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Effects of Cholecystokinin Octapeptide on Neuronal Activities in the Rat Nucleus Tractus Solitarius

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Cholecystokinin (CCK) is a gastrointestinal hormone which plays an important role in satiety and gastric motility. It is also widely distributed throughout the central nervous system, where it appears to be involved in the central control of anxiety, feeding behavior and nociception. Two distinct CCK receptor types, CCK_A and CCK_B, have been found in the brain. Both CCK receptors coexist in the rat nucleus tractus solitarius (NTS), which is the primary center for the coordination of peripheral and central activities related to gastrointestinal, cardiovascular and respiratory functions.

In order to study ionic actions of CCK on each type of receptor, we investigated the effects of CCK-8S on neurons located in the NTS of the rat using whole-cell patch-clamp recordings in brainstem slices. Application of CCK-8S, under current clamp, produced a membrane depolarization accompanied by action potential firing. This CCK-evoked excitation was dose-dependent (10 nM ~ 10 μM) and observed in more than 60% of NTS neurons. Under voltage clamp conditions, CCK-8S induced an inward current with a notably increased spontaneous excitatory synaptic activity. However, CCK-8S did not significantly change the amplitude of pharmacologically isolated and evoked EPSP(C)s. Using selective CCK_A and CCK_B receptor antagonists, we observed two different effects of CCK-8S, which suggest CCK_A receptor-mediated inhibitory and CCK_B receptor-mediated excitatory effects in the NTS. These results may help to explain the ability of CCK to modulate gastrointestinal and other reflex systems in the NTS.

Key Words: Cholecystokinin, CCK receptor, Electrophysiology, Synaptic transmission, Nucleus tractus solitarius

INTRODUCTION

The cholecystokinin (CCK) family of peptides was originally isolated from the mammalian gastrointestinal tract and subsequently discovered in the brain (see review; Benedetti, 1997). Because CCK is found in peripheral organs and in the central nervous system, its functions range from gastrointestinal physiology to neurophysiology. For example, CCK is important for gall bladder contraction, exocrine pancreatic secretion, regulation of esophageal sphincter pressure, and bowel motility. On the other hand, CCK has been reported to have an important role in the brain, being implicated in anxiety, nociception, dopamine regulation, and memory.

CCK has been also known to regulate satiety, food intake, and gastric motility. It is partly by direct effect on the gastrointestinal tract and partly by its action in the central nervous system such as brainstem, pons, and hypothalamus (Silver & Morley, 1991). Anatomical and functional evidence suggests that the brainstem solitary complex, which comprises the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus (DMNV), plays an important role as a relay center for the integration of peripheral and central signals related to digestive and cardiovascular functions. This brainstem region receives an extensive innervation from the primary sensory afferents of the vagus nerves. Vagotomy or lesion of

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the NTS blocks the satiety effects of exogenous CCK (Crawley & Schwaber, 1984; Shillabeer & Davison, 1985). Response to systemic injection of CCK has been described as involving NTS neurons responding to gastric distension (Raybould et al, 1988). Furthermore, CCK acting on receptors in the gut may also stimulate the vagal afferents to the brain, with this signal being relayed in the NTS.

In the NTS, abundant CCK immunoreactivity has been reported (Howes et al, 1989). Moreover, both CCK_A and CCK_B receptors have been demonstrated to exist at this brain site, unlike most other central structures that possess only CCK_B receptors. However, little is known about the cellular and membrane mechanisms that are controlled by CCK receptors in NTS neurons. We, therefore, examined the actions of the sulfated form of CCK octapeptide (CCK-8S) which interacts with both binding sites and the effects of highly selective CCK_A and CCK_B receptor antagonists on neurons located in the rat NTS using the whole-cell clamp recordings in brainstem slices.

