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Characterization of SNF 9007, A Novel Cholecystokinin/Opoid Ligand in Mouse Ileum *in Vitro*: Evidence for Involvement of Cholecystokinin$_A$ and Cholecystokinin$_B$ Receptors in Regulation of Ion Transport[1]

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ABSTRACT

The effects of cholecystokinin (CCK) fragments and Asp-Tyr-D-Phe-Gly-Trp-[N-Me]Nle-Asp-Phe-NH_2 1(SNF 9007), a synthetic CCK analog which binds with high affinity to CCK_B and opioid *delta* receptors, were evaluated in isolated sheets of mouse ileum mounted in Ussing flux chambers. Serosal, but not mucosal, administration of cholecystokinin octapeptide-sulfated [CCK_8(s)] and cholecystokinin tetrapeptide (30-33) [CCK_4(30-33)] produced a brief, concentration-related increase in short circuit current (I_{sc}) without changing tissue conductance. Serosal, but not mucosal, SNF 9007 produced a similar concentration-related increase in I_{sc} which was followed by an immediate concentration-related and sustained decrease in I_{sc}; no decrease in I_{sc} was observed for either CCK_8 or CCK_4(30-33). The increase and subsequent decrease in the SNF 9007 I_{sc} response were respectively classified as phase I (i.e., CCK-like) and phase II (opioid-like) activity. CCK_8(s) and SNF 9007 (phase I) were active at low nanomolar concentrations, whereas CCK_4(30-33) was active only at high nanomolar concentrations: the rank order of potencies to increase I_{sc} was CCK_8(s) > SNF 9007 > CCK_4(30-33). Devazepide (L364,718), a selective antagonist of CCK_A receptors, effectively blocked the action of CCK_8(s), but not that of CCK_4(30-33) or SNF 9007 (phase I). In contrast, 3*R*[+]-N-[2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-benzodiazepin-3-yl]-N′-[3-methyl-phenyl]urea (L365,260), a selective CCK_B receptor antagonist, blocked the action of CCK_4(30-33) and SNF 9007 (phase I), and also antagonized CCK_8(s), though to a lesser degree. The phase II response of SNF 9007 was antagonized by N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864), a selective opioid *delta* receptor antagonist; this opioid antagonist did not influence the phase I response. Neither L364,718 or L365,260 influenced the SNF 9007 phase II response. Serosal pretreatment of tissues with tetrodotoxin, or the ganglionic blocker chlorisondamine, significantly blocked the actions of CCK_8(s) and CCK_4(30-33), and both phase I and phase II responses to SNF 9007. Further, these peptides produced no significant response in mucosal preparations of ileum physically stripped of the enteric ganglia and *muscularis externa*. The data suggest that ileal ion-transport can be modulated by the activation of neural CCK_A or CCK_B receptors which are located partly preganglionically and that these receptors can be selectively activated by derivatives or analogs of CCK. CCK_8(s) appears to produce its effects predominately, but not exclusively, at the CCK_A receptor, whereas SNF 9007 and CCK_4(30-33) selectively activate CCK_B receptors in mouse ileum; SNF 9007 (phase I) is several-fold more potent than CCK_4(30-33) in influencing ion transport at the CCK_B receptor. Finally, SNF 9007 has the unusual profile of acting at opioid *delta* receptors to produce a subsequent decrease in I_{sc}. These data demonstrate the importance of both CCK_A and CCK_B, as well as opioid *delta*, receptors in the regulation of ion transport in the same intestinal segment.

CCK is distributed in many areas of the nervous system and fulfills most of the criteria to be designated as a neurotransmitter (Williams, 1982). CCK exerts a variety of biological responses in the central nervous system and the gastrointestinal tract (Itoh *et al.*, 1982; Cooke, 1987). In the gastrointestinal tract, CCK influences smooth muscle contraction (Vizi *et al.*, 1972; Hutchinson and Dockray, 1981; Gaudreau *et al.*, 1987; Lucaites *et al.*, 1991), gastric acid secretion (Patel and Spraggs, 1992) and intestinal ion-transport (Bussjaeger and Johnson, 1973; Matuchansky *et al.*, 1972; Kachur *et al.*, 1991a,b).

Two distinct CCK-specific receptors, CCK_A and CCK_B, have been described in various tissues. The CCK_A receptor, was first characterized in pancreatic acini (Sankaran *et al.*, 1980) and

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ABBREVIATIONS: CCK, cholecystokinin; SNF 9007, Asp-Tyr-D-Phe-Gly-Trp-[N-Me]Nle-Asp-Phe-NH_2; ICI 174,864, N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH; TTX, tetrodotoxin; L365,260, 3R[+]-N-[2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl]-N-[3-methylphenyl]urea; L364,718, devazepide; CCK_8(s), cholecystokinin octapeptide-sulfated; CCK_4(26–29), cholecystokinin tetrapeptide (26–29); CCK_4(30–33), cholecystokinin tetrapeptide (30–33); CHLS, chlorisondamine; I_{sc}, short circuit current; PD, potential difference.

has been recently cloned from rat pancreas (Wank *et al.*, 1992). This CCK receptor type showed a high affinity for CCK_8(s) and lower affinities for CCK_4(30–33) and gastrin. The CCK_B receptor was first described in mouse brain (Saito *et al.*, 1981) and this cloned rat receptor exhibits high affinities for CCK_8(s) as well as CCK_4 and gastrin (Wank *et al.*, 1992). Although both CCK_A and CCK_B receptors have been demonstrated to be present in brain, the CCK_A receptor appeared to be the only CCK receptor present in the small intestine, and the contractile activity of CCK in guinea pig ileal longitudinal muscle (Chang and Lotti, 1986) was shown to be mediated by CCK_A receptors. However, studies by Gaudreau *et al.* (1987) have suggested the presence of CCK_B receptors in the guinea pig ileum because CCK_4 and gastrin were relatively potent contractile agonists. The presence of CCK_B receptors in the small intestine was further supported in a recent study in the guinea pig ileum using selective antagonists (Lucaites *et al.*, 1991).

