Published in final edited form as:

Neurosci Lett. 2010 August 2; 479(3): 327–331. doi:10.1016/j.neulet.2010.05.090.

Dietary cholesterol modulates the excitability of rabbit hippocampal CA1 pyramidal neurons

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Abstract

Previous work has shown high dietary cholesterol can affect learning and memory including rabbit eyeblink conditioning and this effect may be due to increased membrane cholesterol and enhanced hippocampal amyloid beta production. This study investigated whether dietary cholesterol modulates rabbit hippocampal CA1 neuron membrane properties known to be involved in rabbit eyeblink conditioning. Whole-cell current clamp recordings in hippocampal neurons from rabbits fed 2% cholesterol or normal chow for 8 weeks revealed changes including decreased after-hyperpolarization amplitudes (AHPs) – an index of membrane excitability shown to be important for rabbit eyeblink conditioning. This index was reversed by adding copper to drinking water – a dietary manipulation that can retard rabbit eyeblink conditioning. Evidence of cholesterol effects on membrane excitability was provided by application of methyl- β -cyclodextrin, a compound that reduces membrane cholesterol, which increased the excitability of hippocampal CA1 neurons.

Keywords

Cholesterol; Hippocampus; Pyramidal Neurons; Membrane Property; Excitability; Copper

Introduction

Cholesterol is crucial for normal brain function because it regulates synapse formation, neurotransmission, receptor function, and synaptic plasticity. Recent studies indicate that cholesterol can alter behavioral performance by modifying synaptic plasticity [1], affecting neuronal degeneration [1;2], amyloid beta (A β) production and senile plaques [3]. Our previous experiments show feeding rabbits a high cholesterol diet facilitates classical conditioning of both rabbit nictitating membrane response (NMR) [4;5] and heart rate [6], and this facilitation is a function of cholesterol diet duration [7] and concentration [5]. These findings are supported by clinical observations that high serum total cholesterol is associated with better memory in oldest old community-dwelling individuals who do not have the

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apolipoprotein e4 (APOE4) allele [8], and in healthy middle-aged women based on three years of lipid monitoring [9].

The addition of copper to drinking water in cholesterol-fed rabbits impaired NMR classical conditioning and induced Alzheimer-like neuropathology including elevated A β concentration, increased accumulation of A β and extracellular cortical and hippocampal A β plaques and hippocampus [3]. The cholesterol-induced changes in rabbit NMR conditioning have been corroborated by changes in hippocampal-dependent learning in cholesterol-fed rats [10-12], cholesterol-fed mice [13;14], and transgenic animals [14;15].

To date there are no reports showing whether neuronal membrane properties are modulated by dietary cholesterol. The current experiments address this question, assess the effects of adding copper to drinking water given cholesterol-fed rabbits, and investigate whether acutely reducing membrane cholesterol by applying methyl- β -cyclodextrin to hippocampal slices would affect excitability of CA1 pyramidal neurons.

Materials and Methods

Subjects

Male New Zealand rabbits (*Oryctolagus cuniculus*) weighing 2.0-2.2 kg were supplied by Harlan (Indianapolis, IN), housed in individual cages, given free access to food and ultra pure water (18 M Ω , Millipore Academic) and maintained on a 12-hour light/dark cycle. Rabbits were randomly assigned to three groups, cholesterol chow (Chol, n = 7), cholesterol chow and copper (Chol + Copper n = 3), and normal chow (Control, n = 12). Cholesterol-fed rabbits received 2% cholesterol incorporated into Purina rabbit chow (Dyets Inc., Bethlehem, PA) for 8 weeks before electrophysiological experiments and normal chow control rabbits received standard Purina rabbit chow (0% added cholesterol). Cholesterol-fed rabbits given copper received copper sulfate in distilled drinking water with a final copper concentration of 0.12ppm (0.12mg per liter). Rabbits were maintained in accordance with National Institutes of Health guidelines, and the research was approved by the West Virginia University Animal Care and Use Committee.

