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The excitatory effect of cholecystokinin on rat neostriatal neurons: ionic and molecular mechanisms

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Abstract

Whole-cell patch-clamp recordings were performed to study ionic and molecular mechanisms by which cholecystokinin (CCK) peptides modulate the membrane excitability of acutely dissociated rat neostriatal neurons. Immunohistochemical staining studies indicated that about 95% of acutely isolated neostriatal neurons were GABA(γ-aminobutyric acid)ergic medium-sized cells. During current-clamp recordings, sulfated cholecystokinin octapeptide (CCK-8) depolarized neostriatal neurons and evoked action potentials. During voltage-clamp recordings, CCK-8 induced inward currents at negative membrane potentials by increasing the voltage-insensitive and non-selective cationic conductance. Cholecystokinin tetrapeptide (CCK-4), a selective CCK_B receptor agonist, also evoked cationic currents. The CCK-8-induced cation currents were antagonized by PD135,158 (4-{[2-[[3-(1H-indol-3yl)-2-mehtyl-1-oxo-2-[[[1.7.7.-trimethyl-bicyclo[2.2.1]hept-2-yl)oxy]carbonyl]amino]propyl]amino]-1-phenylethyl]amino-4-oxo-[1S-1α,2β[S*(S*)]4α]}-butanoate *N*-methyl-D-glucamine), a highly specific and potent CCK_B receptor antagonist. The CCK-8-evoked inward currents were blocked by the internal perfusion of 1 mM GDP-β-S. In neostriatal neurons dialyzed with 0.5 mM GTP-γ-S, the cationic currents produced by CCK-8 became irreversible. Pretreating neostriatal neurons with 500 ng/ml pertussis toxin did not prevent CCK-8 from evoking cationic currents. Internal administration of heparin (2 mg/ml), an inositol 1,4,5-trisphosphate (IP_3) receptor antagonist, and buffering of intracellular calcium with the Ca^{2+}-chelator, BAPTA (1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid, 10 mM), suppressed CCK-8-evoked cationic currents. These findings suggest that, by activating CCK_B receptors, CCK-8 excites rat neostriatal neurons through enhancing a non-selective cationic conductance and that pertussis toxin-insensitive G-proteins mediate CCK-8 enhancement of the cationic conductance. The coupling mechanism via G-proteins is likely to involve the production of IP_3, and the subsequent IP_3-evoked Ca^{2+} release leads to the opening of non-selective cation channels.

Keywords: CCK_B receptor; Neostriatal neuron; Cationic current; G-protein; Inositol 1,4,5-trisphosphate; Whole-cell patch-clamp recording

1. Introduction

Most areas of the neocortex send somatopically and topographically organized projections to the basal ganglia (Alexander and Crutcher, 1990; Parent, 1990). The neostriatum serves as the input nuclei of the basal ganglia and receives a dense corticoneostriatal innervation (Alexander and Crutcher, 1990; Graybiel et al., 1994; Wilson, 1990). Within the neostriatum, corticoneostriatal afferents make asymmetrical synapses with GABA(γ-aminobutyric acid)ergic medium spiny neurons, which make up more than 95% of the neostriatal neuronal population and function as the projection neurons of the caudate-putamen (Chang et al., 1982; Wilson, 1990). It is generally believed that the neurotransmitter released by the corticoneostriatal nerve terminals is an excitatory amino acid, most probably glutamate (Graybiel, 1990). However, recent studies suggest the existence of a cholecystokininergic corticoneostriatal pathway (Meyer et al., 1988; Morino et al., 1992, 1994a,b; You et al., 1994).

