stimulation of pancreatic growth

Rats were anesthetized with pentobarbital (40 mg/kg) and a PE-50 catheter was inserted into the jugular vein as previously described (15). Animals were allowed to recover from anesthesia while being maintained unrestrained in individual shoe box cages. They were given free access to food and water. Next day either caerulein (0.3 μg/kg/hr), CCK-JMV-180 (0.3 mg/kg/hr), or vehicle (heparinized saline) was infused for 48 hours at which time the animals were sacrificed. Two hours prior to sacrifice, each animal received an intravenous bolus injection of [^{3}H]thymidine (0.1 μCi/g body weight).

Immediately after sacrifice, the pancreas was removed and trimmed of fat. For determination of pancreatic weight, a portion of the pancreas was weighed immediately (wet weight) and again weighed after desiccation at 160°C for 48 hrs (dry weight). Another portion of the pancreas was homogenized in 50mM phosphate buffer and used for measurement of DNA content as well as incorporation of [^{3}H]thymidine into DNA. The former was accomplished using Hoechst dye 33258 as described by Labarca and Paigen (16) with calf thymus DNA as the standard. To measure [^{3}H]thymidine incorporation, DNA in the pancreas homogenate was precipitated by addition of 2N perchloric acid and the resulting precipitate was washed twice with cold 1 N perchloric acid. The precipitated DNA was dissolved in 1N NaOH and radioactivity measured in a scintillation counter. For quantitation of RNA, another portion of the pancreatic homogenate was precipitated with cold 0.4 N perchloric acid and the resulting precipitate washed with cold 0.2 N perchloric acid. RNA was hydrolyzed by addition of 0.3 N KOH and incubation at 37°C for 90 min. The RNA content was then quantitated using the orcinol reaction (17). Protein content of the homogenate was determined by the Bradford method (18).

In-vitro stimulation of pancreatic growth

Pancreatic acini were prepared and cultured as previously described (19). The in-vitro trophic effects of caerulein and CCK-JMV-180 were evaluated by adding either the CCK analog or vehicle to the individual culture wells one hour after the acinar culture was begun. In-vitro culture was continued for 96 hrs. Twenty four hrs prior to the completion of in-vitro

culture, [^{3}H]thymidine (1 μCi/ml) was added to the culture medium. At the completion of in-vitro culture, the medium was removed, the cells gently washed with phosphate buffered saline, and the acini harvested by scraping the wells. The harvested acini were homogenized by sonication in 1 ml of phosphate buffer. Trichloracetic acid (10% final concentration) was added to the homogenate to precipitate DNA for measuring thymidine incorporation. The precipitated DNA was washed twice in 10% trichloroacetic acid, dissolved in 1 ml of 0.1N NaOH, and radioactivity measured as described above.

Analysis of data

Results reported in this communication are mean $\pm$ SEM. Differences between experimental groups were tested for significance by analysis of variance (ANOVA) using Tukey's method (20).

Results

In-vivo stimulation

As shown in Fig 1, infusion of 0.3 μg/kg/hr caerulein caused an approximately 4-fold stimulation of [^{3}H]thymidine incorporation into pancreatic DNA. This dose of caerulein has been shown to stimulate a maximal rate of digestive enzyme secretion from the pancreas (21). A maximal rate of digestive enzyme secretion also follows in-vivo administration of 0.3 mg/kg/hr CCK-JMV-180 (21). As shown in Fig 1, infusion of this dose of CCK-JMV-180 also increases the [^{3}H]-thymidine incorporation into pancreatic DNA and this response represents an approximately 3-fold increase of the control value. Thus the increased [^{3}H]-thymidine incorporation following caerulein and CCK-JMV-180 infusion were similar ($p>0.05$).