Slice preparation and patch-clamp recordings

Procedures identical to those previously published [16-18] were used for brain slices and patch clamp recordings and data analysis. Briefly, rabbits were anesthetized with 30 mg/kg sodium pentobarbital and then transcardially perfused with chilled artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl 125, KCl 3.0, MgSO₄ 1.2, CaCl₂ 2.0, NaH₂PO₄ 1.2, NaHCO₃ 26 and Dextrose 10. After 2 minutes of perfusion, rabbits were decapitated and the hippocampus was isolated and sliced into 400- μ m transverse slices on a Leica VT1000S Vibratome. Slices were incubated in O₂-CO₂-saturated ACSF a minimum of 1 h at room temperature. The electrophysiological recordings were timed to coincide with the point at which classical conditioning would have begun in our previous behavioral experiments [3-5;7].

Hippocampal CA1 neurons were visualized using an Olympus BX50WI microscope. Whole-cell patch-clamp recordings were performed at 32°C using an Axon MultiClamp 700B. Patch pipettes with tip resistance between 2 and 5 M Ω were filled with a solution containing (in mM) potassium gluconate (C₆H₁₁O₇K) 140, MgCl₂•6H₂O 4.6, HEPES 10, EGTA 10, Na₂ATP 4.0 (pH 7.3). Data were low-pass filtered at 2 kHz and acquired at 5–10 kHz. Membrane properties were measured when the neuron had stabilized for five minutes after whole-cell configuration was achieved. Quantitative analysis included resting membrane potential measured directly upon breakthrough in whole-cell configuration, input resistance based on membrane potential changes to depolarizing current injections

immediately after whole cell configuration, action potential (AP) threshold, current required for eliciting the first AP, half-width of elicited AP including rising and falling phases, amplitude of elicited AP, the number of elicited APs, latency to the first AP elicited by a 250-ms duration depolarizing current injection, and peak amplitude of the after-hyperpolarization (AHP). To obtain an accurate measurement of neuronal excitability independent of membrane potential changes, continuous direct current was applied through the recording electrode to hold the cell at a -70 mV baseline.

Methyl- β -Cyclodextrin (M β CD) was purchased from Sigma-Aldrich (St. Louis, MO). Concentrations up to 5 mM (1, 2, 5 mM) were selected because treatment with 5 mM M β CD can reduce total cellular cholesterol levels in hippocampal CA1 pyramidal cells by 60-70% without affecting cell viability or integrity [19].

Data are represented as means $\pm$ SEM. One way ANOVA was used with $p < 0.05$ as a criterion for significance.

Results

To assess dietary cholesterol effects on membrane properties of rabbit hippocampal CA1 neurons, whole cell current clamp recordings were performed on 60 hippocampal CA1 neurons: 25 from rabbits fed normal chow, 22 from rabbits fed 2% cholesterol for 8 weeks, and 13 from rabbits fed 2% cholesterol with copper added to drinking water for 8 weeks. Each of these neurons was silent at resting membrane potential but showed action potentials in response to depolarizing current. Table 1 shows a summary of membrane properties of hippocampal CA1 neurons from rabbits fed normal chow, cholesterol, and cholesterol plus copper.

As seen in Table 1, hippocampal CA1 neurons from rabbits fed cholesterol or normal chow exhibited similar resting membrane potentials, action potential thresholds and amplitudes. However, analysis of variance of input resistance yielded a significant group effect [$F(2, 54) = 3.568$, $p < 0.05$] and post hoc comparisons confirmed a significant difference between the Chol and Control groups ($p < 0.05$). This suggests dietary cholesterol may alter the intrinsic membrane properties via regulation of ion channel number [20]. The addition of copper decreased the membrane potential (-60.2 ± 3.07 mV) in the Chol + Copper group compared to the Control (-66.5 ± 0.71 mV) and Chol groups (-68.1 ± 1.03 mV). A one-way ANOVA yielded a significant group effect [$F(2, 58) = 5.077$, $p < 0.001$] and post hoc comparisons confirmed the significant difference in membrane potential between the Chol + Copper group and both Control and Chol groups (p 's < 0.05).