The peptide, cholecystokinin (CCK), was originally isolated from the gut and subsequently found to be present in various regions of the brain (Dockray, 1976; Emson et al., 1982; Crawley, 1985; Innis et al., 1979). Mammalian cholecystokinin biosynthesis in the brain involves the post-translational enzymatic cleavage of a propeptide (CCK-33) into several bioactive peptide fragments (Rehfeld et al., 1985), such as cholecystokinin-tetrapeptide (CCK-4) and

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cholecystokinin octapeptide. Sulfated cholecystokinin octapeptide (CCK-8; Asp-Tyr[SO_3]-Met-Gly-Trp-Met-Asp-Phe) is the predominant and major bioactive molecular form of CCK peptides in the brain (Rehfeld et al., 1985; You et al., 1994). CCK peptides have been shown to be released from brain slices and synaptosomes in a calcium-dependent manner (Emson et al., 1980; Pinget et al., 1979). A wide spectrum of biological effects induced by CCK peptides is mediated by the activation of specific CCK receptors. At least two subtypes of CCK receptors have been proposed on the basis of differential affinities for CCK peptides. CCK_A receptors are present in pancreas, vagus nerve and some regions of the brain (nucleus tractus solitarius, substantia nigra and ventral tegmemtal area) (Hill et al., 1987, 1990). Unlike CCK-8, CCK-4 and non-sulfated cholecystokinin octapeptide (CCK-8NS) are weak agonists for CCK_A receptors (Innis and Snyder, 1980; Moran et al., 1986). CCK_B receptors are widely distributed in various regions of the brain (Moran and McHugh, 1990; Van Dijk et al., 1984). CCK-4 and CCK-8NS are almost as potent as CCK-8 to activate CCK_B receptors (Innis and Snyder, 1980; Moran et al., 1986). Both subtypes of CCK receptors are reported to stimulate phospholipase C and phosphatidylinositol turnover through pertussis toxin-insensitive G-proteins (Lee et al., 1993; Wank et al., 1992a,b).

The neostriatum contains a high level of CCK-like immunoreactivity, and CCK has been shown to be released in the caudate-putamen in a Ca^{2+}-dependent manner (Hokfelt et al., 1988; Brog and Beinfeld, 1992). However, immunohistochemical and in situ hybridization studies demonstrated that there are only few CCK-immunoreactive and CCK-mRNA-positive neurons in the neostriatum and that most of the CCK immunoreactivity is present in the axons and nerve terminals (Hokfelt et al., 1988; Burgunder and Young, 1990), suggesting an extrinsic origin for most of the neostriatal CCK. Previous studies showed that CCK-ergic neurons in the midbrain projected to the caudate-putamen (Seroogy et al., 1989). In addition to a mesencephalic origin, several lines of evidence indicate that CCKergic neurons in the neocortex also make projections to the neostriatum and contribute to the corticoneostriatal pathway. Immunohistochemical and in vivo dialysis studies showed that decortication combined with callosotomy results in a marked decrease of CCK immunoreactivity and CCK release in the neostriatum (Morino et al., 1992; You et al., 1994). Antrograde and retrograde tracing studies revealed that CCK-immunoreactive neurons in motor areas of frontal cortex project bilaterally to the neostriatum and that CCKergic corticoneostriatal afferents form asymmetrical synapses with medium spiny neurons (Burgunder and Young, 1990; Morino et al., 1994a,b; Snyder et al., 1993). In agreement with neuroanatomical findings, a high density of CCK_B receptors is expressed in the neostriatum (Moran and McHugh, 1990). Therefore, CCK peptides released by corticoneostriatal nerve fibers could play an important role in motor function by modulating ionic conductances and excitability of neostriatal neurons. To better understand the functional implication of CCKergic corticoneostriatal innervation, whole-cell current- and voltage-clamp recordings were now used to investigate the electrophysiological effects of CCK peptides on acutely dissociated neurons of the rat neostriatum.

2. Materials and methods

2.1. Acute isolation of rat neostriatal neurons

Rat neostriatal neurons were acutely isolated according to procedures similar to those described previously (Wu et al., 1995). Briefly, 3–4-week-old Sprague-Dawley rats were terminally anesthetized with pentobarbital and decapitated. The whole brain was quickly removed, and 400-μm-thick coronal slices were obtained by using a Vibratome slicer in ice-cold PIPES-buffered Ringer's solution containing (in mM): NaCl 120, KCl 5, $NaHCO_3$ 20, $MgSO_4$ 2, $CaCl_2$ 2, KH_2PO_4 1, glucose 10 and PIPES 15 (pH = 7.4). Segments containing the neostriatum were excised, and incubated for 20 min at 32°C in oxygenated PIPES saline (NaCl 125 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgSO_4$ 2 mM, glucose 10 mM, PIPES 15 mM, pH = 7.4) containing pronase E (0.4 mg/ml, Sigma). The enzymatic treatment was terminated by washing tissue fragments twice with PIPES saline containing 2.4 mM EGTA. Subsequently, tissue segments were triturated with a Pasteur pipette, and dissociated neurons were plated onto polylysine-coated coverslips. Dissociated neurons were kept in a 100% O_2 atmosphere for 30 min and then used for the whole-cell recordings.

