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Cholesterol depletion inhibits synaptic transmission and synaptic plasticity in rat hippocampus

C. Frank [a], S. Rufini [b], V. Tancredi [c], R. Forcina [b], D. Grossi [c], G. D'Arcangelo [c,*]

[a] Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, 00161 Rome, Italy
[b] Department of Biology, University of Rome "Tor Vergata", 00133 Rome, Italy
[c] Department of Neuroscience, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome, Italy

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ABSTRACT

Several neurodegenerative disorders are associated with impaired cholesterol homeostasis in the nervous system where cholesterol is known to play a role in modulating synaptic activity and stabilizing membrane microdomains. In the present report, we investigated the effects of methyl-β-cyclodextrin-induced cholesterol depletion on synaptic transmission and on the expression of 1) paired-pulse facilitation (PPF); 2) paired-pulse inhibition (PPI) and 3) long-term potentiation (LTP) in the CA1 hippocampal region. Results demonstrated that cyclodextrin strongly reduced synaptic transmission and blocked the expression of LTP, but did not affect PPF and PPI. The role of glutamatergic and GABAergic receptors in these cholesterol depletion-mediated effects was evaluated pharmacologically. Data indicate that, in cholesterol depleted neurons, modulation of synaptic transmission and synaptic plasticity phenomena are sustained by AMPA-, kainate-and NMDA-receptors but not by GABA-receptors. The involvement of AMPA-and kainate-receptors was confirmed by fluorimetric analysis of intracellular calcium concentrations in hippocampal cell cultures. These data suggest that modulation of receptor activity by manipulation of membrane lipids is a possible therapeutic strategy in neurodegenerative disease.

Introduction

The importance of cholesterol in brain functioning is made clear by the fact that the human brain, while comprising only 2% of total body weight, contains 25% of the total pool of this lipid (Dietschy and Turley, 2001). Cholesterol is crucial to the formation of membrane microdomains, or "lipid rafts", thought to be important for a variety of cellular functions, including receptor signalling, synapse formation and activity. Although doubts about the nature of these microdomains still remain (Shaw, 2006), the existence of "lipid rafts" and the proteins bound to them are generally considered essential for a broad range of activities in the nervous system, including neuronal excitability and synaptic transmission (Tsui-Pierchala et al., 2002). It is not surprising that several human diseases of the nervous system, such as Alzheimer's (AD), Huntington's (HD), Niemann Pick's and prion's Diseases (Vance, 2006; Valenza et al., 2007; Cutler et al., 2004; Hooper, 2005), involve an impairment of lipid metabolism and transport. It has been proposed that the neurotoxic protein, β-amyloid is produced in lipid rafts after hydrolysis of the amyloid precursor protein (APP) by preseniline (Cordy et al, 2006). Since brain cholesterol levels increase in AD (Cutler et al., 2004), and hypercholesterolemia is an early risk factor for its development (Pappolla et al., 2003; Lane and Farlow, 2005), cholesterol-lowering drugs might be a potential treatment for this disease (Wolozin et al., 2000). Recently in the R6/2 mice, a model of HD, it has been reported that cholesterol biosynthesis pathway is reduced in brain (Valenza et al., 2007) showing abnormalities in synaptic plasticity (Picconi et al., 2006).

In Niemann Pick Disease (NPD), gene-related lipidosis induces incorrect development of neuronal networks and, subsequently, the insurgence of neurological disorders, culminating in neurodegeneration (Vance, 2006). The genetic mutation present in NPD may lead to neuronal dysfunction by upsetting the lipid traffic dynamics of cholesterol sphingolipid-enriched microdomains in the plasma membrane of neuron and glia (Tashiro et al., 2004). Similarly, prion protein in transmissible spongiform encephalitis is known to modulate raft-associated signaling cascades, demonstrating a clear link between prion disease insurgence and neuronal membrane cholesterol level (Hooper, 2005). However, the cellular mechanisms that link cholesterol homeostasis to pathological events in the nervous system are poorly understood, and comprehension of the role of cholesterol in the brain is a compelling objective in neurobiology. Several authors have recently proposed an involvement of cholesterol in the stabilization of lipid rafts, the membrane platforms where many neurotransmitter receptors are located. There is evidence for localization of the alpha7-subunit of the nicotinic acetylcholine receptor (Bruses et al., 2001) and for a subpopulation of AMPA and NMDA receptors on lipid rafts in CNS neurons (Hering et al., 2003). In cholesterol enriched microdomains,

* Corresponding author. Fax: +39 0672596407.
E-mail address: darcange@uniroma2.it (G. D'Arcangelo).

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alterations in cholesterol levels directly modulate the activity of ligand gate-ion channels, as observed for the $GABA_A$ receptor (Sooksawate and Simmonds, 1998). Taken together, these findings clearly indicate a common pivotal role of cholesterol impairment (i.e. increase or decrease) in the insurgence of several neurodegenerative diseases by affecting synaptic activity.

The aim of the present work was to investigate how synaptic function in the hippocampal region is modulated by altering the dynamics of lipid microdomains through cholesterol depletion. The hippocampus is involved in learning and memory processes (Bliss and Lomo, 1973) and appears to be a very vulnerable region in several neurodegenerative diseases (Back et al., 2004). For this purpose, we focused on the role exerted by cholesterol on the primary neurotransmitter receptors involved in synaptic transmission and plasticity by using electrophysiological recordings of hippocampal brain slices treated with methyl-β-cyclodextrin (MβCD), a molecule that dissolves the hydrophobic core of lipid rafts (Kilsdonk et al., 1995). This same protocol was subsequently applied to evaluate glutamatergic neurotransmission by fluorimetric analyses of intracellular calcium concentrations.