Figure 1A depicts typical APs elicited in hippocampal CA1 neurons from rabbits in the Control, Chol, and Chol + Copper groups. The recordings show and Table 1 summarizes cholesterol-fed rabbits had shortened AP durations and a significant decrease in the falling phase of the AP suggesting dietary cholesterol may have altered the AP duration by regulating the rapid efflux of potassium ions during repolarization. One-way ANOVA of AP duration and AP falling phase duration yielded significant group effects [$F(2, 58) = 15.143$, $p < 0.001$, and $F(2, 51) = 9.756$, $p < 0.001$, respectively] and post hoc comparisons confirmed significant differences in AP duration and AP falling phase duration between the Control group and both Chol and Chol + Copper groups ($p < 0.05$, and $p < 0.001$, respectively). If compared to recordings from neurons in the Control and Chol groups, neurons in the Chol + Copper group also showed a decrease in the AP rising phase duration. A one-way ANOVA of AP rising phase duration yielded a significant group effect [$F(2, 51) = 6.145$, $p < 0.001$] which a post hoc comparison confirmed to be due to a significant difference between the Chol + Copper and Control groups ($p < 0.001$).

Importantly, Figure 1C and Table 1 show that AHPs in rabbits fed cholesterol were significantly lower than those in the Control group. A reduction in the AHP is recognized as a learning-induced change in the excitability of hippocampal CA1 neurons [21;22]. This suggests dietary cholesterol may be able to enhance learning by its action on the AHP of hippocampal CA1 neurons. Even more interestingly, the decreased AHP found in cholesterol-fed rabbits was reversed by addition of copper to drinking water (Figure 1C). This copper-induced change in the AHP may underlie the retarded learning previously reported in cholesterol-fed rabbits given copper in their drinking water. A one-way ANOVA analysis yielded a significant group effect [$F(2, 58) = 3.447, p < 0.05$] and post hoc comparisons confirmed the significant difference in AHP between the Chol group and the Control and Chol and Chol + Copper groups (p 's < 0.05).

As summarized in Table 1, dietary cholesterol resulted in increased current required to elicit an AP. Analysis of variance of current for eliciting the first AP yielded a significant group effect [$F(2, 58) = 14.199, p < 0.001$] and post hoc comparisons confirmed a significant difference between the Control group and Chol and Chol + Copper groups (all p 's < 0.01). This is consistent with the reduced input resistance observed in the Chol group.

To further explore whether changes in membrane properties of hippocampal CA1 neurons were caused by membrane cholesterol, membrane excitability was assessed after membrane cholesterol was reduced by acute application of soluble M β CD to the bath. The top and middle panels of Figure 2 show typical responses of hippocampal CA1 neurons to a 0.5-nA depolarizing current step, recorded before and after M β CD application. The bottom panel of Figure 2 shows M β CD dose-dependently increased the number of elicited APs and significantly changed the firing pattern. A one-way ANOVA yielded a significant group effect [$F(2, 10) = 5.121, p < 0.05$] for the number of elicited APs after M β CD application, a post hoc comparison confirmed the dose-dependent effect with significantly more APs at both 2 and 5mM than at 1mM (p 's < 0.05). These M β CD data provide confirmation that changes in membrane cholesterol can affect the excitability of hippocampal CA1 neurons.

Discussion

In the present study we found clear and striking effects of dietary cholesterol and copper drinking water on membrane properties of hippocampal CA1 neurons. On the one hand, there was a dietary cholesterol-induced decrease in the amplitude of AHPs – an index of membrane excitability shown to be important for classical conditioning of the rabbit NMR [16;23]. This index was reversed by addition of copper to drinking water – a dietary manipulation we have shown to retard classical conditioning of the rabbit NMR [3]. On the other hand, there were dietary cholesterol-induced reduction in input resistance and increases in the current required to evoke APs not affected by copper in the drinking water, and decreases in AP duration that were exacerbated by copper. Taken together, these cholesterol-induced changes in membrane excitability may help explain some of the effects of dietary cholesterol on learning and memory found in a number of different animal models. Finally, evidence of cholesterol's effects on membrane excitability is provided by application of methyl- β -cyclodextrin which is known to alter membrane cholesterol and that we show increases the excitability of hippocampal CA1 neurons.