2.2. Whole-cell current- and voltage-clamp recordings

Neostriatal neurons were voltage- and current-clamped by using the conventional whole-cell version of patch-clamp techniques (Hamil et al., 1981). Patch electrodes with a resistance of 6–8 MΩ were made from hard borosilicate glass using a pipet puller (P-87, Sutter). Holding potentials, data acquisition and analysis were controlled by an online IBM-PC compatible computer-programmed with Axotape 2 and pCLAMP 5.5 (Axon Instruments). Current and voltage signals obtained with a patch-clamp amplifier (Axopatch-200A, Axon Instruments) were filtered at 2 kHz, digitized (TL-1 DMA interface, Axon Instruments) and stored on the harddisk of the computer for later analysis. The compensation circuitry of the amplifier was used to minimize the series resistance error.

The external solution had the following composition (in mM): NaCl 145, KCl 3, $CaCl_2$ 2, $MgCl_2$ 1, glucose 15, and HEPES 10 (pH 7.3 with NaOH). The patch pipette was filled with (in mM): KF 140, $MgCl_2$ 1, $CaCl_2$ 0.1, EGTA 1.1, ATP 2, GTP 0.3, and HEPES 5 (pH 7.3 with

KOH). In some experiments, GTP was replaced by guanosine-5'-*O*-(2-thiodiphosphate) (GDP-β-S) or guanosine-5'-*O*-(3-thiotriphosphate) (GTP-γ-S) (Boehringer Mannheim). CCK peptides (Peninsula) were dissolved in the external solution and applied to neurons using pressure ejections (Picospritzer, General Valve) from blunt micropipettes (diameter = 20–30 μm). Experiments were performed at room temperature (22–25°C). Student's *t*-test was used to determine whether the difference was statistically significant. All results are expressed as the mean ± S.E.M. for *n* cells.

2.3. Immunohistochemical characterizations of neostriatal neurons

To characterize the chemical identity of acutely isolated neostriatal neurons, immunocytochemical staining was carried out using the monoclonal antibody raised against GABA (Chemicon). Neostriatal neurons on the coverslips were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, followed by two rinses in phosphate-buffered saline (PBS). Then, coverslips were immersed in 10% normal horse serum and 0.1% Triton X-100 for 1 h. Subsequently, the coverslips were incubated with the monoclonal antibody against GABA in PBS. After being washed, the coverslips were incubated with Texas red-conjugated horse antimouse IgG (immunoglobulin G, Vector). Neostriatal neurons were viewed and photographed under a Nikon Diaphot microscope equipped with an Epifluorescence system (Nikon).

2.4. Chemicals and drugs

Tetrodotoxin, heparin and pertussis toxin were obtained from Sigma, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid (BAPTA)-tetrapotassium salt from Molecular Probes and protein kinase C-(19–31) from Bachem. PD135,158 was purchased from RBI.

3. Results

3.1. CCK-8 excites rat neostriatal neurons by increasing non-selective cationic conductance and activating CCK_B receptors

CCK-containing axon terminals have been shown to make synapses with medium spiny neurons of the neostriatum (Snyder et al., 1993). For whole-cell recordings with dissociated neostriatal neurons, it is very important to identify medium spiny neurons. Medium spiny neurons make up more than 95% of neostriatal neurons and are GABA(γ-aminobutyric)ergic cells (Chang et al., 1982; Wilson, 1990). Consistent with previous findings (Hoehn et al., 1993; Stefani et al., 1994; Surmeier et al., 1992, 1995), our immunohistochemical studies indicated that