Methods

Electrophysiological recordings of rat hippocampus

41 adult male Wistar rats (8–12 weeks old) were used according to the procedures established by the European Union Councils of Animal Care. All efforts were made to minimize the number of animals used and their suffering. Under anesthesia with enfluorane, they were decapitated and brains were quickly removed and placed in cold, oxygenated artificial cerebral spinal fluid (ACSF) containing the following (in mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 10. The hippocampus was rapidly dissected out and cut transversely into 450-μm-thick slices with a McIlwain tissue chopper (Mickle Laboratory Engeneering Co., Gomshall, U.K.). Slices were then transferred to a tissue chamber where they were laid in an interface between oxygenated ACSF and humidified gas (95% O2/5% CO2) at 32–34 °C (pH=7.4), constantly superfused at flow rate of 1.2 ml/min. In various experiments ACSF also contained: methyl-β-cyclodextrin (MβCD 0.5 mg/ml), water soluble cholesterol (cyclodextrin-balanced), baclofen (10 μM), bicuculline methiodide (BMI 10 μM), α-amino-3-hydroxy-5-methyl-4-isoxazole proprionate (AMPA 1 μM) and kainic acid (2 μM).

Basal synaptic transmission (BST), paired-pulse stimulation (PPS) and long-term potentiation (LTP) were examined in the Schaffer collateral/commissural CA1 pathways. Extracellular recordings of the population spike (PS) were made in the *stratum* pyramidale of the CA1 subfield with glass microelectrodes filled with 2 M NaCl (resistance 5–10 MΩ). Orthodromic stimuli (10–500 mA, 20–90 ms, 0.1 Hz) were delivered through a platinum electrode placed in the stratum radiatum (Schaffer collaterals). The test stimulus intensity of 50-ms square pulses was adjusted to give a PS of 2–3 mV at 0.03 Hz. The effect of MβCD on basal synaptic transmission (BST) was investigated during a two-hour incubation period by analyzing PS amplitude, which was calculated every minute as the average of six recordings performed every 10 s. Responses were acquired, digitized, and stored using a personal computer with standard acquisition software (Axon, Foster City, CA, U.S.A.). For paired-pulse experiments, responses were recorded at various interstimulus intervals (20–200 ms) for facilitation and at 10 ms for inhibition under control conditions and with drug perfusion. Slices used for paired-pulse experiments were not subjected to tetanic stimulation. Paired-pulse ratios between PS amplitudes in response to the test stimulus and the conditioning stimulus, respectively, were calculated for each slice and interpulse interval by averaging the values of four responses.

In the LTP experiments, a tetanic stimulation (100 Hz, 1 s) was delivered to induce LTP at the same stimulus intensity used for identifying baseline responses. LTP was measured by calculating the PS amplitude before and after the tetanus, with or without a previous 120-minute incubation period with MβCD. Changes in the amplitude of PS after tetanization were expressed as percentages of the basal PS amplitude (PS/basal PS×100, where basal PS was the mean PS amplitude before tetanization). To evaluate impairment of GABAergic transmission, baclofen and BMI were added to the bath for 15 min after the two-hour bathing period with MβCD. Similarly, to evaluate impairment of AMPA or kainic acid transmission, AMPA or kainate were added to the bath for 20 and 25 min respectively, after the two-hour bathing period with MβCD.

Intracellular calcium measures

Cell cultures

Primary cultures of rat hippocampal neurons were prepared as described previously (Frank et al., 2004). Fetuses were removed on embryonic day 17 from maternal rats anesthetized with enfluorane and sacrificed by decapitation. After removal of meninges, the hippocampi were collected in Hank's balanced salt solution (HBSS, purchased by Euroclone) and mechanically fragmented. Hippocampal cells were washed in HBSS and re-suspended in Neurobasal medium supplemented with 0.5 mM L-glutamine, 2% B-27 supplement, 5 U/ml penicillin and 5 μg/ml streptomycin (referred as complete medium [CM]; all products purchased by GIBCO). Aliquots of $2–3\times10^4$ cells were placed in 35 mm Petri dishes coated with poly-L-lysine (5 μg/ml) and maintained at 37 °C in humidified air with 5% CO2. Every 4 days, 0.5 ml of medium was removed and replaced by the same volume of fresh CM.

Drug application

In Fura-2AM experiments, cells were pre-incubated with MβCD for 60 min, Fura-2 was added and the incubation continued for an additional 60 min (120 min of total MβCD incubation time). After removal of Fura, MβCD was kept constant in the bath solution during AMPA and kainic acid stimulation for the duration of the experiments.

Calcium measurements

Experiments took place 13–15 days after plating. Optical fluorimetric recordings with Fura-2AM were used to evaluate the intracellular calcium concentration ($[Ca^{2+}]_i$). Fura-2AM stock solutions were obtained by adding 50 μg of Fura-2AM to 50 μl of 75% DMSO plus 25% pluronic acid. Cells were bathed for 60 min at room temperature with 5 μl of stock solution diluted in 1 ml of extracellular solution (in mM: 125 NaCl, 1 KCl, 5 $CaCl_2$, 1 MgCl2, 8 glucose, and 20 HEPES, pH7.35) for a final Fura concentration of 5 μM. This solution was then removed and replaced with extracellular solution, and the dishes were quickly placed on the microscope (Leica DMIRB) stage. To measure fluorescence changes, a Hamamatsu (Shizouka, Japan) Argus 50 computerized analysis system was used, recording every 6 s the ratio between the values of light intensity at 340 and 380 nm stimulation.

All drugs were purchased at Sigma (St. Louis, MO) and Fura-2AM at Molecular Probes (Eugene, OR). All compounds were stored as stock solutions.

Statistics

Data are expressed as mean measurements ± SEM and n represents the number of slices or cells studied. Data were statistically compared using the Student's t test or the ANOVA test and were considered significantly different if $p<0.05$. Excel 5.0 software was used for statistics and generation of graphs.