Through a synthesis effort aimed at the design of highly selective agonists at the types of CCK receptors. SNF 9007 was identified as binding selectively, and with high affinity to CCK_B (IC_{50}, 0.55 nM), rather than CCK_A (IC_{50} >10,000 nM), receptors (Slaninova *et al.*, 1991). Additionally, SNF 9007 showed the unusual property of also binding with good affinity (IC_{50}, 29 nM) to opioid *delta*, but not *mu* or *kappa* (IC_{50}, >1000 nM), receptors (Slaninova *et al.*, 1991). CCK_8(s) did not show selectivity between CCK_A and CCK_B subtypes and did not bind to opioid receptors (Slaninova *et al.*, 1991).

Both CCK and opioid agonists have been previously shown to modulate ion-transport in guinea pig (Kachur *et al.*, 1980, 1991a,b) and mouse intestine (Sheldon *et al.*, 1990). Opioid mechanisms involving *delta* and *mu* receptors elicit net proabsorptive changes in basal Na^+ and Cl^- transport in mouse intestine (Sheldon *et al.*, 1990), whereas prosecretory changes were elicited by CCK in guinea pig ileum (Kachur *et al.*, 1991a,b). The unusual CCK_B/opioid *delta* receptor binding profile of SNF 9007 led us to characterize its effects, and those of reference fragments of CCK, on ion-transport in the mouse ileum.

Methods

Animals. Male ICR mice (35–40 g), allowed free access to food and water, were used in all studies. Animals were maintained in 12-hr light and 12-hr dark cycles.

Chemicals. SNF 9007 was synthesized by methods previously described (Hruby *et al.*, 1990). ICI 174,864 was purchased from Cambridge Research Biochemicals (Atlantic Beach, NY). Theophylline, TTX, atropine sulfate and inorganic salts were from Sigma chemical Co. (St. Louis, MO) and CCK_8(s) and CCK_4(30–33) were purchased from Peninsula Laboratories (Belmont, CA). L365,260 and L364,718 were gifts from Merck Sharp and Dohme Research Laboratories (Rahway, NJ) and CHLS was a donation from Ciba-Geigy (Summit, NJ).

Preparation of ileal tissues. Mice were sacrificed by cervical dislocation and the small intestine was removed. The small intestine was divided into three equal parts and only the distal third (ileum) was used in these studies. The ileum was cut along the mesenteric border, rinsed free of luminal contents and placed in oxygenated Krebs-Ringer buffer. Small segments (2–3 cm) of ileal sheets were mounted into Ussing chambers (0.65 cm^2 exposed surface area) as previously described (Sheldon *et al.*, 1989). In some experiments, ileal segments were physically stripped of their smooth muscle and neural plexuses (Sheldon *et al.*, 1989).

Bioelectric measurements. After mounting the ileal sheets, mucosal and serosal surfaces were bathed independently with 10 ml of Krebs-Ringer solution containing 10 mM of either α-D-glucose (serosal buffer) or mannitol (mucosal buffer) and gassed constantly with 95% O_2/5% CO_2 at 37°C. Ileal tissues were equilibrated under short circuit conditions for 30 min. Agonists and antagonists were added to the medium bathing the serosal surface and the maximum change in I_{sc} and PD were recorded using DVC-1000 voltage/current clamp (World Precision Instruments, New Haven, CT). Concentration-effect curves were constructed noncumulatively, whereby each tissue was exposed to only one concentration of agonist. Antagonists, CHLS or TTX were applied to the serosal medium 6 to 10 min before the addition of agonists. In all experiments, tissue viability was confirmed by assessing the I_{sc} response to theophylline (1 mM) applied to the mucosal and serosal medium. Values from tissues which produced a theophylline response of less than 50 μA/cm^2 were discarded (usually less than 5% of tissues).

Statistics. Concentrations of compounds eliciting 50% of the maximal response (A_{50}), 95% confidence limits and relative potency values were obtained from least-square analysis of the linear portions of the concentration-response curves using a computer program (Tallarida and Murray, 1987). Comparisons between groups were made using analysis of variance followed by Student's *t* test for grouped data. Comparisons of two values from a single tissue were made by Student's *t* test using paired data. Statistical significance in all tests was derived at the 95% or greater confidence level.

Results

Effects of CCK derivatives and analogs. Administration of CCK_8(s) or CCK_4(30–33) to the serosal side of the ileal sheets mounted in Ussing flux chambers produced an immediate, although relatively brief, increase in I_{sc} and PD without altering tissue conductance (fig. 1). SNF 9007 produced a biphasic effect with an initial increase in I_{sc} and PD (phase I) followed by a sustained decrease in basal I_{sc} and PD (phase II). The activities of CCK_8(s), and CCK_4(30–33), and both phase I and phase II responses of SNF 9007 were concentration dependent (fig. 2). The A_{50} (95% confidence limits) values obtained from noncumulative dose-response relationships were 0.56 (0.45–0.70), 63.6 (41.8–96.8), 4.85 (2.1–11.3) and 6.27 (3.7–10.7) nM for CCK_8(s), CCK_4(30–33) and phase I and phase II responses of SNF 9007, respectively. CCK_4(26–29) produced no response even at concentrations as high as 10 μM; this concentration of CCK_4(26–29) also did not antagonize the activities of CCK_8(s) or SNF 9007. All of these peptides were ineffective when added to the mucosal medium.