Infusion of caerulein was associated with a 62.6 $\pm$ 9.2% increase in the dry weight of the pancreas ($p<0.05$) while a 52.9 $\pm$ 13.8% increase in pancreatic weight followed infusion

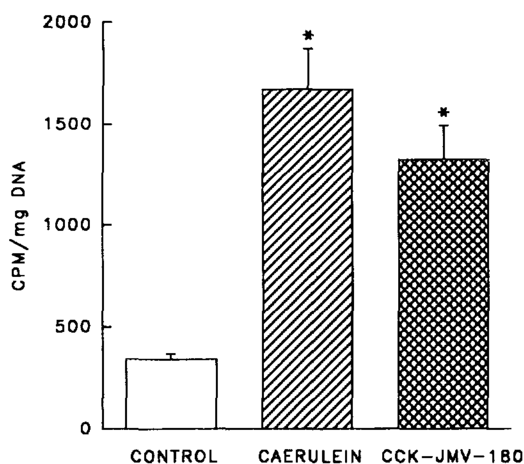

Figure 1. [^{3}H]-thymidine incorporation into pancreatic DNA. Rats were infused with either saline, caerulein (0.3 μg/Kg/hr), or CCK-JMV-180 (0.3 mg/Kg/hr) and [^{3}H]-thymidine incorporation into DNA quantitated as described in the text. Results shown are mean $\pm$ SEM values from at least four animals in each group. Asterisks denote values that are significantly different ($p<0.05$) from the saline-infused group.

of CCK-JMV-180 ($p < 0.05$) (Fig 2). The increases following infusion of caerulein and CCK-JMV-180 were similar ($p > 0.05$). Also as shown in Fig 2, infusion of caerulein was associated with significant ($p < 0.05$) increases in pancreatic DNA content (32.4 $\pm$ 8.5%), RNA content (63.4 $\pm$ 17.0%), and protein content (40.5 $\pm$ 8.6%). Infusion of CCK-JMV-180 was also associated with significant ($p < 0.05$) increases in DNA (26.1 $\pm$ 3.3%),RNA (75.7 $\pm$ 9.2%) and protein content (35.5 $\pm$ 7.5%). These increases were all similar ($p > 0.05$) in the caerulein and CCK-JMV-180 treated groups.

In vitro stimulation

Inclusion of 10 nM caerulein in the acinar culture medium resulted in an increased rate of cell spreading and [^{3}H]-thymidine incorporation (not shown). When 1 μM CCK-JMV-180 was included in the culture medium, a significant increase in the rate of cell spreading (not shown) and of [^{3}H] thymidine incorporation into DNA was also noted (Fig 3)($p < 0.05$).

Discussion

Pancreatic acinar cells possess two functionally distinct classes of receptors for CCK: those with high affinity and those with lower affinity for the hormone. Transfection of the cloned CCK receptor gene into COS7 cells has recently been shown to result in expression of both affinity states of the receptor (22). This observation strongly suggests that both the high and low affinity receptors for CCK represent products of a single gene.

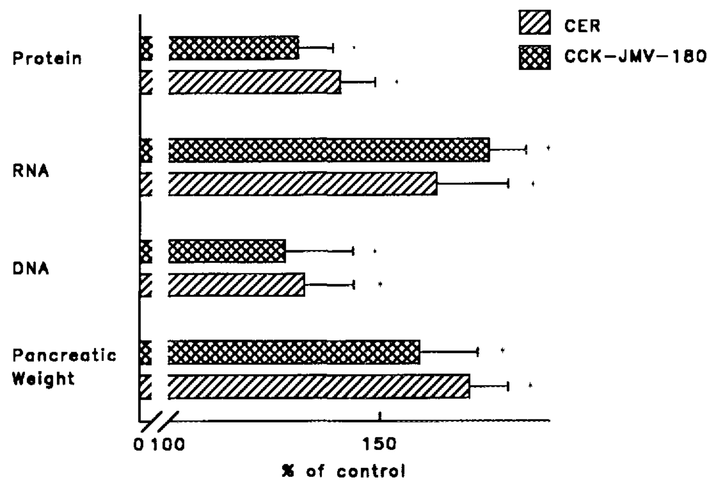

Figure 2. Effect of caerulein and CCK-JMV-180 on the weight, DNA, RNA and protein content of rat pancreas. Pancreatic weight, DNA, RNA, and protein content measured as mg/100g body weight were determined after infusion of saline, caerulein (0.3 μg/Kg/h) or CCK-JMV-180 (0.3 mg/Kg/h) and results expressed as percent of the values obtained for the saline-infused group. Data shown represent mean $\pm$ SEM values for at least four animals in each group and asterisks denote $p < 0.05$ when compared to the saline-infused group.