Results

Cyclodextrine effects on basal synaptic transmission and paired-pulse stimulation

The effect of a MβCD 0.5 mg/ml bath on population spike amplitudes (PS) in the pyramidal cell layer of the CA1 hippocampal region of adult

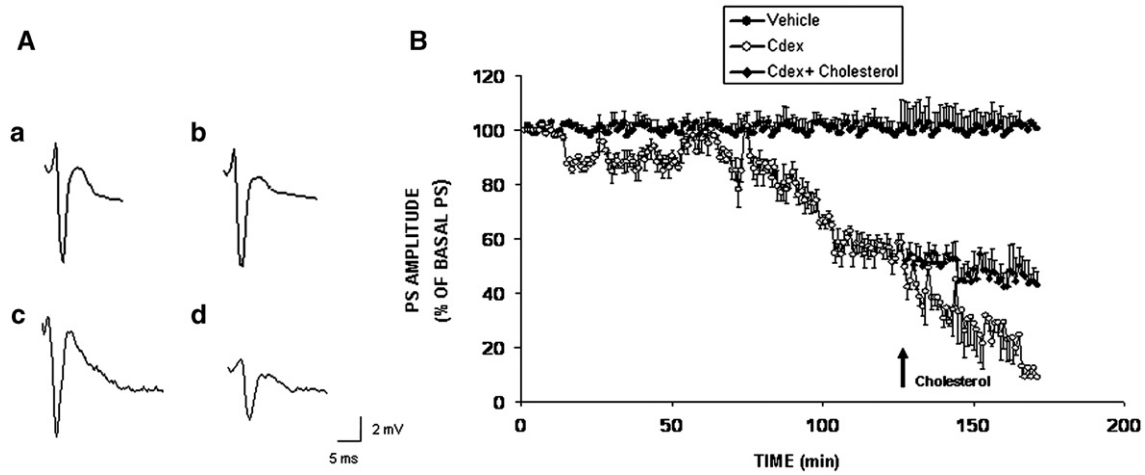

Fig. 1. Effects of MβCD on basal synaptic transmission (BST) in the CA1 region of the hippocampus. A: Representative recordings were obtained from slices treated with vehicle (a–b–c) or 0.5 mg/ml MβCD (d). Curve (a) was recorded at time $t = 10$ and curve (b) at time $t = 120$, while curves (c) and (d) refer respectively to the basal population spikes (PS; $t = 10$ and 120) before and after 120 min MβCD treatment. Curves represent the average of 6 recordings. B: % PS amplitude as a function of time is shown under control conditions (vehicle ●, $n = 10$), during treatment with MβCD (○, $n = 11$) or cholesterol-balanced cyclodextrin (◆, $n = 8$). The PS amplitude, calculated every minute, corresponds to an average of 6 recordings/min. Points in the plot are means ± SEM of values obtained from different slices. Note the marked reduction of basal PS induced by MβCD perfusion ($p < 0.01$) and how the decrease was abolished by cyclodextrin-cholesterol treatment ($p < 0.01$).

rats was investigated. After 1 h of treatment, basal synaptic transmission (BST) showed a statistically significant slow and progressive decrease to a value close to 60% that of controls (Figs. 1A–B). Some experiments were carried out using cholesterol-balanced cyclodextrin to avoid possible a specific effects of cyclodextrin, as recently suggested by Zidovetzki and Levitan (2007). After 2 h of cyclodextrin treatment, slices were perfused with 0.1 mg/ml of cyclodextrin-cholesterol. As shown in Fig. 1B, the cyclodextrin-dependent progressive decrease of BST was abolished by cyclodextrin-cholesterol treatment, indicating that this BST decrease was induced by cholesterol depletion rather than any a specific cyclodextrin effect.

Since the effect observed may have depended on the involvement of presynaptic and/or postsynaptic mechanisms, a homosynaptic paired-pulse facilitation (PPF) paradigm was used to further address the site of action of MβCD. PPF is a short lasting presynaptic alteration in synaptic efficacy determined by neurotransmitter release. A high PPF ratio indicates a high release of neurotransmitter from presynaptic nerve terminals. If the MβCD-induced reduction in field PSs had involved a presynaptic mechanism, it would have been associated with alterations in this PPF ratio. In these experiments, MβCD did not significantly affect PPF at any interpulse interval used (20–200 ms, Fig. 2B). These results indicated that MβCD did not modify glutamate release at a presynaptic site.

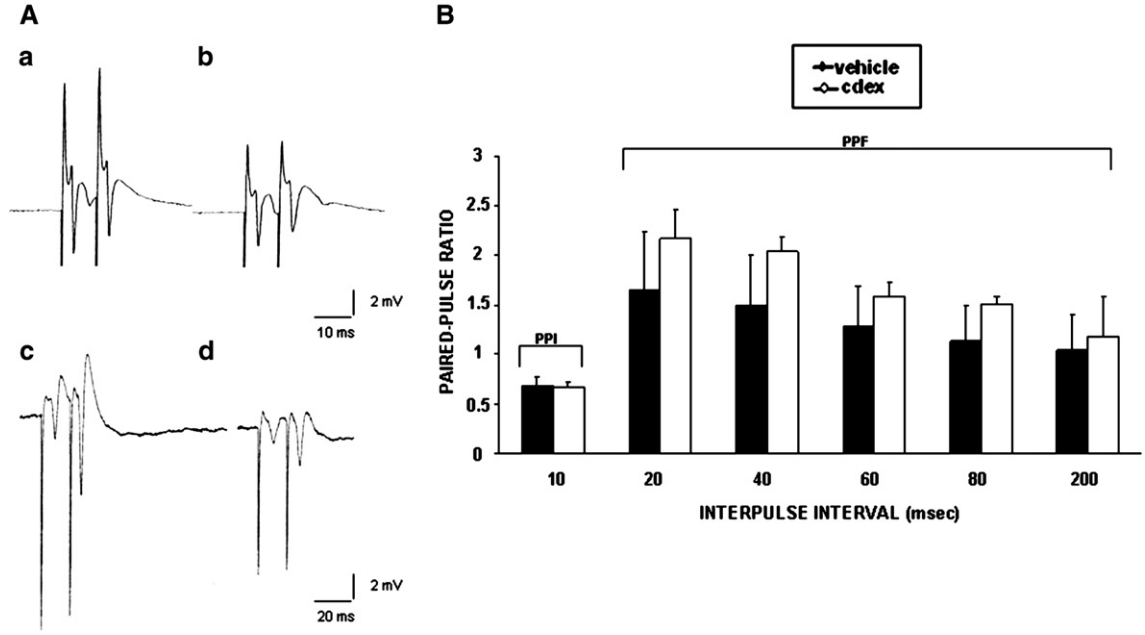

Fig. 2. Effects of MβCD on paired-pulse inhibition (PPI) and on paired-pulse facilitation (PPF) in the CA1 hippocampal region. A: Representative PS recorded from slices in response to paired stimuli delivered at interpulse intervals of 10 ms (a, b) and 20 ms (c–d) under control conditions (a–c) and with 0.5 mg/ml MβCD (b–d). B: Paired-pulse ratios related to PPI and PPF as a function of the interpulse interval. Data represent means ± SEM of the amplitude ratio between the PS evoked by the testing impulse and the PS evoked by the conditioning impulse. Recordings were obtained from different slices subjected to paired-pulse inhibition under control conditions (vehicle black bar; $n = 6$) or after 120 min of perfusion with MβCD (white bar, $n = 6$) at 10 ms interpulse interval; the following interpulse intervals refer to recordings obtained from various slices subjected to paired-pulse facilitation in control conditions (vehicle black bar, $n = 6$) or after 120 min of perfusion with MβCD (white bar, $n = 6$).