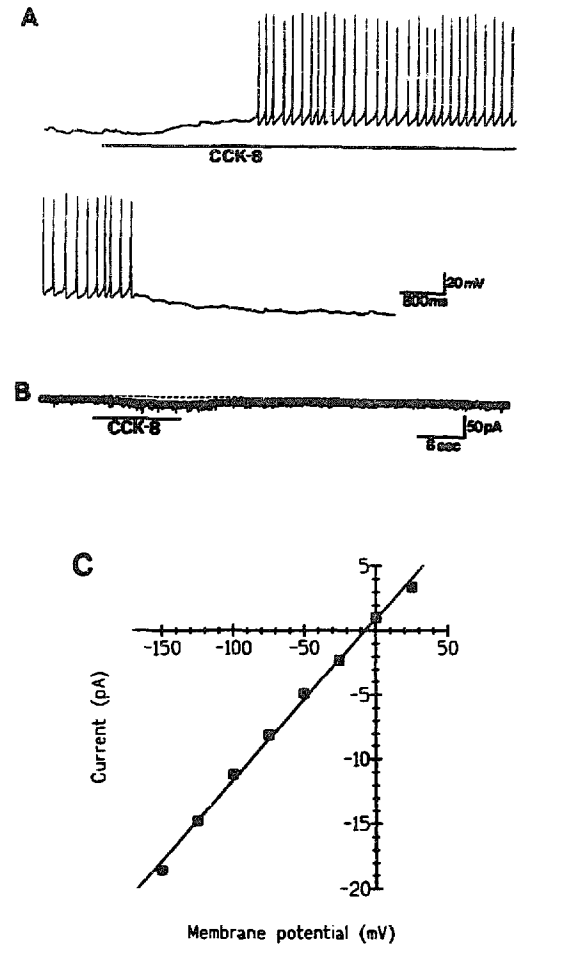

Fig. 1. CCK-8 depolarizes and excites medium-sized neostriatal neurons by enhancing voltage-insensitive and non-selective cationic conductance. (A) During the current-clamp recording, CCK-8 (5 μM) depolarized a neostriatal neuron and triggered action potentials in a reversible manner. The resting membrane potential of this neuron was −70 mV. (B) During voltage-clamp recordings, CCK-8 (5 μM) reversibly evoked an inward current at the holding potential (V_H) of −70 mV. (C) CCK-8 (5 μM)-evoked currents were obtained at various holding potentials (−150 to 30 mV) and current (I)–voltage (V) curves were constructed from 6 neostriatal neurons. One representative I–V curve is shown here. Note that CCK-8-evoked currents reversed polarity at about −10 mV and were linear over the membrane potentials studied.

about 95% of acutely isolated neostriatal neurons are GABAergic medium-sized cells with a diameter of 10-20 μm across their long axis (n = 336 neurons). Whole-cell patch-clamp recordings were obtained from GABAergic medium-sized neostriatal neurons with long processes.

During current-clamp recordings, medium-sized neostriatal neurons did not exhibit spontaneous firing and had a resting membrane potential more negative than −65 mV (Jiang and North, 1991; Kawaguchi, 1993). Application of CCK-8 (5 μM) to neostriatal neurons resulted in a reversible membrane depolarization and the induction of action potentials (Fig. 1A, n = 7 out of 10 neurons). In the presence of 2 μM tetrodotoxin, the mean depolarization induced by CCK-8 (5 μM) was 17 ± 2 mV (n = 7 neurons). During voltage-clamp recordings at the holding

potential (V_H) of −70 mV, CCK-8 (5 μM) evoked an inward current reversibly (Fig. 1B) and dose-dependently (1 μM, 5 ± 1 pA, $n = 5$; 5 μM, 10 ± 2 pA, $n = 20$; 15 μM, 11 ± 2 pA, $n = 3$). These findings suggest that CCK-8 depolarizes neostriatal neurons by evoking inward currents at negative membrane potentials.