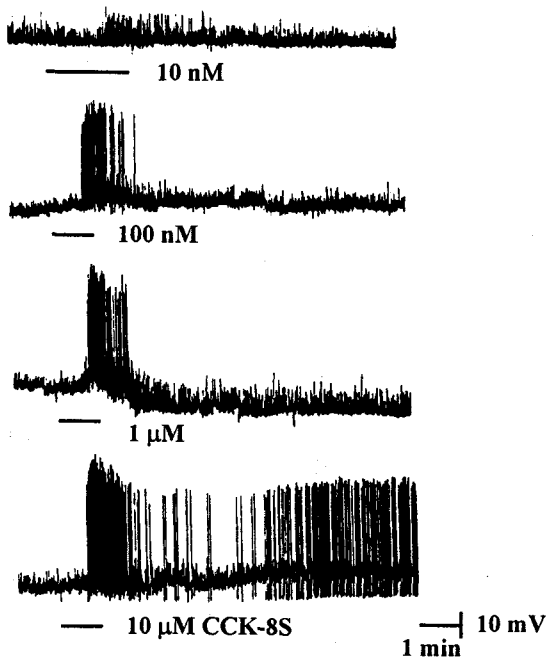

Fig. 1. CCK-8S shows excitatory effects in a dose dependent manner. At holding potential (V_{hold})= −53 mV, CCK-8S starts showing its excitatory effects, increasing synaptic activity at 10 nM. At 100 nM CCK-8S, depolarization is sufficient to fire action potentials. This cumulative dose response for CCK-8S was obtained from one tested cell. The application of drugs is indicated by bars under the traces in this and following figures.

METHODS

Slice preparation and whole-cell recordings were done as previously described (Brooks et al, 1992; Rhim et al, 1993). Briefly, 300 μM transverse slices were prepared from the brainstem of Sprague-Dawley rats (2−4 weeks old) and placed in a holding chamber filled with 32°C artificial cerebrospinal fluid (ACSF) which contained (in mM): NaCl, 126; $NaHCO_3$, 26.2; NaH_2PO_4, 1; KCl, 3; $MgSO_4$, 1.5; $CaCl_2$, 2.5; glucose, 10; gassed with 95% O_2, 5% CO_2. After 1 hr recovery, one slice was transferred to the recording chamber and continuously perfused with ACSF at room temperature.

Whole-cell patch electrodes with resistance of 2−5 MΩ were filled with a solution containing (in mM): potassium gluconate, 145; $MgCl_2$, 2; HEPES, 5;

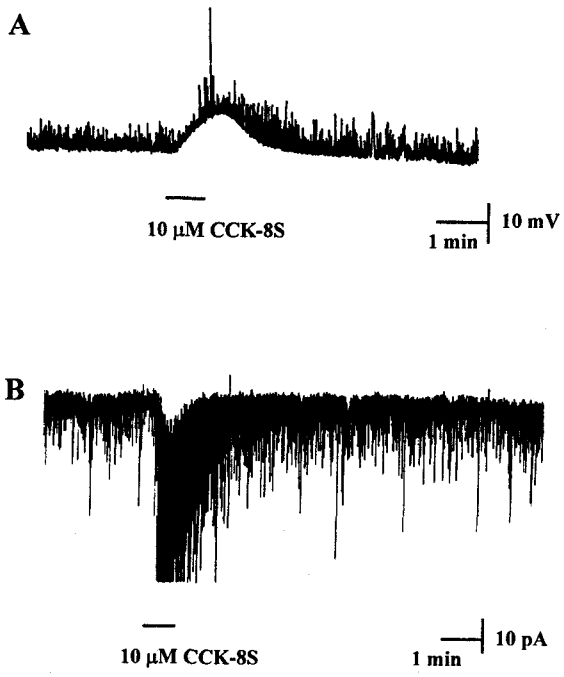

Fig. 2. CCK-8S produces depolarization and an inward current in more than 60% of the NTS neurons. (A) Under current clamp conditions, CCK-8S produces depolarization from V_{hold} of −80 mV. The mean change of membrane potential observed in 18 from 29 tested cells cells with 10 μM CCK-8S was 11.4±6.2 mV. (B) Under voltage-clamp condition, 10 μM CCK-8S induces an inward current. At V_{hold}= −70 mV, the mean peak current was 8.9±3.7 pA (n=12). Increasing levels of spontaneous synaptic activity was noted to the CCK-8S responses and was truncated due to enormous change of activities in this trace.