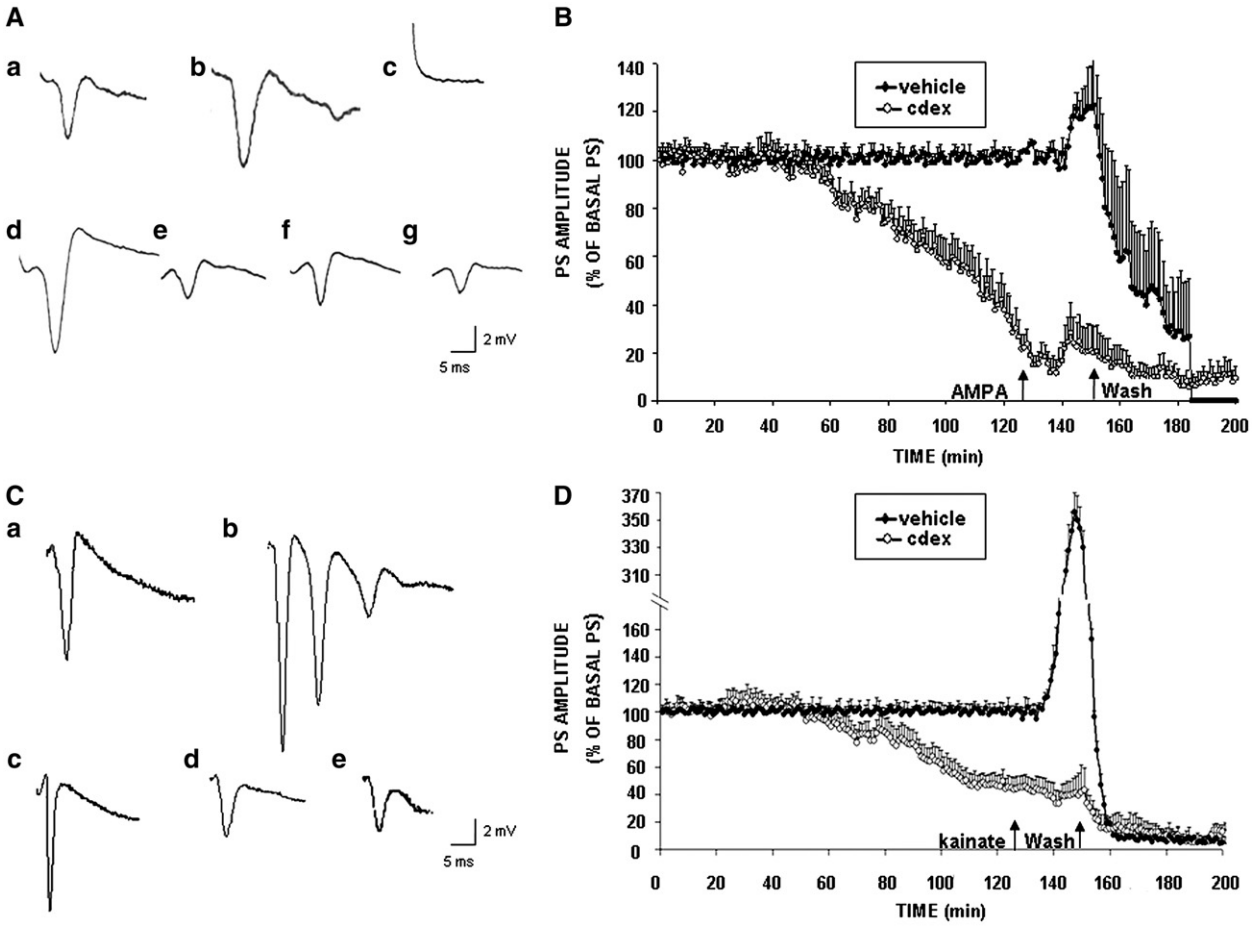

Fig. 3. Effects of AMPA and kainate on BST under control conditions and during MβCD perfusion of the CA1 hippocampal region. A: Representative recordings were obtained from slices before, during 20 min of AMPA 1 μM perfusion and after washout in control (a–c) and 0.5 mg/ml MβCD pretreated samples (e–g). Curve (d) refers to PS at $t=1$ before MβCD perfusion. B: % PS amplitude as a function of time is shown under control conditions (vehicle ●; $n=4$) and in MβCD-treated slices (○, $n=4$) during AMPA perfusion. In both cases, AMPA (first arrow) increased, within about 15 min, the amplitude of PS. During washout, a progressive reduction in PS until complete signal disappearance was observed in control slices, while in MβCD-treated slices the PS was always detectable. The amplitude, calculated every minute, corresponds to an average of 6 recordings/min. Points in the plot are means ± SEM of values obtained from different slices. C: Representative PS recorded from slices before and after 25 min of treatment with kainate 2 μM in control (a–b) and in 0.5 mg/ml MβCD-treated slices (d–e). PS in (c) refers to basal PS before MβCD application. D: % PS amplitude as a function of time is shown under control conditions (vehicle ●; $n=4$) and in the presence of MβCD (○, $n=5$) during perfusion of kainate 2 μM. Kainate (first arrow) significantly increased PS amplitude only in control slices. PS amplitude, calculated every minute, corresponds to the average of 6 recordings/min. Points in the plot are means ± SEM of values obtained from different slices.