To investigate the ionic mechanism by which CCK-8 induces the inward currents, a current-voltage curves was made by measuring CCK-8-evoked currents at various holding potentials. CCK-8-induced currents reversed direction at −12 ± 5 mV ($n = 6$ neurons) and were linear over holding potentials between −150 mV and 30 mV (Fig. 1C). These observations suggest that CCK-8 depolarizes and excites neostriatal neurons by enhancing a voltage-insensitive and non-selective cationic conductance.

CCK-4, a selective agonist for CCK_B receptors, also evoked cationic currents from neostriatal neurons (9 ± 1 pA, $V_H = -70$ mV, $n = 5$). PD 135,158 (300 nM), a highly potent and selective CCK_B receptor antagonist (Hughes et al., 1990), completely blocked CCK-8-induced inward currents (Fig. 2, $n = 6$ neurons). These findings show that the excitatory effects of CCK peptides on neostriatal neurons are mediated by CCK_B receptors.

3.2. Pertussis toxin-insensitive G-proteins couple CCK_B receptors to cation channels of neostriatal neurons

The CCK_B receptor is a member of the superfamily of G-protein-coupled receptors, thus, G-proteins could be involved in CCK-8 modulation of non-selective cation channels. This hypothesis was tested by dialyzing neostriatal neurons with the GTP analogues, GDP-β-S and GTP-γ-S (Hille, 1992). GDP-β-S binds to G-proteins and causes an irreversible inactivation of G-proteins. CCK-8 (5 μM) failed to evoke cationic currents in neostriatal neurons ($n = 8$) perfused with internal solution containing 1 mM GDP-β-S for 9–10 min (Fig. 3A,B). When neostriatal neurons ($n = 4$) were internally perfused with 0.5 mM GTP-γ-S, an irreversible activator of G-proteins, for 10 min, CCK-8 (5 μM) evoked cationic currents irreversibly (data not shown). When neostriatal neurons ($n = 28$) were studied with a pipet solution containing 0.3 mM GTP, the CCK-8-evoked cationic currents were totally reversible and did not display any significant rundown for 10–15 min after the start of whole-cell recordings. CCK-8 (5 μM) still evoked inward cationic currents (10 ± 1 pA, $n = 7$, $V_H = -70$ mV; control response = 10 ± 2 pA, $n = 20$, Fig. 3C) from neostriatal neurons pretreated with 500 ng/ml pertussis toxin for 6 h under a 100% O_2 atmosphere. The effectiveness of pertussis toxin treatment was indicated by our previous finding that dopamine failed to evoke potassium outward currents from pertussis toxin-pretreated substantia nigra dopaminergic neurons (Wu and Wang, 1994; Wu et al., 1995). These results suggest that

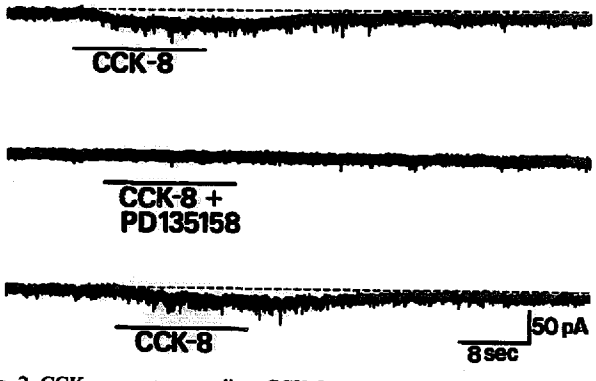

Fig. 2. CCK_B receptors mediate CCK-8 excitation of neostriatal neurons. The CCK-8 (5 μM)-induced cationic current was reversibly blocked by 300 nM PD135,158 (middle and lower traces), a potent and specific CCK_B receptor antagonist. $V_H = -70$ mV.