The AHP has previously been reported to be important for regulating spike frequency accommodation in hippocampal CA1 neurons, and therefore regarded as a general mechanism engaged in the process of hippocampal-dependent learning and memory [16;23;24]. Conditioning-specific AHP reduction can result in increased excitability in hippocampal CA1 neurons from conditioned animals versus controls, and changes in AHP, encoded by calcium-activated potassium channels, are independent of modification in

synaptic properties [23]. Consistent with our behavioral and slice recording copper data, AHP increases have been reported in old animals [24-26] and correlated with impaired learning and memory in aged rats [24;26], mice [27], and rabbits [23;28]. The AHP reductions seen here are presumably cholesterol-specific because dietary cholesterol can increase membrane cholesterol levels in hippocampal CA1 neurons [29], and cholesterol itself can regulate both the calcium-activated potassium channels [30;31] and the interaction between large-conductance and intermediate conductance calcium-activated potassium channels [32]. In fact, increased membrane cholesterol can suppress calcium-activated potassium channel activity [33] providing a direct mechanistic link between dietary cholesterol, AHP reduction, and increased learning-specific membrane excitability.

Another interesting finding was the decreased input resistance and increased current required to evoke APs in the Chol group compared with the Control group, which represents another index of membrane excitability. This provides further evidence that dietary cholesterol can modulate the intrinsic membrane properties by regulating a number of ion channels [20] and reiterates the complexity cholesterol effects have on different indices of membrane excitability. Levitan et al. (2010) have documented a range of cholesterol effects on voltage-dependent and inwardly-rectifying potassium channels as well as voltage-dependent sodium currents responsible for regulating membrane excitability [33].

Copper is an essential trace element and serves as a modulator of neuronal excitability under both physiological and pathophysiological conditions. Addition of copper to water given cholesterol-fed rabbits reversed cholesterol-induced AHP reduction suggesting copper may directly regulate calcium-activated potassium channels [34;35]. Addition of copper also resulted in decreases in AP duration, AP amplitude, and resting membrane potential (Table 1). These changes suggest the combination of cholesterol and copper may directly affect the function of a number of different voltage- and ligand-gated ion channels including inhibition of sodium channels [36;37], transient A-type potassium currents [36;38], delayed rectifier-type potassium channels [36], and voltage-gated calcium channels [36]. The addition of copper to cholesterol may also influence clearance of excessive A β caused by the dietary cholesterol [3;7;39] and result in beta amyloid plaques [3].

In contrast to previous studies demonstrating a shortened AP duration and increased AP peak amplitude resulting from cholesterol level decreases in rat hippocampal neurons [40], and a down-regulated gene transcription of transient outward and delayed rectified potassium channel in the hearts of rabbits fed with 1.5% dietary cholesterol for 8 weeks [41], we found dietary cholesterol shortened AP duration but did not alter AP amplitude. This indicates shortened AP duration in our cholesterol-fed rabbits may function as a result of a molecular cascade secondary to dietary cholesterol but not to cholesterol itself.

Finally, our study shows membrane cholesterol depletion by M β CD increased the excitability of hippocampal CA1 neurons (Figure 2). This increased excitability may be due to the decrease in calcium-activated potassium channel by M β CD-induced cholesterol depletion rather than a direct inhibitory action of M β CD [31].

Taken together, the present patch clamp recordings in hippocampal slices from cholesterol-fed rabbits indicate there are clear effects of cholesterol on the excitability of CA1 pyramidal cells that can be further altered by adding copper to the water. Changes in excitability mediated by altered after-hyperpolarization can explain the behavioral effects of cholesterol and copper on classical conditioning of the rabbit NMR.

Acknowledgments

This project was supported by NIH grant AG023211 and BRNI funds to Bernard G. Schreurs. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the NIA.

We are grateful to Carrie L. Smith-Bell for animal care, Dr. Lauren B. Burhans, Jimena Gonzales-Joekes, and Deya S. Darwish for assistance during transcatheter perfusion, Wen Zheng for reading the manuscript.