EGTA, 1.1; $CaCl_2$, 0.1; K_2ATP, 5. Most recordings were obtained from cells with a stable resting membrane potential and with overshooting action potentials. However, in spontaneously firing neurons, membrane potential (V_m) was adjusted by direct current injection. Electrophysiological experiments were performed at room temperature with the use of EPC-9 amplifier and Pulse/Pulsefit software (HEKA, Germany). For current-clamp data, the signal was recorded on a chart record (Gould, Cleveland, OH) and videocassette recorder tape with the use of a VR-10 Instrutech digital data recorder (Elmont, NY). Synaptic activity was evoked by positioning a bipolar tungsten stimulating electrode in the region of the ipsilateral tractus solitarius (TS), which contains the projections of autonomic peripheral afferents. In experiments examining evoked activity, the postsynaptic potential (PSP) or postsynaptic current (PSC) amplitude was averaged over 5 consecutive values obtained prior to drug application and compared to the averaged value obtained at the apparent peak of the response. The excitatory PSP/PSC was pharmacologically separated by using bicuculline (10 μM) to block the evoked inhibitory PSP/PSC. All data were expressed as the mean$\pm$S.E.M. A single experiment was performed per slice and all drugs were applied in the perfusate.

RESULTS

The effects of CCK on neuronal activities in the NTS were initially investigated under current clamp conditions. The major response of NTS neurons to CCK-8S, the most predominant form of CCK peptide family, was a depolarization of the membrane potential (Fig. 1). The excitatory effect of CCK-8S on the membrane potential was dose-dependent between 10 nM and 10 μM and accompanied by action potential firing from 100 nM. The depolarization produced by 10 μM CCK-8S was observed in 62% of NTS neurons (n=18/29, Fig. 2A) and not blocked by tetrodotoxin treatment (TTX, 0.5$-$1 μM; data not shown). From a holding potential (V_{hold}) of $-$80 mV, the mean change of membrane potential produced by 10 μM CCK-8S was 11.4$\pm$6.2 mV (n=18). In some