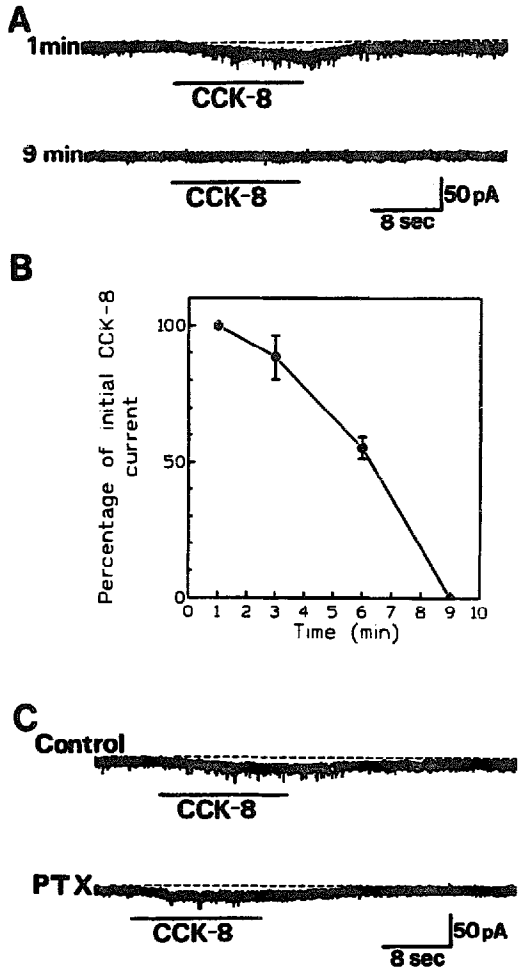

Fig. 3. CCK-8 enhances cationic conductance through activation of pertussis toxin-insensitive G-proteins. (A) 1 min after the start of the whole-cell recording, CCK-8 (5 μM) evoked an inward current (upper trace). After perfusion of the neuron with 1 mM GDP-β-S for 9 min, CCK-8 failed to induce membrane currents (lower trace). $V_H = -70$ mV. (B) The time course for the blocking effect of 1 mM GDP-β-S. CCK-8-induced currents obtained 1 min after the start of recordings are represented as initial currents. Each data point is the mean ± S.E.M. value from 8 neurons. (C) In both control and pertussis toxin-pretreated neostriatal neurons, CCK-8 (5 μM) evoked inward cationic currents. $V_H = -70$ mV.

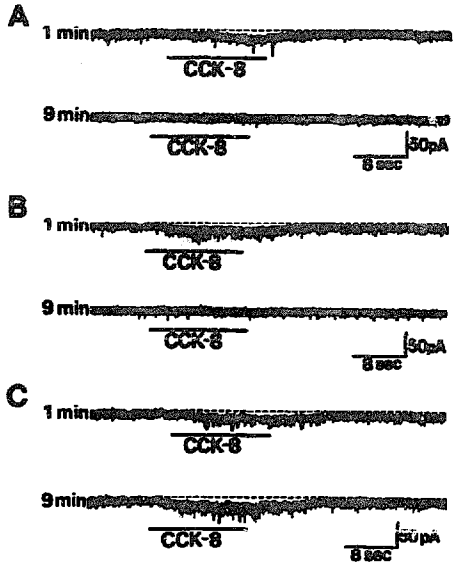

Fig. 4. The IP_3–Ca^{2+} pathway is involved in CCK-8 modulation of cation channels. (A) 1 min after internal dialysis of neostriatal neurons with heparin, an IP_3 receptor antagonist, CCK-8 (5 μM) evoked a cationic current. Within 9 min, the CCK-8-induced inward current was blocked (lower trace). $V_H = -70$ mV. (B) When neostriatal neurons were dialyzed with an internal solution containing 10 mM BAPTA for 9 min, CCK-8 (5 μM) failed to evoke the cationic current (lower trace). $V_H = -70$ mV. (C) CCK-8 (5 μM) still induced an inward cationic current in a neostriatal neuron dialyzed with 5 μM protein kinase C-(19–31) for 9–10 min (lower trace). $V_H = -70$ mV.

pertussis toxin-insensitive G-proteins, most likely G_q or G_{11}, couple CCK_B receptors to non-selective cation channels of neostriatal neurons.

