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Cholecystokinin inhibits endocannabinoid-sensitive hippocampal IPSPs and stimulates others

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Abstract

Cholecystokinin (CCK) is the most abundant neuropeptide in the central nervous system. In the hippocampal CA1 region, CCK is co-localized with GABA in a subset of interneurons that synapse on pyramidal cell somata and apical dendrites. CCK-containing interneurons also uniquely express a high level of the cannabinoid receptor, CB_1, and mediate the retrograde signaling process called DSI. Reported effects of CCK on inhibitory post-synaptic potentials (IPSPs) in hippocampus are inconsistent, and include both increases and decreases in activity. Hippocampal interneurons are very heterogeneous, and these results could be reconciled if CCK affected different interneurons in different ways. To test this prediction, we used sharp microelectrode recordings from pyramidal cells with ionotropic glutamate receptors blocked, and investigated the effects of CCK on pharmacologically distinct groups of IPSPs during long-term recordings. We find that CCK, acting via the CCK_2 receptor, increases some IPSPs and decreases others, and most significantly, that the affected IPSPs can be classified into two groups by their pharmacological properties. IPSPs that are increased by carbachol (CCh-sIPSPs), are depressed by CCK, ω-conotoxin GVIA, and endocannabinoids. IPSPs that are enhanced by CCK (CCK-sIPSPs) are blocked by ω-agatoxin IVA, and are unaffected by carbachol or endocannabinoids. Interestingly, a CCK_2 antagonist enhances CCh-sIPSPs, suggesting normally they may be partially suppressed by endogenous CCK. In summary, our data are compatible with the hypothesis that CCK has opposite actions on sIPSPs that originate from functionally distinct interneurons.

Keywords: CCK_2; $GABA_B$; Neuronal rhythms; Endocannabinoid; Muscarinic; LY225910

1. Introduction

Cholecystokinin (CCK) is the most abundant neuropeptide in the central nervous system (CNS) (Beinfeld et al., 1981), and is highly expressed in a subset of GABAergic interneurons of the hippocampus (Dockray, 1976; Innis et al., 1979). CCK is released mainly as CCK8-S, and also, at low concentrations, as CCK4 or CCK8-U (Rehfeld, 1985). CCK-releasing interneurons in the hippocampus all contain GABA (Somogyi et al., 1984) and most express cannabinoid receptors (Katona et al., 1999; Freund, 2003). The axons of many CCK-positive neurons terminate on hippocampal pyramidal cell somata in stratum (s.) pyramidale and their proximal dendrites of s. radiatum (Freund and Buzsaki, 1996). Central (CCK_2) receptors are widely distributed throughout the CNS (Zarbin et al., 1983) and modulate stress, anxiety and exploratory behaviors (Singh et al., 1991; Matto et al., 1997).

Physiological actions of CCK in the hippocampus have been attributed to CCK_2 (Bohme et al., 1988; Carlberg et al., 1992); however CCK_2 has not yet been localized to specific neuronal sub-types in hippocampus or elsewhere (cf. Mercer et al., 2000). Reports of CCK's physiological actions are inconsistent, with both excitation (Dodd and Kelly, 1979; Boden and Hill, 1988; Bohme et al., 1988; Shinohara and Kawasaki, 1997), and inhibition (MacVicar et al., 1987; Perez de la Mora et al., 1993) of pyramidal cells having been demonstrated. CCK may inhibit pyramidal cells indirectly (Perez de la Mora et al., 1993) by increasing GABA release from

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interneurons (Miller and Lupica, 1994; Miller et al., 1997; Ferraro et al., 1999; Deng and Lei, 2006). Some discrepancies have been ascribed to dosage and application method, or to different effects of CCK on interneurons and pyramidal cells (Miller et al., 1997). The close association of CCK only with certain interneurons suggests that some of the reported discrepancies in CCK effects might reflect its actions on distinct classes of interneurons (Freund and Buzsaki, 1996).

The primary aim of the present study was to test the hypothesis that CCK affects different interneurons in different ways, by using pharmacological tools to identify classes of interneuron outputs. Focusing on the rat hippocampal CA1 region, we show that CCK_2 activation mediates the effects of CCK agonists, and directly stimulates persistent spontaneous (sIPSP) activity in control conditions. However, other sIPSPs are initiated in the presence of carbachol (CCh) and CCK_2 activation inhibits the CCh-sIPSPs. This does not represent opposing effects of CCK and CCh on the same interneurons however, because the IPSPs in these two different conditions are sharply distinguished by their sensitivity to endocannabinoids, calcium channel antagonists, muscarinic agonists, and $GABA_B$ agonists. We also report the first evidence that endogenously released CCK suppresses CCh-sIPSPs. Our data are consistent with the hypothesis that the disparate actions of CCK on inhibition reflect opposite effects on distinct interneuron classes. Indeed, the pharmacological profiles of these two classes of sIPSPs correspond well with the properties of PV- and CCK-expressing interneurons as described in the literature (see Table 1). We suggest that CCK could thereby link the actions of different interneurons, a hypothesis that may have implications for understanding some of the oscillatory electrical activity in hippocampus (Buzsaki, 2002; Baraban and Tallent, 2004; Freund, 2003; Whittington and Traub, 2003).