Antagonist studies. Serosal L364,718 or L365,260 (100 nM) produced no effects on basal I_{sc} in mouse ileum. Pretreatment with L364,718 completely antagonized the effects of CCK_8(s) as shown by the marked rightward shift with decreased maximal effect in the concentration-effect curve for this peptide (fig. 3). Pretreatment with L365,260 produced a small rightward shift in the CCK_8(s) concentration-effect curve. The activity of CCK_4(30–33) was unaffected by pretreatment with L364,718, but was effectively antagonized by L365,260 resulting in a 6-fold rightward shift of the concentration-response curve (fig. 4). Similar to CCK_4(30–33), the phase I response to SNF 9007 was also unaffected by L364,718 and completely antagonized by L365,260 (fig. 5). Serosal pretreatment of tissues with an opioid *delta* receptor selective concentration of ICI 174,864 (0.4 μM; Sheldon *et al.*, 1991), had no effect alone but completely blocked the phase II response to SNF 9007 (fig. 6). ICI 174,864 had no effect on the phase I response to SNF 9007 (fig. 5), and L364,718 and L365,260 had no effect on the phase II response to SNF 9007 (fig. 6). The A_{50} value for CCK_8(s) was greatly

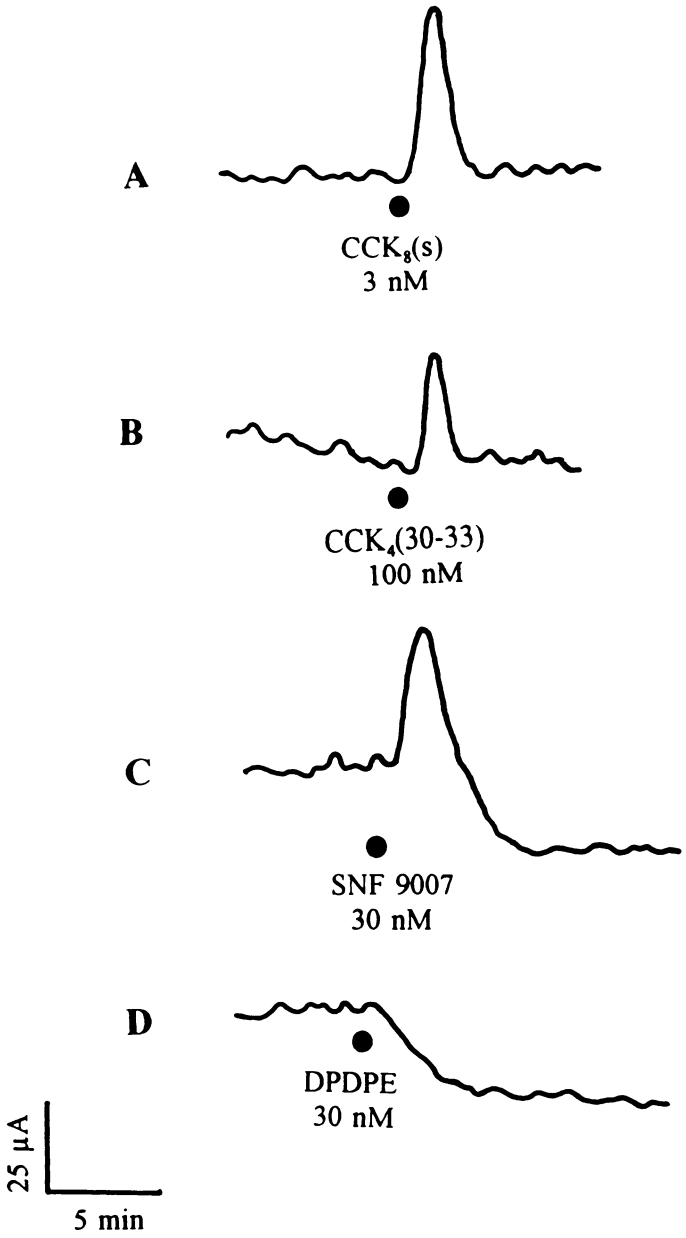

Fig. 1. Representative I_{sc} responses elicited by serosal application of $CCK_8(s)$ (3 nM, A), $CCK_4(30–33)$(100 nM, B), SNF 9007 (30 nM, C) and DPDPE (30 nM, D), in mouse ileum mounted in Ussing flux chambers.

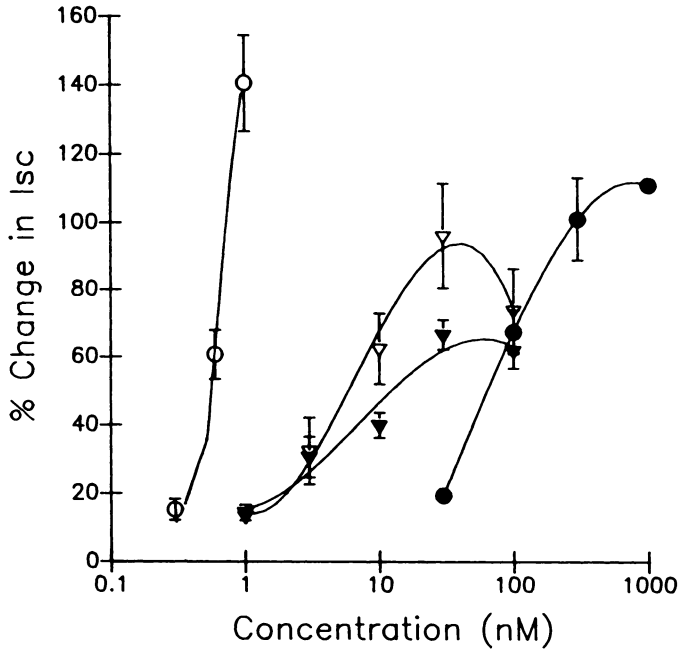

Fig. 2. Noncumulative concentration-effect curves for the elevation of basal I_{sc} by $CCK_8(s)$(○), CCK4(30–33)(●) and SNF 9007 (phase I)(▽), and the reduction of basal I_{sc} by SNF 9007 (phase II)(▼) in intact preparations of mouse ileum. Ordinate, maximal change of I_{sc} (ΔI_{sc}) was normalized to the basal I_{sc} measured before the addition of agonist. Data points are mean ± S.E.M. for responses observed in tissues obtained from 3 to 15 mice.