3.3. IP_3-evoked Ca^{2+} release mediates CCK-8 enhancement of cationic conductance

Activation of CCK_B receptors causes the stimulation of phospholipase C through G-proteins, and phospholipase C hydrolyzes phosphatidylinositol 4,5-bisphosphate and generates second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. Subsequently, IP_3 evokes Ca^{2+} release from intracellular stores and diacylglycerol activates protein kinase C (Berridge, 1993). To investigate the role of IP_3 in the mediation of the enhancement of cationic conductance by CCK-8, heparin (2 mg/ml), an IP_3 receptor antagonist (Thorn and Petersen, 1993), was included in the internal solution during whole-cell recordings. The amplitude of the CCK-8-induced cationic current was significantly diminished 5 min after the intracellular perfusion of heparin ($P < 0.01$). CCK-8 (5 μM) failed to induce inward currents after dialysis of neostriatal neurons ($n = 6$) with heparin for 9–10 min (Fig. 4A, lower trace). This finding shows that IP_3 is essential for the CCK-8 activation of cationic conductance.

IP_3 induces Ca^{2+} release from intracellular stores, and the subsequent rise in the intracellular calcium concentration evokes various cellular responses (Berridge, 1993). The control pipet solution containing 1 mM EGTA, a slow Ca^{2+} chelator, could not rapidly buffer cytoplasmic calcium released from intracellular stores and prevent the opening of Ca^{2+}-activated ion channels (Wakui et al., 1991). Therefore, the hypothesis that IP_3-evoked Ca^{2+} release mediates CCK-8 modulation of cation channels was tested by buffering intracellular calcium with a high concentration of fast Ca^{2+} chelator, BAPTA [1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid]. In neostriatal neurons ($n = 6$) internally perfused with 10 mM BAPTA for 9–10 min, CCK-8 failed to evoke cationic currents (Fig. 4B, lower trace). Together with the finding that the intracellular administration of heparin abolishes CCK-8-evoked cationic currents, these results suggest that IP_3-induced Ca^{2+} release is responsible for the enhancement of cationic conductance by CCK-8.

The involvement of protein kinase C in CCK-8 activation of cationic conductance was investigated by including protein kinase C-(19–31) (Yang and Tsien, 1993), a specific pseudo substrate inhibitor of protein kinase C (IC_{50} = 0.2 μM), in the pipet solution during whole-cell recordings. In neostriatal neurons internally perfused for 9–10 min with 5 μM protein kinase C-(19–31), CCK-8 (5 μM) still evoked cationic currents at the holding potential of -70 mV (Fig. 4C; 10 ± 1 pA, $n = 7$; control response = 10 ± 2 pA, $n = 20$). These finding suggest that protein kinase C does not mediate CCK-8 modulation of cation channels in neostriatal neurons.

4. Discussion

To gain insight into the functional importance of CCK-ergic corticoneostriatal pathway, we investigated the electrophysiological effects of CCK-8 on acutely dissociated neostriatal neurons. The present study demonstrated that by activating CCK_B receptors, CCK-8 excites GABAergic medium-sized neurons by enhancing voltage-independent cationic conductance. This observation is consistent with previous autoradiographic studies that only one subtype of cholecystokinin receptors, the CCK_B receptors, is expressed in the neostriatum (Hill et al., 1987, 1990). In agreement with our findings, activation of CCK_B receptors in the supraoptic nucleus also causes an increase in cationic conductance and membrane depolarization (Jarvis et al., 1992). However, it has been reported that CCK_B receptor-mediated excitation of nucleus tractus solitarius, hippocampal and hypothalamic ventromedial neurons results from a reduction of potassium conductance (Boden and Hill, 1988; Boden, 1991; Branchereau et al., 1992, 1993). Either the existence of subtypes of CCK_B receptors or cell-specific receptor-effector coupling mechanisms could explain the finding that different ionic mechanisms are responsible for CCK_B receptor-induced excitation in various regions of the brain.