To investigate a possible increase in presynaptic GABA release, paired-pulse inhibitions (PPI) were determined (10 ms, Fig. 2B). Inhibition in the hippocampus arises from feedforward and feedback connections via inhibitory interneurons. Activation of inhibitory neurons by CA1 neurons is demonstrated by recurrent inhibition of subsequent responses when a second stimulus is delivered shortly (10 ms) after the first. This type of pairing of two pulses in rapid succession leads to an inhibition known as a paired-pulse inhibition (PPI). Statistically significant differences in PPI under control and MβCD-treated conditions were not observed, indicating that cholesterol depletion did not interfere with GABA release.

Cyclodextrine effects on synaptic transmission of hippocampal slices and intracellular calcium concentrations in cultures cells when treated with glutamatergic agonists

Electrophysiology

Results reported thus far appeared to indicate that the reduction observed in synaptic transmission was due to an involvement of receptors lying in postsynaptic cell membrane lipid rafts. It is known that AMPA and kainate receptors play a primary role, and the NMDA receptor, a secondary role, in basal synaptic transmission (Collingridge et al., 1983). This known, we evaluated if AMPA and kainate receptor activities were affected by lipid raft deregulation using both electrophysiology and intracellular calcium fluorescence.

After 2 h of recording basal synaptic transmission, perfusion with 1 μM AMPA (Figs. 3A–B) for 20 min initially induced an increase in PS (by about 20% with respect to baseline), followed by a progressive reduction of signal up to its complete disappearance. In comparison, application of AMPA after 2 h of MβCD perfusion, induced a similar increase of PS amplitude (by about 30% with respect to baseline) that lasted only 1 min, followed by a reduction but not a complete disappearance of response (Figs. 3A–B).

Conversely application of 2 μM kainate for 25 min, induced a 250% increase of PS and an epileptiform pattern indicating cellular distress (Figs. 3C–D). The two-hour MβCD pre-treatment significantly quenched this dramatic kainate effect (Figs. 3C–D).

Fluorescence

Electrophysiological data were then confirmed by fluorescence experiments performed on hippocampal cultured cells. With or without a pre-treatment with MβCD, hippocampal cultured neurons were treated with AMPA and kainic acid and the increases in $[Ca^{2+}]_i$ measured by fluorescent analysis. Under control conditions, perfusion of cells with 100 μM AMPA or 100 μM kainic acid (Roychowdhury et al, 2006; Angehagen et al., 2004), markedly raised $[Ca^{2+}]_i$ (Fig. 4). The

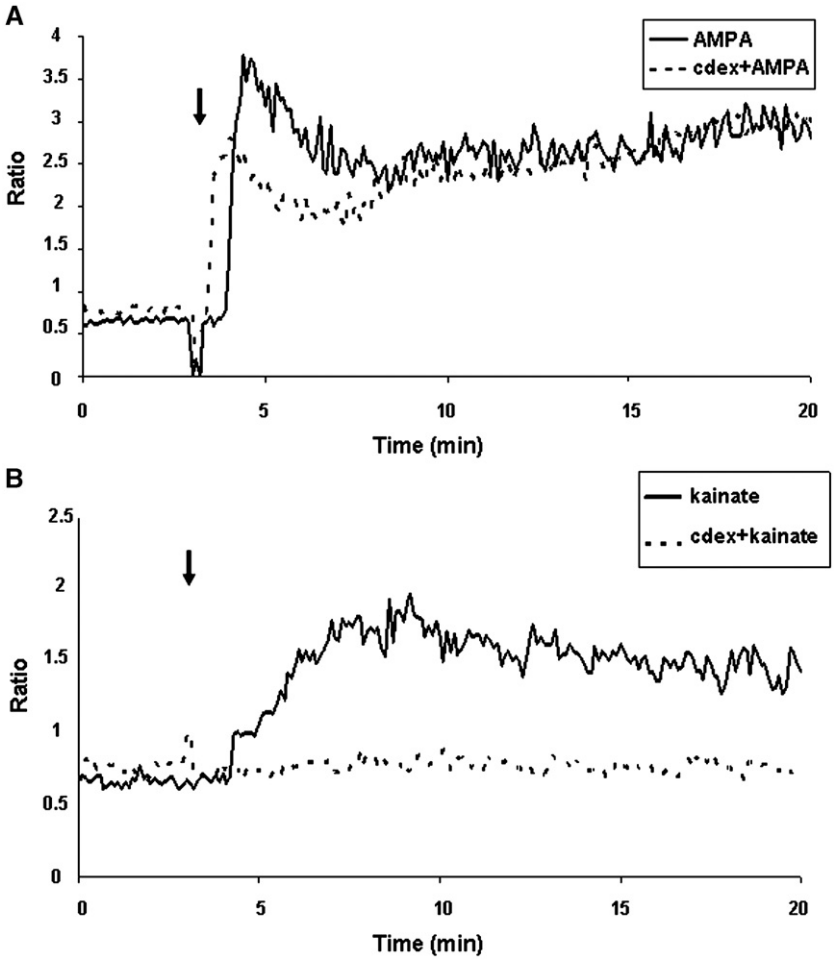

Fig. 4. Effects of MβCD on intracellular calcium influx induced by AMPA and kainate perfusion of cultured hippocampal neurons. A: After 3 min of basal recording, perfusion with AMPA 100 μM (arrow) induced a rapid increase of $[Ca^{2+}]_i$, as revealed by an increased 340/380 ratio in fluorimetric analysis by Fura-2AM. This remained at a constant level for at least the following 15 min (black line). A 2-hour pretreatment with MβCD 0.5 mg/ml reduced the early phase of the AMPA-induced $[Ca^{2+}]_i$ increase (dotted line) by 26%. B: Kainate 100 μM perfusion (arrow) induced within 5 min a significant increase of $[Ca^{2+}]_i$ (black line). Pre-treatment with MβCD 0.5 mg/ml totally prevented the effect of kainate (dotted line, $p<0.01$). In this figure, representative experiments are shown.

peak ratio values were respectively 3.77±0.3 (average of 93 cells from 8 different experiments) and 1.92±0.7 (average of 80 cells from 5 different experiments). Performing these same experiments after a two-hour pre-treatment with, and in the presence of, MβCD (0.5 mg/ml) dampened the AMPA-induced $[Ca^{2+}]_i$ increase to only 26% with respect to baseline (Fig. 4A, ratio 2.80±0.5: mean of 79 cells from 7 different experiments). In contrast, MβCD treatment completely blocked the kainic acid-induced $[Ca^{2+}]_i$ increase (mean of 76 cells from 5 different experiments, Fig. 4B). MβCD, by itself, failed to influence basal levels of $[Ca^{2+}]_i$ within the two-hour observation period (data not shown). When MβCD and kainic acid were applied together, no dampening was observed in cultured neurons or hippocampal slices, thus demonstrating that the effect of MβCD was due to its direct interaction with the agonist and/or its receptor.