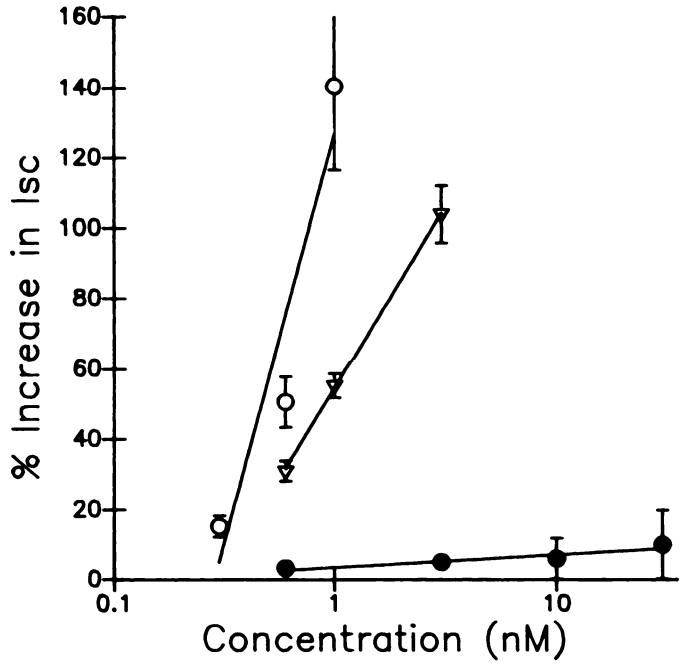

Fig. 3. Noncumulative concentration-effect curves for the response of $CCK_8(s)$ applied alone (○) or after pretreatment with L364,718 (●) or L365,260 (▽) in intact preparations of mouse ileum. Ordinate, maximal increase in I_{sc} (ΔI_{sc}) was normalized to the basal I_{sc} measured before the addition of agonist. Data points are mean ± S.E.M. for responses observed in tissues obtained from 3 to 10 mice.

increased by pretreatment of tissue with L364,718, whereas it was increased only 2.5-fold by L365,260 (table 1). On the other hand, the A_{50} values for $CCK_4(30–33)$ and SNF 9007 (phase I) were increased by L365,260, but L364,718 had no effect. The A_{50} value for phase II response to SNF 9007 was unaffected by both L364,718 and L365,260, whereas it was significantly increased by ICI 174,864.

The I_{sc} effects of $CCK_8(s)$ and $CCK_4(30–33)$, and both phase I and phase II responses to SNF 9007 were significantly blocked by the pretreatment of tissues with CHLS (3 μM) or TTX (0.02 μM) (figs. 7 and 8)(Sheldon *et al.*, 1989). $CCK_8(s)$, $CCK_4(30–33)$ and SNF 9007 responses were significantly blocked in mucosal sheets of ileum that were stripped of smooth muscle and enteric plexuses (fig. 9). The effects of $CCK_8(s)$, $CCK_4(30–33)$ and SNF 9007 were partially blocked by pretreatment of tissues with 1 μM atropine (fig. 10).

Discussion

The results demonstrate that ion transport across mouse ileum can be selectively modulated through either CCK_A or

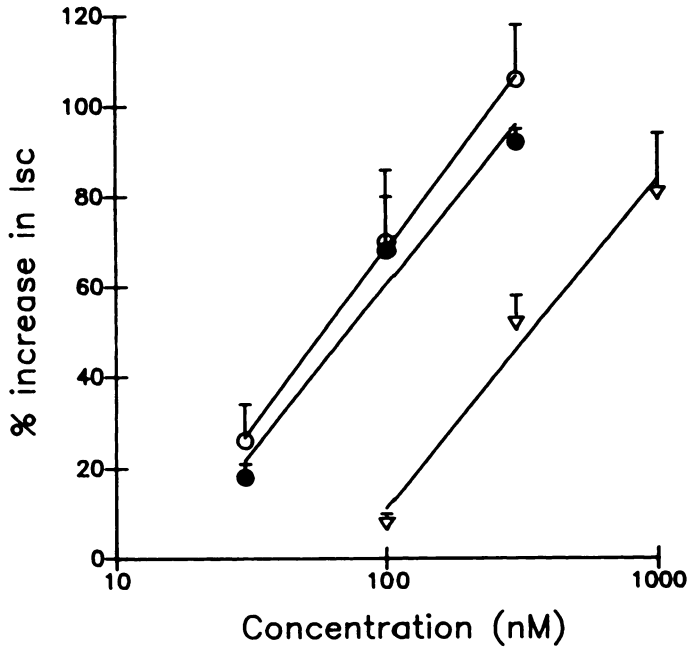

Fig. 4. Noncumulative concentration-effect curves for the response of $CCK_4(30–33)$ applied alone (○) or after pretreatment with L364,718 (●) or L365,260 (▽) in intact preparations of mouse ileum. Ordinate, maximal increase in I_{sc} (ΔI_{sc}) was normalized to the basal I_{sc} measured before the addition of agonist. Data points are mean ± S.E.M. for responses observed in tissues obtained from 3 to 10 mice.