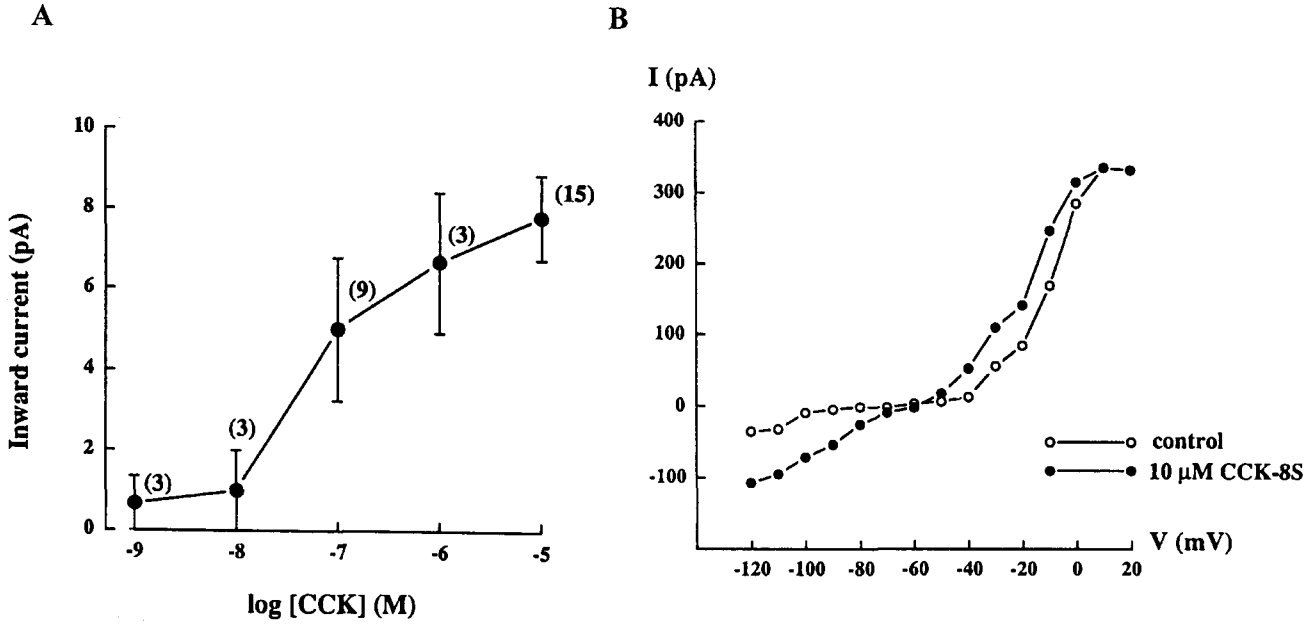

Fig. 3. The dose dependence and steady state current-voltage (I$-$V) relationship of CCK-8S-induced inward current. (A) Cumulative dose response for CCK-8S under voltage clamp conditions at $V_{hold}=-70$ mV. A significant inward current was observed at 100 nM CCK-8S or greater. Numbers beside data point indicate the number of cells treated at each concentration. The cells tested at a low concentration (1$-$10 nM) were subsequently tested at high concentrations to check the responsiveness of cells. (B) The steady state current-voltage (I$-$V) for an NTS neuron. CCK-8S induces an inward current reversed at -55.3 ± 2.0 mV (n=3) in the presence of 0.5 μM TTX. Cells were held at -70 mV and given 1 sec voltage steps to the indicated potentials. Steady state currents were evaluated at the end of the voltage step.

neurons (n=11/29), CCK-8S (10 μM) was without effect. Under voltage-clamp condition, 10 μM CCK-8S induced an inward current at V_{hold}= −70 mV (Fig. 2B). The mean peak current induced by 10 μM CCK-8S was 8.9±3.7 pA (n=12). This CCK-8S-induced inward current was also dose-dependent (Fig. 3A). The steady-state current-voltage (I−V) relationship in control $[K^+]_o$ (3 mM) is shown in Fig. 3B. The CCK-8S induced inward current reversed at −55.3 ±2.0 mV (n=3) in the presence of 0.5 μM TTX.

As shown in Fig. 2B, increasing levels of spontaneous synaptic activity was also noticed in the CCK-8S responses from 10 nM. In order to examine potential presynaptic effects of CCK receptor activation, current and voltage clamp recordings were carried out in combination with stimulation of the ipsilateral tractus solitarius (TS), which contains the projections of autonomic peripheral afferents. In most cells, the effects of CCK-8S (at the concentration of 100 nM) were examined on the amplitude of the evoked excitatory postsynaptic potentials (EPSPs, Fig. 4A) or postsynaptic currents (EPSCs, Fig. 4B & 4C) in the presence of bicuculline (10 μM). These evoked EPSP(C)s are due to the release and action of glutamate. Application of CCK-8S produced insignificant changes in the amplitude of the EPSP(C) (<10%, n=3 and n=5 for EPSPs and EPSCs, respectively). However, CCK-8S produced a notable increase in spontaneous synaptic activity while it did not significantly alter the amplitude of the evoked EPSCs in the same cell (Fig. 4C), suggesting different modulating mechanisms of CCK on synaptic transmission.

To determine the types of CCK receptor mediating CCK-8S-induced excitatory effects in the NTS neurons, selective CCK_A and CCK_B receptor antagonists were used. Fig. 5 illustrates the effects of pretreatment and co-treatment of CCK receptor antagonists on the CCK-8S action. Pretreatment of the CCK_A receptor antagonist L-364,718 (10 μM for 10 min) for complete blockade of the activation of CCK_A receptor did not block depolarization or inward current induced by CCK-8S (1 μM, Fig. 5A), suggesting that the excitatory effect of CCK-8S occurs via an activation of CCK_B receptor. The excitatory effect of CCK-8S via CCK_B receptor activation was also confirmed using a selective CCK_B receptor antagonist, LY288513. In the presence of LY288513 (1 μM), CCK-8S-mediated excitatory effects were partially blocked and completely recovered after antagonist washout (Fig. 5C). However, pretreatment of the CCK_B receptor antagonist L-365,260 (10 μM) transformed the excitatory CCK action to an inhibitory one; CCK-8S inhibited spontaneous synaptic transmission (Fig. 5B). These results suggest that CCK_A and CCK_B receptors are implicated in CCK-8S-induced inhibitory and excitatory effects, respectively in the NTS, although the inhibitory effect was rarely observed in the absence of CCK_B receptor antagonist.