Cyclodextrine effects on synaptic transmission in hippocampal slices treated with GABAergic agonist and/or antagonist

To verify if the reduction in PS observed during MβCD perfusion was due to potentiation of GABAergic transmission, two series of experiments were performed with perfusion of a $GABA_A$ receptor antagonist, BMI, and a $GABA_B$ receptor agonist, baclofen. $GABA_B$ antagonists were not used since the $GABA_B$ antagonist, CGP55845, has been shown to not induce significant changes in PS amplitude (data not shown) according to Chen et al. (2006), that indicates a low tonic level of $GABA_B$ receptor mediated modulation of synaptic excitation. In the first series of experiments, control and MβCD-pretreated slices were perfused with ACSF containing BMI, 10 μM for 15 min. This caused a progressive increase in PS intensity, up to an epileptogenic pattern that was reversible by washout (Figs. 5A–B). In subsequent experiments, slices were perfused with the $GABA_B$ receptor agonist, baclofen, which inhibited PS amplitude by reducing GluR-mediated excitatory synaptic transmission. These data are in agreement with previous findings that GABA-BR activation inhibits excitatory synaptic transmission by presynaptic inhibition of glutamate release (Davies et al., 1993; Isaacson et al., 1993; Ziakopoulos et al., 2000), as well as postsynaptic hyperpolarization of CA1 cells (Newberry and Nicoll, 1984). In these experiments, no difference between control and MβCD-treated slices was observed. Figs. 5C–D illustrates the effect of a 15-minute perfusion with baclofen 10 μM on PS. With and without MβCD pretreatment, a significant reduction of PS followed by a fast recovery was observed. We concluded that GABAergic neurotransmission was not affected by MβCD perfusion.

Cyclodextrine effects on long term potentiation (LTP) in hippocampal slices

In a previous study (Frank et al., 2004), we had demonstrated that lipid raft deregulation affected NMDA receptor activity, i.e., a two hour pretreatment with MβCD blocked the intracellular calcium influx