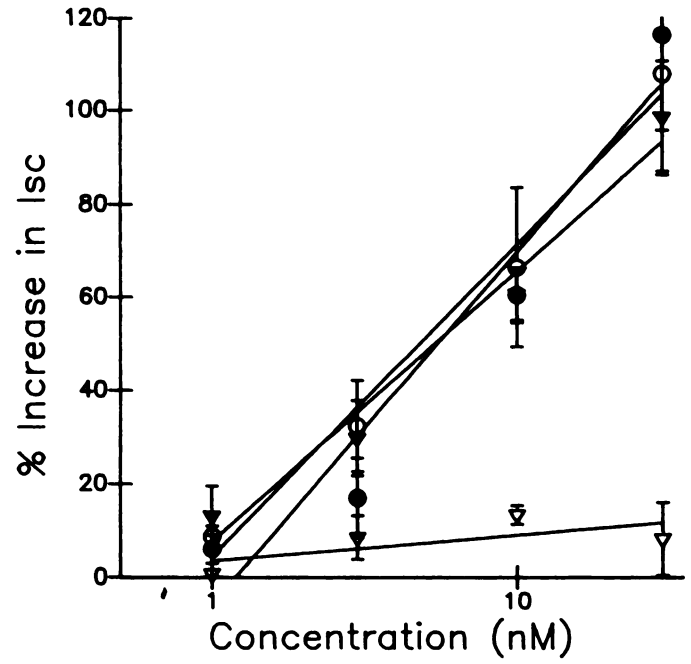

Fig. 5. Noncumulative concentration-effect curves for the phase I response of SNF 9007 applied alone (○) or after pretreatment with L364,718 (●), L365,260 (▽) or ICI 174,864 (▼) in intact preparations of mouse ileum. Ordinate, maximal increase in I_{sc} (ΔI_{sc}) was normalized to the basal I_{sc} measured before the addition of agonist. Data points are mean ± S.E.M. for responses observed in tissues obtained from 3 to 10 mice.

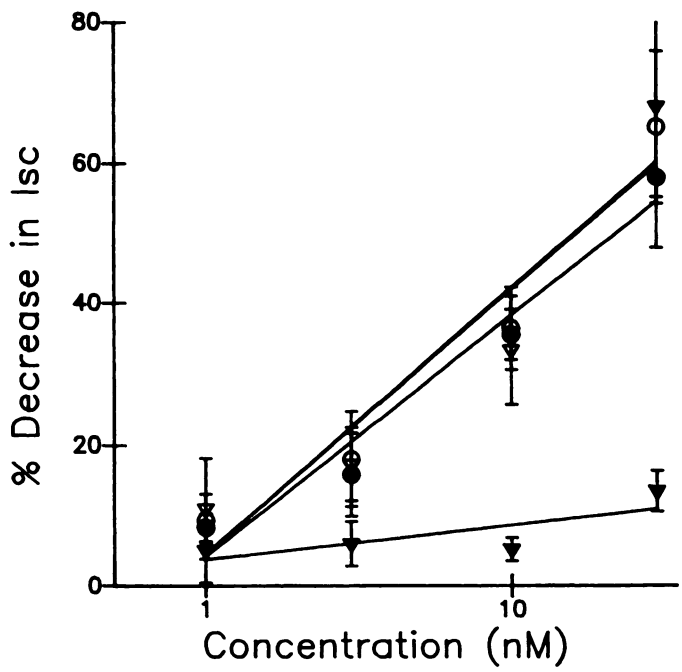

Fig. 6. Noncumulative concentration-effect curves for the phase II response of SNF 9007 applied alone (○) or after pretreatment with L364,718 (●), L365,260 (▽) or ICI174,864 (▼) in intact preparations of mouse ileum. Ordinate, maximal decrease in I_{sc} (ΔI_{sc}) was normalized to the basal I_{sc} measured before the addition of agonist. Data points are mean ± S.E.M. for responses observed in tissues obtained from 3 to 10 mice.

TABLE 1

Effects of different antagonists on A_{50} values of $CCK_8(s)$, $CCK_4(30-33)$ and SNF 9007

Values are mean A_{50} in nanomolar units with 95% confidence limit given in parentheses. Values in brackets represent relative potency in relation to that of $CCK_8(s)$. A_{50} values were calculated from the concentration-response relationship studies for above peptides performed in tissues with or without pretreatment with different antagonists as described under "Methods."

Peptides	None	L364,718	L365,260	ICI 174,864
$CCK_8(s)$	0.56 [1] (0.45–0.70)	>10,000	1.41 (1.23–1.62)	
$CCK_4(30-33)$	63.6 [114] (41.8–96.8)	72.0 (44.8–115.8)	378 (241–592)	
SNF9007				
Phase I	4.85 [9] (2.1–11.3)	6.36 (3.9–10.4)	>10,000	6.54 (4.3–9.9)
Phase II	6.27 (3.7–10.7)	6.71 (3.9–11.5)	4.82 (2.0–11.9)	990 (119–8277)

CCK_B receptors using derivatives and analogs of CCK. The intestinal role of $CCK_8(s)$ has been more thoroughly characterized in smooth muscle contraction in guinea pig ileum (Hender and Rossman, 1968; Vizi *et al.*, 1972; Zetler *et al.*, 1979; Hutchinson and Dockray, 1981; Lucaites *et al.*, 1991) than in modulation of ion transport. Recent studies by Kachur *et al.*, (1991a,b), however, demonstrated that $CCK_8(s)$ induced a transient increase in transepithelial PD and I_{sc} upon application to the serosal side of guinea pig ileum *in vitro*, although $CCK_8(s)$ failed to produce an effect on intestinal electrolyte transport *in vivo* (Hubel, 1972; Barbezal and Grossman, 1971). $CCK_8(ns)$, $CCK_4(30–33)$ and gastrin also produced such prosecretory effects but with very low potencies (Kachur *et al.*, 1991b). Our present studies show that $CCK_8(s)$, $CCK_4(30–33)$ and SNF 9007, an analog of CCK, also produced transient increases in PD and I_{sc} across mouse ileum *in vitro*. The potency of $CCK_8(s)$