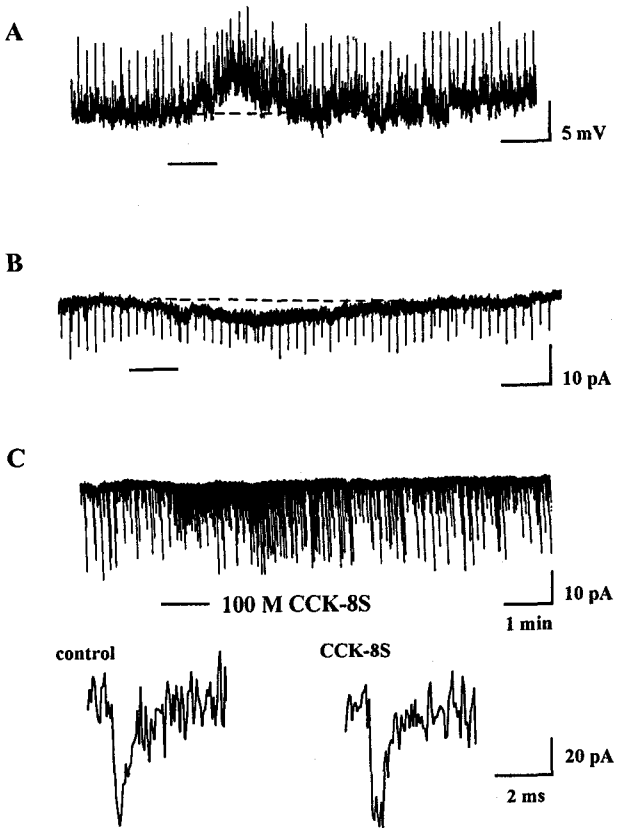

Fig. 4. CCK-8S also mediates excitatory action through an increase in spontaneous synaptic transmission. (A, B) The effects of 100 nM CCK-8S were examined on the amplitude of the evoked excitatory postsynaptic potential (EPSP, A) or postsynaptic current (EPSC, B) by the stimulation of the tractus solitarius (TS) in the presence of bicuculline (10 μM). These evoked EPSP(C)s are due to the release and action of glutamate. CCK-8S produces insignificant changes in the amplitude of the EPSP(C) (<10%, n=3 and n=5 for A and B, respectively). Both cells were hold at V=−70 mV. (C) CCK-8S produces an increase in spontaneous synaptic activities in a cell showing insignificant changes on the evoked EPSCs at V_{hold}=−80 mV. Lower traces are high resolution, averaged 5 evoked EPSPs from the control and peak of the response.