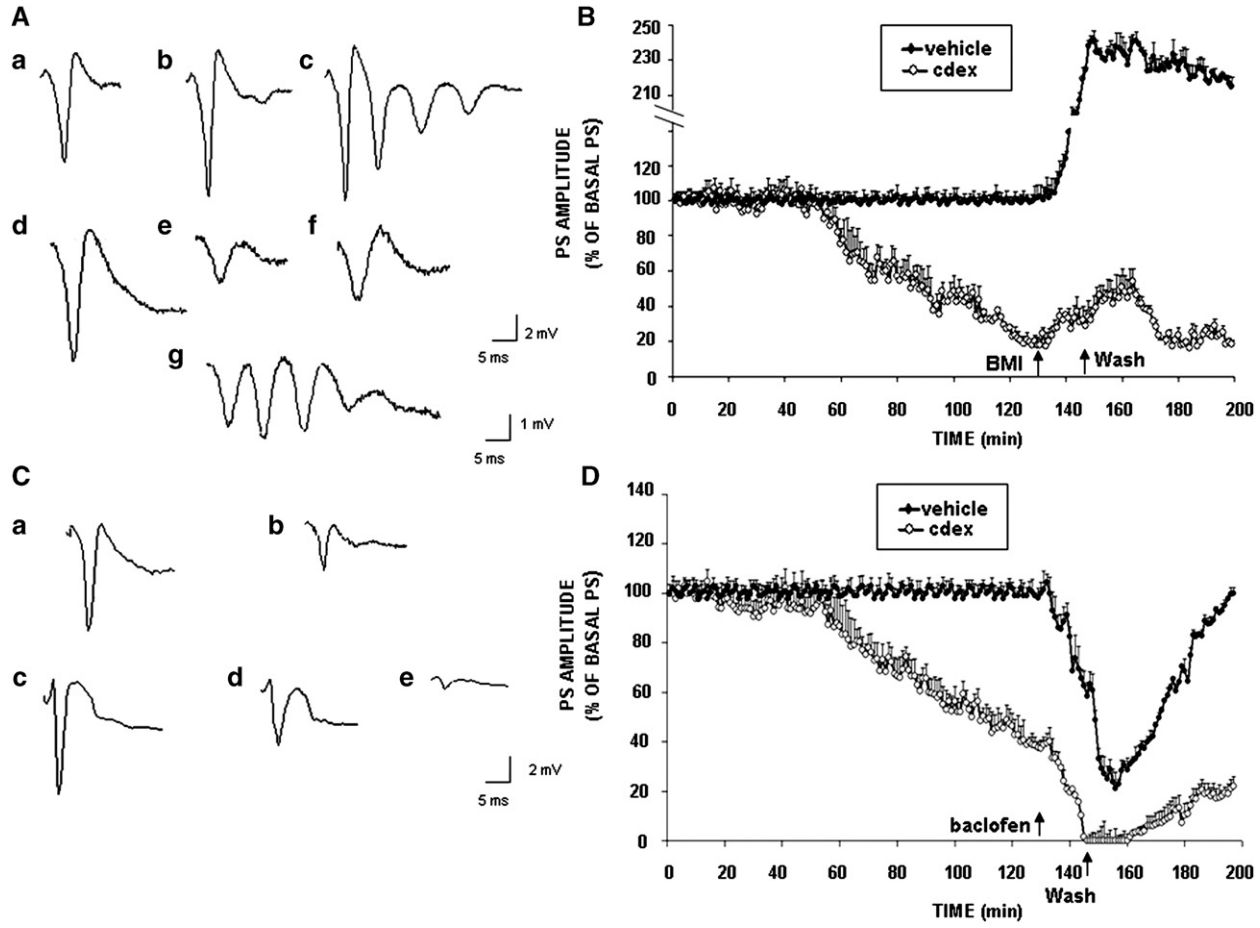

Fig. 5. Effects of bicuculline methiodide (BMI) and baclofen on BST in the CA1 region of the hippocampus in control and MβCD-treated slices. A: Representative recordings were obtained from slices before and after 10 and 15 min of BMI 10 μM perfusion under control conditions (a–c) and in the presence of 0.5 mg/ml MβCD (e–g). Curve (d) refers to PS at $t=1$ before MβCD perfusion. Curves represent the average of 6 recordings. B: % of PS amplitude as a function of time is shown under control conditions (vehicle ●; $n=4$) and in MβCD-treated slices (○, $n=4$) during perfusion with BMI. In both cases, treatment with BMI (first arrow) significantly increased, within several minutes, the amplitude of PS, inducing the generation of an epileptiform pattern. During washout (second arrow), this effect was maintained for about 30 min. The PS amplitude, calculated every minute, corresponds to an average of 6 recordings/min. Points in the plot are means±SEM of values obtained from different slices. C: Representative PS recorded from slices before and after 15 min of treatment with baclofen 10 μM in control (a–b) and in 0.5 mg/ml MβCD-treated slices (d–e). PS in (c) refers to basal PS before MβCD application. D: % of PS amplitude as a function of time is shown under control conditions (vehicle ●; $n=4$) or in the presence of MβCD (○, $n=5$) during perfusion of baclofen 10 μM. In both cases, application of baclofen (first arrow) significantly reduced PS amplitude. Washout (second arrow) reverted this effect during the observation period. The PS amplitude, calculated every minute, corresponds to an average of 6 recordings/min. Points on the plot are means±SEM of values obtained from different slices.

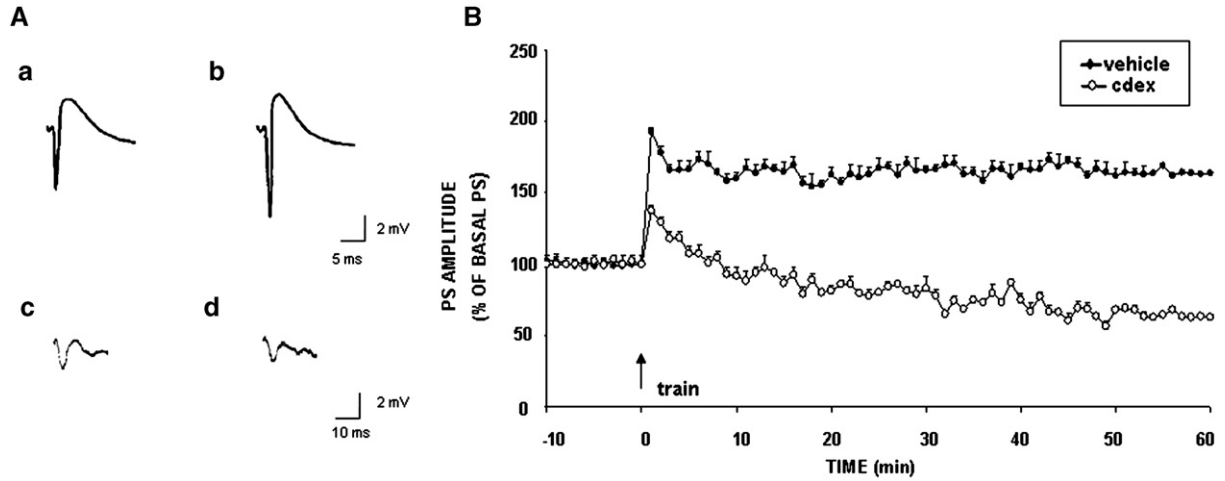

Fig. 6. Effects of MβCD on long-term potentiation (LTP) in the CA1 region of the hippocampus. A: Representative recordings were obtained from slices treated with vehicle (a–b) or 0.5 mg/ml MβCD (c–d). Curves (a) and (c) were recorded at time $t=-5$ while curves (b) and (d) at time $t=40$. Curves represent the average of 6 recordings/min. B: % PS amplitude as a function of time is shown after tetanic stimulation applied at time $t=0$ (arrow). Treatment with vehicle (●, $n=8$) or with 0.5 mg/ml MβCD (○, $n=6$) started 120 min before tetanic stimulation (starting values are referred as 100%). The PS amplitude, measured every minute, corresponds to an average of 6 recordings/min. Points in the plot are means±SEM of values obtained from different slices. Note that LTP induction and expression are blocked by MβCD perfusion.

provoked by perfusion of hippocampal cell cultures with NMDA. In the present study, we verified in the CA1 region of hippocampal slices the effect of cholesterol depletion on the activity of NMDA and AMPA responsive receptors, involved respectively in the induction and maintenance of LTP (Bliss and Collingridge, 1993; Malenka, 2003). After recording stable PS signals, a 100 Hz train stimulation was applied for 1 s (Fig. 6) to slices pretreated with MβCD for 120 min and to control, not pretreated slices. Results showed that in the presence of MβCD, the induction as well the expression of LTP were blocked.

Discussion

It is well known that an increase as well a decrease of cholesterol levels is involved in the pathogenesis of some neurodegenerative diseases (i.e. AD and HD) (Cutler et al., 2004; Valenza et al., 2007). The aim of the present work was to explore the effect of MβCD-induced cholesterol depletion on synaptic transmission and synaptic plasticity in the CA1 pyramidal cell layer of rat hippocampal slices, thus elucidating how altered lipid homeostasis might mediate pathogenic mechanisms responsible for neurodegeneration. MβCD-induced cholesterol depletion was shown to reduce basal synaptic transmission in the CA1 area in a time-dependent manner. The mechanisms responsible for this effect act at the postsynaptic level, as neither glutamate's nor GABA's mediation of paired-pulse facilitation and inhibition was altered by cholesterol depletion. Several studies carried out in synaptosomes and PC12 cells suggest that lipid rafts are highly enriched with SNARE proteins (Lang, 2007) and that cholesterol depletion greatly reduces calcium evoked neurotransmitter release from synaptosomes (Chamberlain et al, 2001; Gill et al., 2005). This divergence from our data is likely due to the different experimental protocol used in the vesicular release study. In synaptosomes, mediator release was studied after long-term depolarization by ionophore-mediated calcium influx, while in our model a low stimulation intensity that recruits different mechanisms was used.