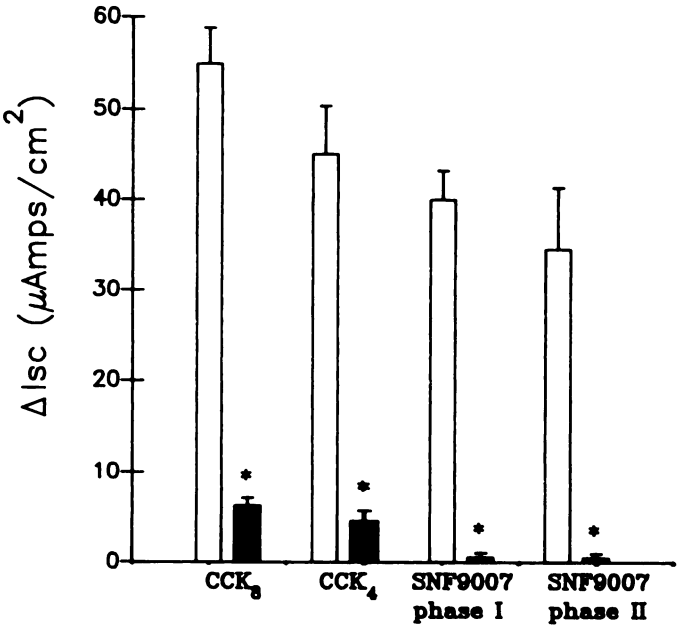

Fig. 7. Effects of $CCK_8(s)$, $CCK_4(30–33)$ and SNF 9007 (phase I, increase in I_{sc} and phase II, decrease in I_{sc}) on I_{sc} in control (open bars) or TTX-treated (20 nM, filled bars) intact preparations of mouse ileum. Ordinate, maximal change in I_{sc} (ΔI_{sc}) is presented as $\mu A/cm^2$. Values are mean ± S.E.M. for responses observed in tissues obtained from three to ten mice. *Values that are significantly different ($P < .05$) from corresponding control values.

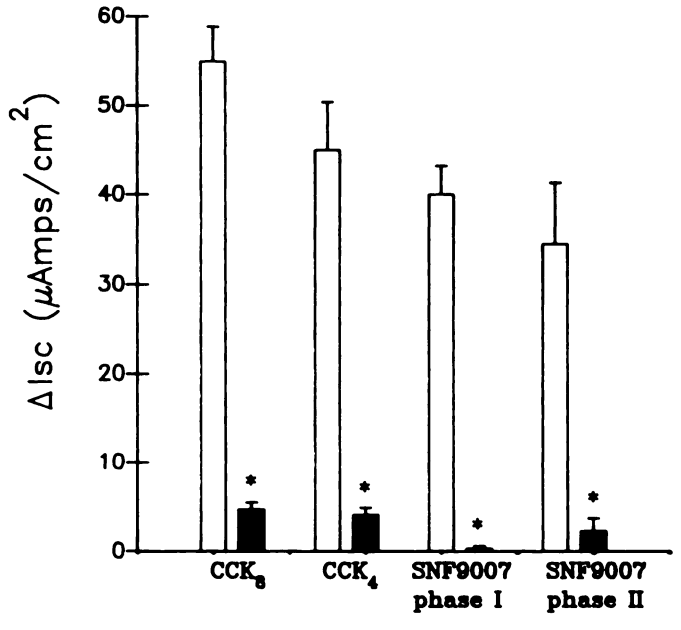

Fig. 8. Effects of $CCK_8(s)$, $CCK_4(30–33)$ and SNF 9007 on I_{sc} in control (open bars) or CHLS-treated (3 μM, filled bars) intact preparations of mouse ileum. Ordinate, maximal change in I_{sc} (ΔI_{sc}) is presented as $\mu A/cm^2$. Values are mean ± S.E.M. for responses observed in tissues obtained from 3 to 10 mice. *Values that are significantly different ($P < .05$) from corresponding control values.

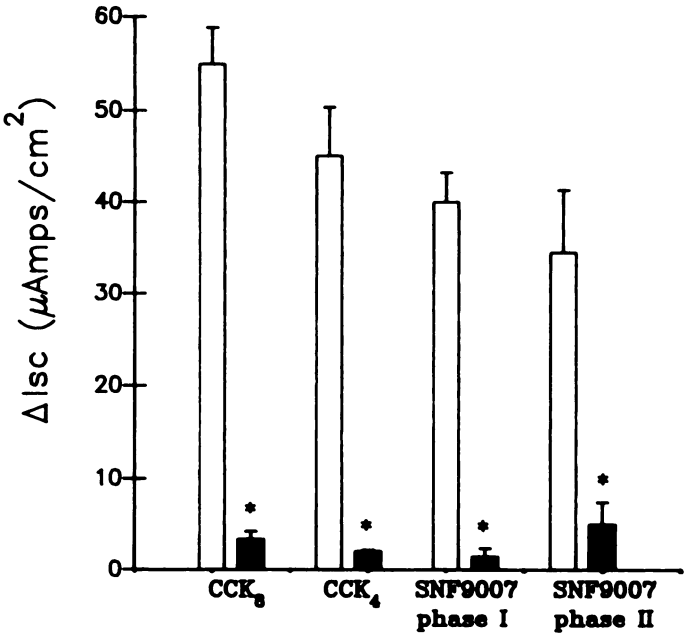

Fig. 9. Effects of $CCK_8(s)$, $CCK_4(30–33)$ and SNF 9007 on I_{sc} in intact (open bars) or mucosal (filled bars) preparations of mouse ileum. Ordinate, maximal change in I_{sc} (ΔI_{sc}) is presented as $\mu A/cm^2$. Values are mean ± S.E.M. for responses observed in tissues obtained from 3 to 10 mice. *Values that are significantly different ($P < .05$) from corresponding control values.