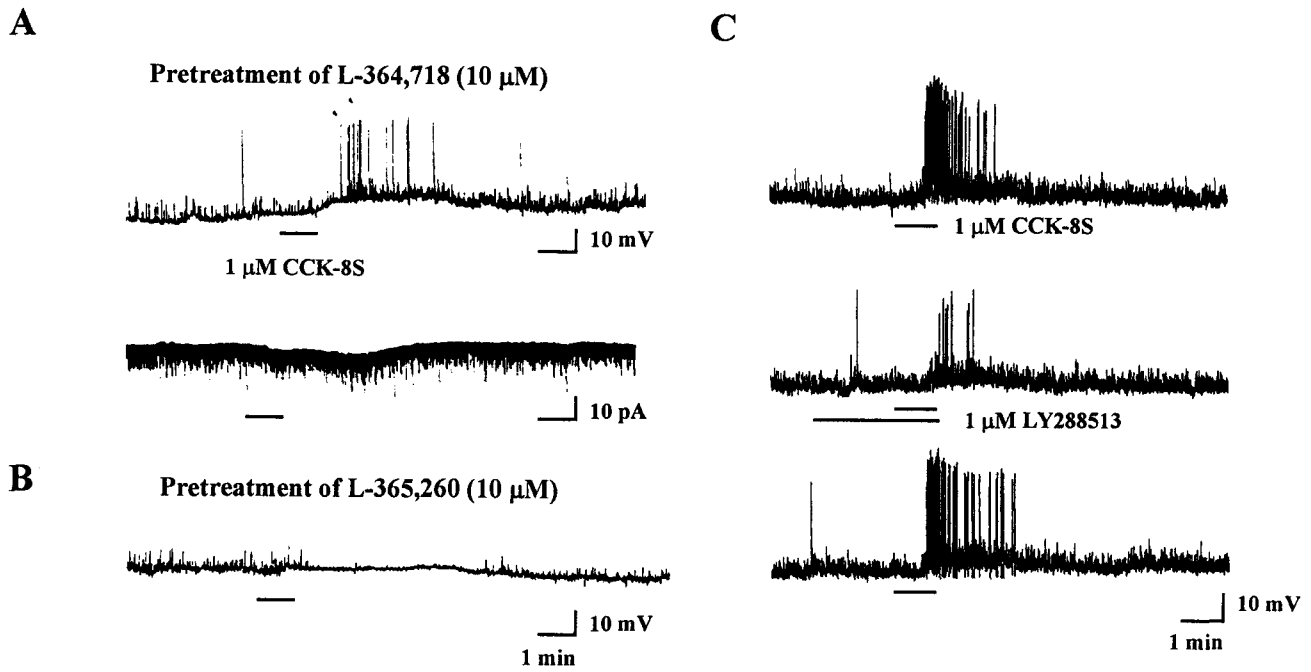

Fig. 5. Selective CCK_A and CCK_B receptor antagonist differentiate CCK-8S effects in the NTS. After 10-min pretreatment of selective CCK_A and CCK_B receptor antagonist in A and B, respectively, (A) Depolarization (upper trace) or inward current (lower trace) induced by 1 μM CCK-8S are still observed by pretreatment of the CCK_A receptor antagonist L-364,718 (10 μM). (B) By pretreatment of the CCK_B receptor antagonist L-365,260 (10 μM), CCK-8S inhibits spontaneous synaptic transmission. (C) In the presence of another selective CCK_B receptor antagonist, LY288513 (1 μM), 1 μM CCK-8S-mediated excitatory effects were partially blocked and completely recovered after antagonist washout.

DISCUSSION

In the present investigation, we examined the effects of CCK-8S on neurons located in the NTS at the cellular level and identified two major effects. The majority of NTS neurons tested were excited by CCK-8S and this excitation was associated with a membrane depolarization and firing action potentials. The second effect noted was an increase in spontaneous synaptic activities with no significant change in evoked synaptic transmission. This CCK-mediated postsynaptic effect, a membrane depolarization, was consistent with previous reports in a variety of CNS tissues (Boden & Hill, 1988a, b; Boden et al, 1991; Liu et al, 1994). However, modulation of spontaneous synaptic activities by CCK-8S has not been described elsewhere in the CNS of mammals using electrophysiological recordings.

Although a depolarizing action of CCK has been observed in a variety of CNS tissues, the effects of this peptide on target ion channels are not uniform. For example, even via an activation of the same type of CCK receptor (A-type), CCK-8S excited substantia nigra neurons by increasing a cationic conductance while it depolarized thalamus reticular neurons by suppressing K^+ conductance (Wu & Wang, 1994; Cox et al, 1995). In hippocampal neurons, it was also reported that CCK depolarized neurons through the inhibition of voltage-dependent K^+ current or of a resting K^+ conductance via activation of CCK_B receptors (Buckett & Saint, 1989; Miller et al, 1997). However, in most cases, the excitatory effect of CCK-8S persisted in the presence of tetrodotoxin and was not blocked either by reduction of extracellular Ca^{2+} or by addition of high Mg^{2+}, suggesting the involvement of postsynaptic receptors (Boden et al, 1991; Branchereau et al, 1992, Miller et al, 1997). In the present experiments, CCK increased the membrane conductance, and the reversal potential (-55.3 ± 2.0 mV) occurred at a potential more positive than the theoretical K^+ equilibrium potential E_K. This suggests an increase in permeability to cations such as Na^+ and Ca^{2+}. However, as we neither tested NTS cells in Na^+-free medium nor blocked Ca^{2+} channels,