Reference List

1. Koudinov AR, Koudinova NV. Essential role for cholesterol in synaptic plasticity and neuronal degeneration. *FASEB J*. 2001; 15:1858–1860. [PubMed: 11481254]
2. Sparks DL. Cholesterol metabolism and brain amyloidosis: evidence for a role of copper in the clearance of A β through the liver. *Curr Alzheimer Res*. 2007; 4:165–169. [PubMed: 17430242]
3. Sparks DL, Schreurs BG. Trace amounts of copper in water induce beta-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2003; 100:11065–11069. [PubMed: 12920183]
4. Schreurs BG, Smith-Bell CA, Lochhead J, Sparks DL. Cholesterol modifies classical conditioning of the rabbit (*Oryctolagus cuniculus*) nictitating membrane response. *Behav Neurosci*. 2003; 117:1220–1232. [PubMed: 14674842]
5. Schreurs BG, Smith-Bell CA, Darwish DS, Stankovic G, Sparks DL. High dietary cholesterol facilitates classical conditioning of the rabbit's nictitating membrane response. *Nutr Neurosci*. 2007; 10:31–43. [PubMed: 17539481]
6. Schreurs BG, Smith-Bell CA, Darwish DS, Wang D, Burhans LB, Gonzales-Joekes J, Deci S, Stankovic G, Sparks DL. Cholesterol enhances classical conditioning of the rabbit heart rate response. *Behav Brain Res*. 2007; 181:52–63. [PubMed: 17466388]
7. Schreurs BG, Smith-Bell CA, Darwish DS, Stankovic G, Sparks DL. Classical conditioning of the rabbit's nictitating membrane response is a function of the duration of dietary cholesterol. *Nutr Neurosci*. 2007; 10:159–168. [PubMed: 18019398]
8. West R, Beeri MS, Schmeidler J, Hannigan CM, Angelo G, Grossman HT, Rosendorff C, Silverman JM. Better memory functioning associated with higher total and low-density lipoprotein cholesterol levels in very elderly subjects without the apolipoprotein e4 allele. *Am J Geriatr Psychiatry*. 2008; 16:781–785. [PubMed: 18757771]
9. Henderson VW, Guthrie JR, Dennerstein L. Serum lipids and memory in a population based cohort of middle age women. *J Neurol Neurosurg Psychiatry*. 2003; 74:1530–1535. [PubMed: 14617710]
10. Dufour F, Liu QY, Gusev P, Alkon D, Atzori M. Cholesterol-enriched diet affects spatial learning and synaptic function in hippocampal synapses. *Brain Res*. 2006; 1103:88–98. [PubMed: 16814755]
11. Granholm AC, Bimonte-Nelson HA, Moore AB, Nelson ME, Freeman LR, Sambamurti K. Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat. *J Alzheimers Dis*. 2008; 14:133–145. [PubMed: 18560126]
12. Micale V, Scapagnini G, Colombrita C, Mazzola C, Alkon DL, Drago F. Behavioral effects of dietary cholesterol in rats tested in experimental models of mild stress and cognition tasks. *Eur Neuropsychopharmacol*. 2008; 18:462–471. [PubMed: 18222653]
13. Mogi M, Tsukuda K, Li JM, Iwanami J, Min LJ, Sakata A, Fujita T, Iwai M, Horiuchi M. Inhibition of cognitive decline in mice fed a high-salt and cholesterol diet by the angiotensin receptor blocker, olmesartan. *Neuropharmacology*. 2007; 53:899–905. [PubMed: 18028965]
14. Thirumangalakudi L, Prakasam A, Zhang R, Bimonte-Nelson H, Sambamurti K, Kindy MS, Bhat NR. High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice. *J Neurochem*. 2008; 106:475–485. [PubMed: 18410513]
15. Hutter-Paier B, Huttunen HJ, Puglielli L, Eckman CB, Kim DY, Hofmeister A, Moir RD, Domnitz SB, Frosch MP, Windisch M, Kovacs DM. The ACAT inhibitor CP-113,818 markedly reduces