We also investigated the signal transduction pathway through which CCK-8 enhances the cationic conductance of neostriatal neurons. Our results suggest that pertussis toxin-insensitive G-proteins couple CCK_B receptors to cation channels and that the coupling mechanism through G-proteins is likely to involve the IP_3-induced Ca^{2+} mobilization that enhances non-selective cationic conductance. Interestingly, our recent studies showed that CCK-8, acting on CCK_A receptors, also excites substantia nigra dopaminergic neurons by increasing non-selective cationic conductance via the IP_3–Ca^{2+} pathway (Wu and Wang, 1994)

The present results suggest that pertussis toxin-insensitive G-proteins transduce CCK-8 modulation of cation channels indirectly through the IP_3–Ca^{2+} pathway. The most likely pertussis toxin-insensitive G-protein to mediate CCK-8 activation of the cationic conductance of neostriatal neurons is G_q or G_{11} (Helper and Gilman, 1992; Simon et al., 1991). Our recent single-cell reverse transcriptase-polymerase chain reaction assays showed that both $G_{\alpha q}$ and $G_{\alpha 11}$ are expressed in substantia nigra dopaminergic neurons (Wang and Wu, in press). Furthermore, internally perfusing substantia nigra dopaminergic neurons with a specific antibody against $G_{\alpha q/11}$ suppressed CCK-8-evoked cationic currents (Wu and Wang, unpublished observations). The same types of experiments are required to identify pertussis toxin-insensitive G-proteins that couple CCK_B receptors to non-selective cation channels of neostriatal neurons.

Several lines of evidence suggest that, in addition to having a direct excitatory effect on neostriatal neurons, CCK might coexist with glutamate in some corticoneostriatal neurons and could regulate the physiological functions of medium spiny neurons by modulating corticoneostriatal glutamatergic neurotransmission. Immunocytochemical studies demonstrated that some corticoneostriatal CCKergic neurons have a pyramidal shape, which is the typical morphology of corticoneostriatal glutamatergic neurons (Morino et al., 1994a,b). Decortication caused a parallel decrease in CCK and glutamate release in the neostriatum (You et al., 1994). These results suggest that CCK and glutamate are likely to co-localize in a subpopulation of corticoneostriatal neurons. In the neostriatum, forskolin induces the phosphorylation of 32-kDa phosphoprotein (DARPP-32) of medium spiny neuron. Conversely, glutamate, by activating NMDA receptors, induces the dephosphorylation of DARPP-32 (Snyder et al., 1993). Interestingly, although CCK-8 alone does not affect the phosphorylation state of DARPP-32, CCK-8 has been shown to reduce the forskolin-stimulated phosphorylation of DARPP-32. This effect is blocked by the NMDA receptor antagonist MK-801 [(5S,10R)-(−)5-methyl-10,11-dihydro-5H-dibenzo[a,d]cylohepten-5,10-imine] (Snyder et al., 1993), suggesting that CCK-8 inhibits the forskolin-stimulated phosphorylation of DARPP-32 indirectly by increasing glutamate release from corticoneostriatal nerve terminals. Whole-cell voltage-clamp recordings of medium spiny neurons in neostriatal slices are required to provide direct evidence that CCK peptides regulate glutamatergic transmission in the neostriatum.

The basal ganglia play an essential role in the execution of voluntary movements and are also involved the motor learning (Delong, 1990; Graybiel et al., 1994). The neostriatum functions as the input nuclei of the basal ganglia, receives diverse inputs from the entire neocortex and sends processed information, through globus pallidus, substantia nigra pars reticulata, ventroanterior and ventrolateral nuclei of thalamus, to premotor cortex and supplementary motor area. This motor circuit plays a vital role in the execution and preparation of voluntary movements (Graybiel et al., 1994). Dysfunction of this motor loop caused by damage to the neostriatum results in grave defects in motor functions, exemplified in Huntington's disease and hemiballismus (Delong, 1990). It is generally believed that corticoneostriatal neurons utilize an excitatory amino acid, most probably glutamate, as the only neurotransmitter and send an excitatory projection to medium spiny neurons of the neostriatum (Graybiel, 1990). Glutamate evokes excitatory postsynaptic currents by activating kainate receptors, AMPA receptors, and NMDA receptors (Kotter, 1994). However, taken together with results of previous immunohistochemical and in vivo dialysis studies, the present results suggest that CCK peptides released from corticoneostriatal nerve fibers also exert an excitatory effect on neostriatal neurons and that there could be a synergistic interaction between CCKergic and glutamatergic corticoneostriatal innervations.

Acknowledgements

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