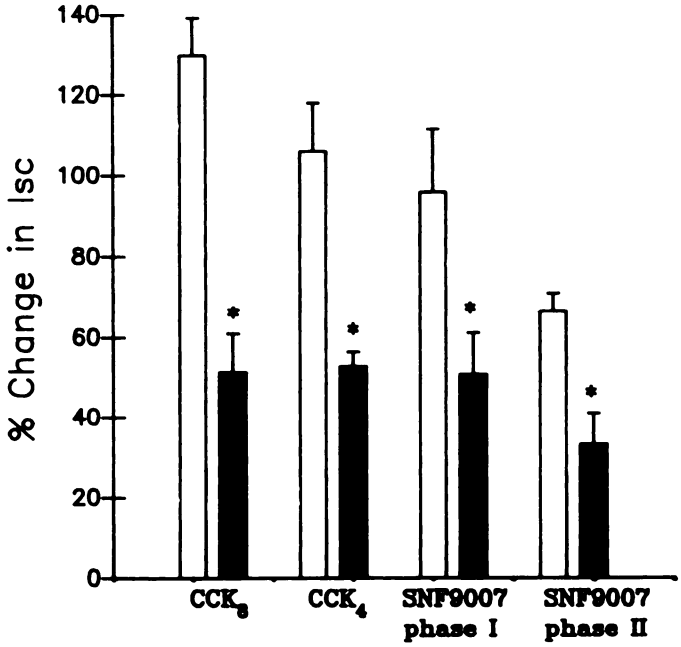

Fig. 10. Effects of $CCK_8(s)$, $CCK_4(30–33)$ and SNF 9007 on I_{sc} in control (open bars) or atropine-treated (1 μM, filled bars) intact preparations of mouse ileum. Ordinate, maximal change in I_{sc} (ΔI_{sc}) is presented as $\mu A/cm^2$. Values are mean ± S.E.M. for responses observed in tissues obtained from 3 to 10 mice. *Values that are significantly different ($P < .05$) from corresponding control values.

was virtually identical to that reported by Kachur *et al.* (1991a) for this compound in guinea pig ileum. The potency of $CCK_4(30–33)$, however, was found to be greater in the present experiments than that reported earlier (Kachur *et al.*, 1991b). One possible interpretation of this discrepancy in potency may be due to the slightly different experimental procedure of the present studies, and those of Kachur *et al.* (1991b) who used a cumulative dosing technique. In this regard, Lucaites *et al.* (1991) have demonstrated that CCK derivatives produced tachyphylaxis in the guinea pig ileal smooth muscle contraction studies. A similar discrepancy was apparent in the potencies of CCK derivatives in guinea pig ileal smooth muscle contraction.

Gaudreau *et al.* (1987), in a cumulative dose-response study, observed that CCK_8(s) was 1000-fold more potent than CCK_8(ns), whereas in a noncumulative dose-response study Lucaites *et al.* (1991) demonstrated that CCK_8(s) was only 30-fold more potent than CCK_8(ns).

L364,718 and L365,250, selective and competitive antagonists of CCK_A and CCK_B receptors (Lotti and Chang, 1989; Iversen *et al.*, 1991; Mercer and Lawrence, 1992), respectively, were used to characterize the receptor subtypes involved in the activities of CCK peptides. The effect of CCK_8(s) was effectively antagonized by L364,718, a selective antagonist of CCK_A receptors. L365,260, a selective antagonist of CCK_B receptors, produced only a slight effect on the potency of CCK_8(s). This is consistent with the previous reports that CCK_8(s) stimulates ion transport by the activation of CCK_A as well as CCK_B receptors in guinea pig ileum (Kachur *et al.*, 1992). In contrast to the activity of CCK_8(s), the activity of CCK_4(30–33) was effectively blocked by L365,260, but was unaffected by L364,718, suggesting that CCK_4(30–33) increased mouse ileal I_{sc} by selective action at CCK_B receptors. Such selective activation of intestinal CCK_A and CCK_B receptors was previously demonstrated by Lucaites *et al.* (1991) using contractile responses of longitudinal smooth muscle from guinea pig ileum. It is also interesting to note that CCK_4(26–29) did not show either agonist or antagonist properties in this preparation, suggesting that this N-terminal fragment of CCK_8(s) is not sufficient to bind to the CCK_A receptor.