- amyloid pathology in a mouse model of Alzheimer's disease. *Neuron*. 2004; 44:227–238. [PubMed: 15473963]
16. Schreurs BG, Gusev PA, Tomsic D, Alkon DL, Shi T. Intracellular correlates of acquisition and long-term memory of classical conditioning in Purkinje cell dendrites in slices of rabbit cerebellar lobule HVI. *J Neurosci*. 1998; 18:5498–5507. [PubMed: 9651230]
 17. Wang D, Schreurs BG. Characteristics of IA currents in adult rabbit cerebellar Purkinje cells. *Brain Res*. 2006; 1096:85–96. [PubMed: 16716270]
 18. Moyer JR Jr, Brown TH. Methods for whole-cell recording from visually preselected neurons of perirhinal cortex in brain slices from young and aging rats. *J Neurosci Methods*. 1998; 86:35–54. [PubMed: 9894784]
 19. Simons M, Keller P, De SB, Beyreuther K, Dotti CG, Simons K. Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci U S A*. 1998; 95:6460–6464. [PubMed: 9600988]
 20. Luebke JI, Rosene DL. Aging alters dendritic morphology, input resistance, and inhibitory signaling in dentate granule cells of the rhesus monkey. *J Comp Neurol*. 2003; 460:573–584. [PubMed: 12717715]
 21. Matthews EA, Weible AP, Shah S, Disterhoft JF. The BK-mediated fAHP is modulated by learning a hippocampus-dependent task. *Proc Natl Acad Sci U S A*. 2008; 105:15154–15159. [PubMed: 18799739]
 22. Matthews EA, Linardakis JM, Disterhoft JF. The fast and slow afterhyperpolarizations are differentially modulated in hippocampal neurons by aging and learning. *J Neurosci*. 2009; 29:4750–4755. [PubMed: 19369544]
 23. Coulter DA, Lo Turco JJ, Kubota M, Disterhoft JF, Moore JW, Alkon DL. Classical conditioning reduces amplitude and duration of calcium-dependent afterhyperpolarization in rabbit hippocampal pyramidal cells. *J Neurophysiol*. 1989; 61:971–981. [PubMed: 2542473]
 24. Landfield PW, Pitler TA. Prolonged Ca²⁺-dependent afterhyperpolarizations in hippocampal neurons of aged rats. *Science*. 1984; 226:1089–1092. [PubMed: 6494926]
 25. Hemond P, Jaffe DB. Caloric restriction prevents aging-associated changes in spike-mediated Ca²⁺ accumulation and the slow afterhyperpolarization in hippocampal CA1 pyramidal neurons. *Neuroscience*. 2005; 135:413–420. [PubMed: 16112472]
 26. Tombaugh GC, Rowe WB, Rose GM. The slow afterhyperpolarization in hippocampal CA1 neurons covaries with spatial learning ability in aged Fisher 344 rats. *J Neurosci*. 2005; 25:2609–2616. [PubMed: 15758171]
 27. Kaczorowski CC, Disterhoft JF. Memory deficits are associated with impaired ability to modulate neuronal excitability in middle-aged mice. *Learn Mem*. 2009; 16:362–366. [PubMed: 19470651]
 28. Power JM, Wu WW, Sametsky E, Oh MM, Disterhoft JF. Age-related enhancement of the slow outward calcium-activated potassium current in hippocampal CA1 pyramidal neurons in vitro. *J Neurosci*. 2002; 22:7234–7243. [PubMed: 12177218]
 29. Ghribi O, Larsen B, Schrag M, Herman MM. High cholesterol content in neurons increases BACE, beta-amyloid, and phosphorylated tau levels in rabbit hippocampus. *Exp Neurol*. 2006; 200:460–467. [PubMed: 16696972]
 30. Chang HM, Reitstetter R, Mason RP, Gruener R. Attenuation of channel kinetics and conductance by cholesterol: an interpretation using structural stress as a unifying concept. *J Membr Biol*. 1995; 143:51–63. [PubMed: 7714888]
 31. Shmygol A, Noble K, Wray S. Depletion of membrane cholesterol eliminates the Ca²⁺-activated component of outward potassium current and decreases membrane capacitance in rat uterine myocytes. *J Physiol*. 2007; 581:445–456. [PubMed: 17331986]
 32. Romanenko VG, Roser KS, Melvin JE, Begenisich T. The role of cell cholesterol and the cytoskeleton in the interaction between IK1 and maxi-K channels. *Am J Physiol Cell Physiol*. 2009; 296:C878–C888. [PubMed: 19176762]
 33. Levitan I, Fang Y, Rosenhouse-Dantsker A, Romanenko V. Cholesterol and ion channels. *Subcell Biochem*. 2010; 51:509–549. [PubMed: 20213557]
 34. Ma Z, Wong KY, Horrigan FT. An extracellular Cu²⁺ binding site in the voltage sensor of BK and Shaker potassium channels. *J Gen Physiol*. 2008; 131:483–502. [PubMed: 18443360]