the question of whether Na^+, Ca^{2+} or both channels are involved in the excitatory action of CCK on NTS neurons remains to be addressed. Similar value of the reversal potential induced by CCK-8S was also reported in periaqueductal gray (PAG) neurons (Liu et al, 1994), but this effect was mediated via an activation of CCK_A receptor. On the other hand, the specificity of CCK excitatory action in NTS neurons was strongly supported by using selective CCK_A and CCK_B receptor antagonists. CCK-8S-induced excitatory effects in NTS neurons were blocked by selective B-type antagonist, LY288513, but not by selective A-type antagonist L-364,718, suggesting the excitatory effect of CCK in the NTS neurons occurs via an activation of CCK_B receptor.

In contrast to preponderant excitatory effect of CCK on membrane potentials, the effects of CCK on synaptic transmission are not consistent. In electrophysiological studies, excitation or inhibition of evoked synaptic transmission by CCK was reported in hippocampal neurons (Jaffe et al, 1987; MacVicar et al, 1987). An insignificant change on evoked excitatory synaptic transmission by CCK-8S was also noticed in the present study. In biochemical studies, no effect or moderate increase of electrical- or KCl-evoked neurotransmitter release by CCK was reported using slices and synaptosomes (Barnes et al, 1991; Migaud et al, 1994; Breukel et al, 1997). On the other hand, CCK was found to increase basal release of endogenous neurotransmitter in hypothalamus, striatum and hippocampal neurons (Barnes et al, 1991; Migaud et al, 1994; Breukel et al, 1997). This CCK-mediated increase in basal neurotransmitter release was dose-dependent and blocked by the CCK_B receptor antagonist L365,260. The present results on spontaneous synaptic activity are well consistent with these biochemical studies, suggesting important roles of CCK in regulating basal release of neurotransmitter. However, more investigations are required to elucidate where (pre- or postsynaptic locus) and which types of receptor are involved in CCK-8S-mediated regulation of spontaneous synaptic transmission in NTS neurons.

Both CCK_A and CCK_B receptors coexist in the NTS of rats and humans (Howes et al, 1989, Mailleux & Vanderhaeghen, 1990), and both receptors could be involved, at this level, in the CCK-induced satiety, although peripheral CCK_A receptors seem to play a major role in this effect (Crawley et al, 1991). The possible role of CCK_B receptor controlling vegetative reactions in the brainstem also comes from the anxiolytic effects of CCK_B antagonist administered at very low doses (Hughes et al, 1990). From present data and other previous studies (Branchereau et al, 1992, 1993), the action of CCK-8S in the NTS neurons appears to be multiphasic. Both excitatory and inhibitory effects, transmitted through different receptors, are observed. The variable I-V relationship and time course of CCK action are also reported (Branchereau et al, 1992, 1993). Multiphasic effects of CCK on neurons have to be taken into account to explain the variety of responses observed, in the intact animal, after an administration of CCK agonist. However, it is not known why responses to CCK vary among neurons of the NTS. A possible explanation may come from the localization of the CCK receptors on a selective neuronal population. Another explanation for the variety of the CCK effects might be that regulatory processes control responsiveness to CCK and thus determine the actual pattern of impulses transmitted from the NTS. Different regulatory mechanisms in the NTS may, in fact, determine the actual response (excitation or inhibition) that predominates according to the particular physiological conditions. Regulation may also involve cellular processes that control the different membrane permeabilities gated by CCK-8S. Altogether, our results show that CCK-8S exerts, on neurons of the NTS, mixed effects due to simultaneous activation of CCK_A inhibitory and CCK_B excitatory binding sites. From a functional point of view, inhibitory CCK_A receptors may be linked to the inhibition of gastrointestinal motility observed after a meal, whereas excitatory CCK_B receptors may be linked to the anxiogenic effects mediated by CCK.

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