2. Materials and methods

Male Sprague–Dawley rats, 5–7 weeks old (Charles River Laboratories) were deeply anaesthetized with halothane and decapitated in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee of the University of Maryland, School of Medicine. The brain was rapidly removed from the skull and both hippocampi dissected free. Transverse hippocampal sections (400 μm thick) were cut on a Vibratome (Series 1000, Technical Products International). Slices were kept in a holding chamber at room temperature at the interface of artificial cerebro-spinal fluid (ACSF) and a humidified gas mixture of 95% O_2 and 5% CO_2 for ≥1 h and then transferred to a submersion chamber (Nicoll and Alger, 1981) that was continuously perfused with ACSF at 29–31 °C, and positioned under a dissecting microscope. The submerged chamber permits rapid, thorough access of bath-applied drugs to the entire slice, and cells remain in healthy condition (as judged by active and passive cell properties) for 6–8 h. ACSF contained (in mM): 120 NaCl, 3 KCl, 2 $MgSO_4$, 1 NaH_2PO_4, 25 $NaHCO_3$, 10 glucose, and 2.5 $CaCl_2$, and was continuously bubbled with 95% O_2 and 5% CO_2 (pH 7.4). The ionotropic glutamate antagonists D-AP5 (20 μM) and NBQX (10 μM, both from Tocris) were present in all experiments to block EPSPs. CCK8-S, LY225910, YM022 CGP55845 and WIN55212-2 were obtained from Tocris. Carbachol (CCh), CCK4, ω-agatoxin IVA (agatoxin), ω-conotoxin GVIA (conotoxin) and all other chemicals were obtained from Sigma. All drugs were bath applied. To avoid desensitization induced by repeated applications of CCK, each slice was limited to a single application of CCK.

2.1. Electrophysiology

Conventional high resistance intracellular (“sharp”) electrode recordings were carried out in CA1. The CA1 stratum pyramidale was visualized under a dissecting microscope at 4× (the objective does not touch the solution, and the tip of the electrode can readily be positioned directly over the layer), the pyramidal cells were then impaled by lowering the electrode “blindly” into the layer. Microelectrodes (50–150 MΩ) were filled with a 3 M KCl solution to facilitate the observance of $GABA_A$-mediated sIPSPs. Acceptable cells had resting potentials of >−60 mV. When necessary, a modest holding current (<−0.5 nA) was used to maintain a slightly hyperpolarized membrane potential at −70 mV, to suppress action potential firing, and enhance sIPSP size. The negative holding potential also prevented CCh from depolarizing the cell because many of the currents affected by CCh are activated only at more depolarized levels. The holding current used was constant during the experiment. In some cases, as noted, we stimulated cells with current injections through the microelectrode in a ‘theta burst’ pattern, where one theta burst equaled 5 depolarizing pulses, 10 ms each in duration, given at 100 Hz; bursts separated by 200 ms. Signals were digitized at 5 kHz (Digidata 1200A, Axon Inst., Foster City, CA), filtered at 2 kHz, and analyzed with pClamp 8.0 or 9.0 software (Axon Inst.). For miniature IPSC (mIPSC) experiments, whole-cell patch clamp recordings were performed. Pyramidal cells were held under whole-cell voltage clamp at −70 mV and cells with low, stable holding current (<300 pA) were used. Whole cell intracellular solution contained in mM: 90 $CsCH_3SO_3$, 1 $MgCl_2$, 1 CsCl, 2 MgATP, 0.2 Cs_4-BAPTA, 10 HEPES, 0.3 Tris-GTP, and 5 QX-314 (lidocaine-*N*-ethyl bromide). The electrode access resistance measured in a cell was ≤30 MΩ and did not change by more than 15% in acceptable experiments.

For analysis of theta rhythms, the data were filtered at 200 Hz with a low-pass, eight pole Bessel filter (Frequency Devices, Haverhill, MA). Power spectrum analysis and autocorrelations were done in Clampfit 9.0. We calculated a value of “relative theta power” for each cell by summing the spectral power between 4 and 14 Hz, and dividing this by the total spectral power between 1 and 50 Hz during 10 s of sIPSP activity (Reich et al., 2005). We also measured peak theta power as the largest peak spectral power within the theta range of

Table 1
Comparison of hippocampal CCK and PV basket interneurons

Properties	PV	CCK	References
Spike timing	Fast spiking	Regular spiking	Reviewed in Freund, 2003; Hefft and Jonas, 2005; Glickfeld and Scanziani, 2006
GABA release	Quantal release synchronous	Quantal release asynchronous	Hefft and Jonas, 2005
Ca channels mediating GABA release	P/Q-type	N-type	Reviewed in Freund, 2003
CB_1R	Absent	Present	Tsou et al., 1998; Marsicano and Lutz, 1999; Freund, 2003
mAChR	M2 on terminals (no M1 or M3)	M1 and M3 on soma, (no M2)	Reviewed in Freund, 2003; Fukudome et al., 2004
Pre-synaptic GABABRs	Low concentration	High concentration	Reviewed in Freund, 2003