The reduction of basal synaptic transmission in MβCD-treated slices indicated that depletion of plasma membrane cholesterol plays a critical role in glutamatergic rather than GABAergic neurotransmission. Replacement of cyclodextrin with a cholesterol-cyclodextrin complex reversed the effects of MβCD on synaptic transmission, thus proving that cholesterol depletion in neuronal membranes, rather than a direct effect of MβCD on neurotransmitter receptors, was responsible for the effects of MβCD on neurotransmission.

Since NMDA receptors are only partially involved in basal synaptic transmission (Collingridge et al., 1983), other possible targets for cholesterol modulation were investigated. The involvement of the excitatory amino acids kainate and AMPA receptors was evaluated and results demonstrated that the kainate receptor impairment had a pivotal role in basal synaptic transmission decrease. In control conditions, indeed, we showed that kainate perfusion, according to other authors (Sari and Kerr 2001; Hesp et al., 2004), induced in hippocampal slices transient neuronal hyperexcitability followed by pronounced depression of PS, while in MβCD-treated slices, these effects were totally prevented. These results were confirmed by the present finding that the kainate-induced intracellular calcium influx in pyramidale cell cultures was blocked by MβCD pretreatment, indicating for the first time that cholesterol directly affects kainate receptor activity. This sheds light on the strict link between plasma membrane cholesterol and kainate receptors in human neurodegenerative diseases. Cholesterol depletion more subtly modified AMPA-mediated effects on PS amplitude. AMPA perfusion initially caused potentiation of PS in both normal and cholesterol depleted slices; this potentiation was longer lasting in normal slices, and rapidly quenched in MβCD-pretreated slices. During washout, a progressive reduction of PS amplitude until its complete disappearance was observed in control slices, while loss of PS activity was not observed in MβCD-treated slices. Cholesterol depletion was also shown to modulate AMPA receptor activity in cultured cells, where a 26% reduction in early-AMPA-mediated calcium influx was shown after MβCD pretreatment. Hence, the present findings indicate that: 1) at least one AMPA receptor subpopulation activity is affected by cholesterol depletion; and 2) cholesterol does not directly influence AMPA receptor channel kinetics, but it may regulate other mechanisms such as the surface expression of AMPA receptors. Our conclusions are in agreement with Hering et al. (2003) and Allen et al. (2007), who suggested the presence of molecular subtypes of AMPA and kainate receptors in lipid rafts, and the reduction of their surface stability when lipid rafts are disrupted.

Overall, our data also indicate that the GABAergic system is not directly affected by disruption of membrane rafts, suggesting that cholesterol does not play a role in regulating its activation. The effect of MβCD on the $GABA_A$ receptor is still under debate (Dalskov et al., 2005; Pyytel et al., 2006). We observed that cholesterol depletion failed to prevent the PS amplitude upsurge induced by bicucullin. According to Sooksawate and Simmonds (2001), although GABA receptors appear to be embedded in membrane lipid rafts, GABA antagonists (i.e. bicuculline and picrotoxin) are not affected by membrane cholesterol depletion. Furthermore, these authors observed a decrease of GABA receptor agonist efficacy in cholesterol depleted neurons, suggesting that the reduction in synaptic transmission observed in our study was not due to an overmodulation of GABAergic transmission. Our results are also in line with those of the Dalskov group who demonstrated that the GABA receptors in cerebellar cells are located on an “unusual” type of cholesterol independent lipid rafts only marginally affected by MβCD (Dalskov et al., 2005). In our model, $GABA_B$ receptors were still active in the presence of MβCD, as shown by the further reduction of synaptic transmission induced by baclofen. Until now, a lipid raft localization of $GABA_B$ receptors in the hippocampus has never been demonstrated, although a previous biochemical study provided evidence for the association of a fraction of the $GABA_B$ receptors with lipid raft domains in the rat cerebellum (Becher et al, 2001). Nevertheless, it is still possible that a more extreme cholesterol depletion, induced by incubating slices with higher doses of MβCD, may have led to a complete suppression of synaptic activity.

Lastly, we verified in this study if in the CA1 region of hippocampal slices cholesterol depletion affects the induction and maintenance of LTP. We have previously demonstrated that MβCD pretreatment prevents the NMDA-dependent calcium influx in hippocampal pyramidal cultured cells (Frank et al., 2004). The present results also provide evidence that in the CA1 pyramidal region, cholesterol depletion inhibits LTP, probably by affecting NMDA and AMPA receptors, which are respectively involved in its induction and consolidation (Bliss and Collingridge, 1993; Malenka, 2003; Plant et al., 2006). This indicates that NMDA receptors are located in lipid rafts, an assertion in agreement with results of Koudinov and Koudinova (2001, 2005), who reported that cholesterol depletion greatly affected NMDA-induced long term potentiation in the hippocampal CA1 *stratum radiatum*. The present results also indicate involvement of the AMPA receptor in inhibition of LTP expression after cholesterol depletion, presumably due either to an altered function of the receptor pool on plasma membranes or to a reduced delivery of intracellular AMPA receptors into the postsynaptic membrane.

Data reported in the literature clearly provide evidence for a correlation between cholesterol homeostasis and neurotransmitter receptor dysfunction in the pathogenesis of several neurodegenerative diseases (Maxfield and Tabas, 2005). A possible unifying hypothesis could reside in cholesterol's role in stabilizing lipid rafts, whose presence has been demonstrated in several brain areas using different purification procedures (Allen et al., 2007).

A failure in the dynamics of cholesterol sphingolipid-enriched microdomains in the plasma membrane of neurons and glia may in fact trigger the insurgence and development of neurological symptoms by altering synaptic function. In conclusion, manipulation of the lipid components of plasma membrane rafts in neurons might provide a new approach to the treatment of neurodegenerative diseases,

leading not only to amelioration of neurological symptoms, but also to disease modification by directly targeting their molecular pathogenic mechanisms.

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