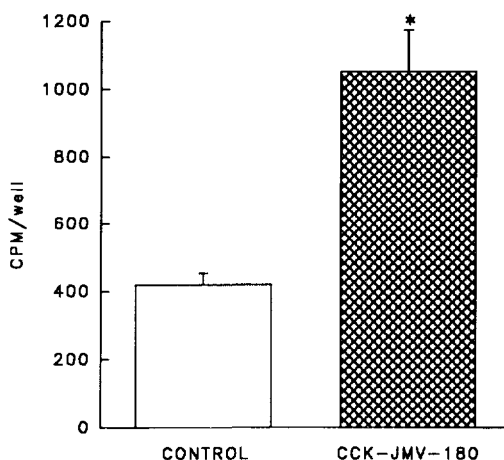

Figure 3. Effect of CCK-JMV-180 on in vitro [^{3}H]-thymidine incorporation.
Rat pancreatic acini in culture media were incubated in the presence or absence (control) of 0.1 μM CCK-JMV-180, and [^{3}H] thymidine incorporation into DNA was quantitated as described in the text. Results shown are mean $\pm$ SEM from 4 separate experiments. Asterisk denotes a value that is significantly different ($p<0.05$) from the control.

The results of the present study confirm those of several others which indicate that the CCK analog caerulein can exert a trophic effect on the pancreas. In addition, and for the first time, we report that the CCK analog CCK-JMV-180 exerts a similar trophic effect on the pancreas. In vivo, the response to each of these agonists is manifested by a significant increase in DNA and RNA content, pancreas dry weight, and [^{3}H]thymidine incorporation into pancreatic DNA. In-vitro, each of the CCK analogs accelerates the rate of [^{3}H]thymidine incorporation into DNA of cultured acini. It is likely that both the in-vivo and the in-vitro trophic effects that we have noted represent acinar cell as opposed to non-acinar cell responses to the CCK analogs since, as noted by others, infusion of caerulein for two days results in an increased rate of DNA labeling with [^{3}H]thymidine exclusively in acinar cells while other cell types within the pancreas do not appear to participate in this process (4). Our finding that both caerulein and CCK-JMV-180 can stimulate acinar cell growth in-vitro as well as in vivo indicates that this trophic response reflects a direct rather than an indirect effect on acinar cells.

The observation that both caerulein and CCK-JMV-180 exert a similar trophic effect on the pancreas indicates that this response is mediated via the high affinity class of CCK receptors since both analogs are known to act as agonists at those high affinity receptors. In contrast, the two analogs have different effects mediated via the low affinity receptors with caerulein acting as an agonist and CCK-JMV-180 as an antagonist. Thus, if the trophic effect was mediated via the low affinity CCK receptors, either caerulein or CCK-JMV-180 but not both analogs would be expected to elicit that response.

It should be noted that, in our studies, only a single concentration of either caerulein or CCK-JMV-180 was tested and the trophic response was measured at only one time interval. Thus, although the magnitude of the response we observed with CCK-JMV-180 was similar to that found with caerulein, a complete dose-response or time-dependence study might have shown that these two ligands have differing potencies as trophic agents. Our conclusion that CCK stimulate growth via high affinity receptors, however, is not dependent upon the relative affinities of caerulein and CCK-JMV-180 as trophic agents but, rather, on the finding that both ligands do, in fact, stimulate pancreatic growth.

The results reported in this communication add to the growing list of acinar cell processes that can be attributed to CCK interaction with one or the other of the two CCK receptor affinity states. Agonist interaction with high affinity receptors results in an oscillatory rise in cytoplasmic Ca^{2+}(23), digestive enzyme secretion, and, as reported here, in stimulation of acinar cell growth. On the other hand, agonist interaction with low affinity receptors results in a sustained rise in cytoplasmic [Ca^{2+}], inhibition of CCK receptor-mediated digestive enzyme secretion, cyclic AMP generation (24), and protein kinase C-independent CCK receptor phosphorylation (25).

Growth promoting effects of exogenous CCK on pancreatic tumors has been reported (26,27) and the suggestion made that CCK receptor antagonists may be of value in the clinical management of pancreatic cancer. Based upon the results reported in this communication, future studies exploring this issue should focus on agents that preferentially interact with the high affinity state of the acinar cell CCK receptor.

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