We have previously demonstrated that SNF 9007 binds with high affinity to CCK_B receptors in the mouse brain, whereas it showed very low affinity for CCK_A receptors (Slaninova *et al.*, 1991). Interestingly, SNF 9007 specifically binds also to opioid *delta* receptors (Slaninova *et al.*, 1991). In support of the receptor binding studies, the response of SNF 9007 on I_{sc} of mouse ileum could be separated into two distinct phases. After serosal application, SNF 9007 produced a transient increase (3–6 min) in I_{sc} (phase I), a response identical to that of CCK_8(s), which was immediately followed by a sustained decrease in I_{sc} (phase II), a response similar to that of opioid agonists. The phase I response to SNF 9007 was blocked by L365,260 but not by L364,718, suggesting selective activation of CCK_B receptors in the mouse ileum by SNF 9007. CCK_4(30–33) and SNF 9007 are, therefore, selective agonists at CCK_B receptors in this system, and SNF 9007 was calculated to be approximately 13-fold more potent than CCK_4(30–33) in increasing I_{sc}. In our assay system, K_b values calculated for L365,718 are 66 and 20 nM against CCK_8(s) and CCK_4, respectively. CCK_4 is less affected in our assay system by L365,260 in comparison to the reports of Lucaites *et al.* (1991) regarding CCK-mediated contraction of guinea pig ileal smooth muscle. The reason for this discrepancy is not clear at present. However, it can possibly be attributed to the differences in animal species and assay models used. Lucaites *et al.* (1991) have used guinea pig ileal longitudinal muscle with attached myenteric plexuses, whereas our assay used mouse ileal tissues with all myenteric, submucosal and mucosal plexuses intact. As described below the differences in CCK_4 mediated activities in these two different assay systems were also observed in their mechanism of action. Lucaites *et al.* (1991) have reported a complete attenuation of CCK_4-induced contraction by atropine, whereas in our study atropine blocked only a portion of CCK_4-induced changes in ileal I_{sc}. The accurate values of K_b for L364,260 cannot be calculated from the data available.

The antagonism of the phase II response to SNF 9007 by receptor selective doses of ICI 174,864, an opioid *delta* antagonist (Sheldon *et al.*, 1991), together with the demonstrated affinity of SNF 9007 for opioid *delta*, but not *mu* or *kappa*, receptors suggests that the phase II response is mediated by the activation of opioid *delta* receptor in mouse ileum. The role of opioid *delta* receptors in antisecretory effects has previously been demonstrated in mouse jejunum (Sheldon *et al.*, 1991). Although both *delta* and *mu* opioid receptors have been shown to be involved in the neural regulation of mucosal ion transport in mouse jejunum, DPDPE, a selective opioid *delta* agonist, was shown to be 41- and 476-fold more potent than the *mu* receptor agonists, [D-Ala^2, NMPhe^4, Gly-ol]enkephalin or morphine (Sheldon *et al.*, 1990). The potency of SNF 9007 in mouse ileum is very close to that of DPDPE in mouse jejunum (Sheldon *et al.*, 1990).

The significance of dual CCK/opioid effects of SNF 9007 is not clear at this time. CCK_8(s) has been shown to antagonize opioid-induced analgesia (Itoh *et al.*, 1982; Faris *et al.*, 1983; Han *et al.*, 1985; Watkins *et al.*, 1985; Wiesenfeld-Hallin and Duranti, 1987), whereas CCK antagonists enhance morphine analgesia (Watkins *et al.*, 1984, 1985; Katsuura and Itoh, 1982; Dourish *et al.*, 1990; Lavigne *et al.*, 1992) and may prevent or reverse morphine tolerance (Watkins *et al.*, 1984; Tang *et al.*, 1984; Panerai *et al.*, 1987; Dourish *et al.*, 1990; Xu *et al.*, 1992). The mechanism of action of CCK in the modulation of compounds acting at opioid receptors is not clear, but our previous work has demonstrated that opioid *delta*, *mu* or *kappa* opioid receptors have no affinity for CCK_8(s)(Slaninova *et al.*, 1991). Further studies are underway to characterize different CCK analogs which may provide insights to possible similarities in the ligand structure requirements for CCK_B and opioid *delta* receptors.

The results of this study suggest that the effects of CCK derivatives and SNF 9007 (*i.e.*, activation of CCK_A and CCK_B receptors) were mediated by the stimulation of intrinsic neurons in the mouse ileum and not by direct action at epithelial cells. First, the activities of all these peptides were abolished by the pretreatment of tissues with TTX, a neural toxin, which is consistent with similar observations of Kachur *et al.* (1991a) with respect to CCK_8(s). Second, none of the CCK derivatives and SNF 9007 produced an effect on I_{sc} in mucosal tissues of mouse ileum in which myenteric and submucosal plexuses are physically removed by dissection. Such a mucosal preparation failed to respond to DMPP, a ganglionic stimulating agent (Sheldon *et al.*, 1989), suggesting that this preparation was virtually aganglionic. The observation of a lack of activity of the CCK-like compounds in the stripped, mucosal preparation is consistent with reports which show a lack of CCK receptors in columnar or goblet cells of intestinal epithelium (Rosselin, 1981). Third, the effects of CCK_8(s), CCK_4(30–33) and SNF 9007 on I_{sc} in mouse ileum were blocked by CHLS, a ganglionic blocker, suggesting that these CCK peptides have a predominately preganglionic site of action. Finally, the present studies also demonstrate that the effects of CCK peptides on I_{sc}, in part, are mediated by the release of acetylcholine. The effects of CCK_8(s), CCK_4(30–33) and SNF 9007 were partially blocked by the pretreatment of tissue with 1 μM atropine, a muscarinic antagonist. This is in agreement with previous reports of the effect of atropine on mucosal ion-transport (Kachur *et al.*, 1991a) and smooth muscle contraction (Lucaites *et al.*, 1991) in guinea pig ileum. Thus, CCK appears to involve two different

neural pathways in its effects on ileal I_{sc}. Similar to the I_{sc} responsiveness to DPDPE (Sheldon *et al.*, 1990) in mouse jejunum, the phase II response to SNF 9007 was also blocked by TTX and CHLS, suggesting a neural site of action of these opioid agonists.

In summary, these studies demonstrate that intestinal ion-transport can be modulated by the activation of either CCK_A or CCK_B receptors, as well as opioid *delta* receptors, in the same intestinal segment, and that these receptors can be selectively activated by different CCK derivatives or analogs.

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