35. Morera FJ, Wolff D, Vergara C. External copper inhibits the activity of the large-conductance calcium- and voltage-sensitive potassium channel from skeletal muscle. *J Membr Biol.* 2003; 192:65–72. [PubMed: 12647034]
36. Horning MS, Trombley PQ. Zinc and copper influence excitability of rat olfactory bulb neurons by multiple mechanisms. *J Neurophysiol.* 2001; 86:1652–1660. [PubMed: 11600628]
37. Xiong W, Li RA, Tian Y, Tomaselli GF. Molecular motions of the outer ring of charge of the sodium channel: do they couple to slow inactivation? *J Gen Physiol.* 2003; 122:323–332. [PubMed: 12913092]
38. Alshuaib WB, Mathew MV. Inhibition of transient K⁺ current by copper in *Drosophila* neurons. *Neurochem Res.* 2004; 29:785–789. [PubMed: 15098942]
39. Sparks DL, Petanceska S, Sabbagh M, Connor D, Soares H, Adler C, Lopez J, Ziolkowski C, Lochhead J, Browne P. Cholesterol, copper and Abeta in controls, MCI, AD and the AD cholesterol-lowering treatment trial (ADCLT). *Curr Alzheimer Res.* 2005; 2:527–539. [PubMed: 16375656]
40. Guo J, Chi S, Xu H, Jin G, Qi Z. Effects of cholesterol levels on the excitability of rat hippocampal neurons. *Mol Membr Biol.* 2008; 25:216–223. [PubMed: 18428037]
41. Varga A, Bagossi P, Tozser J, Peitl B, Szilvassy Z. Effect of experimental hypercholesterolaemia on K⁺ channel alpha-subunit mRNA levels in rabbit hearts. *Eur J Pharmacol.* 2007; 562:130–131. [PubMed: 17346699]

Fig. 1. High dietary cholesterol reduced AHP amplitude and shortened the duration of elicited AP in hippocampal CA1 neurons

A represents typical APs evoked in hippocampal CA1 neurons from rabbits in Control, Chol, and Chol + Copper groups. **B**, **C**, and **D** represent AP measurements taken, AHP amplitude, and half width of AP, respectively. Note dietary cholesterol markedly decreased AHP amplitude, but copper addition reversed this change. There was a shortening of APs observed in rabbits fed cholesterol and cholesterol + copper.

Fig. 2. Membrane cholesterol removal increased the excitability of hippocampal CA1 neurons

Top and middle panels show typical recordings from hippocampal CA1 neurons before and after bath application of 5mM M β CD. Note that M β CD significantly changed both the firing frequency and firing pattern.

Histogram in the bottom panel shows M β CD significantly increased the number of elicited APs as a function of concentration.

Table 1

Summary of membrane properties of rabbit hippocampal CA1 neurons

Membrane property	Control	Chol	Chol + Copper
V _m (mV)	-66.5 ± 0.71	-68.1 ± 1.03	-60.2 ± 3.07 [*]
Input resistance (MΩ)	72.87 ± 6.95	52.46 ± 4.63 [*]	55.13 ± 7.86
AP threshold (mV)	-44.08 ± 1.72	-43.66 ± 1.76	-43.87 ± 1.23
AP amplitude (mV)	87.3 ± 2.89	89.24 ± 2.91	69.23 ± 2.63 ^{□□□□}
AP duration (ms)	1.66 ± 0.10	1.19 ± 0.10 ^{**}	0.81 ± 0.07 ^{***,□}
AP rising duration (ms)	0.46 ± 0.03	0.38 ± 0.03	0.31 ± 0.03 ^{***}
AP falling duration (ms)	1.09 ± 0.1	0.81 ± 0.08 [*]	0.5 ± 0.05 ^{***,□□}
AHP (mV)	-5.14 ± 1.04	-2.31 ± 0.79 [*]	-6.25 ± 1.45 [□]
Latency (ms)	69.85 ± 14.60	57.96 ± 14.64	37.71 ± 12.36
Current required for first elicited AP (nA)	0.17 ± 0.02	0.35 ± 0.03 ^{***}	0.32 ± 0.04 ^{***}

Cell number (n) for Control, Chol, Chol + Copper was 25, 22, and 13, respectively.

AP: Action potential; AP duration was measured at the width of 50% AP duration.

^{*}, ^{**}, ^{***} indicated $p < 0.05$, 0.01, 0.001, respectively, as compared to Control.

[□], ^{□□}, ^{□□□□} indicated $p < 0.05$, 0.01, 0.001, respectively, as compared to Chol.