Cholesterol:

is it that bad biophysically?

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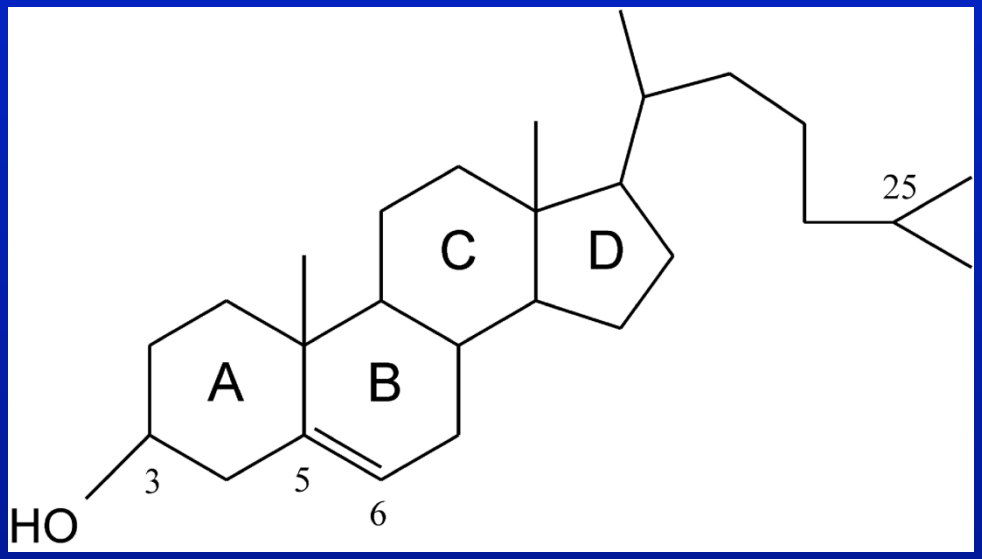
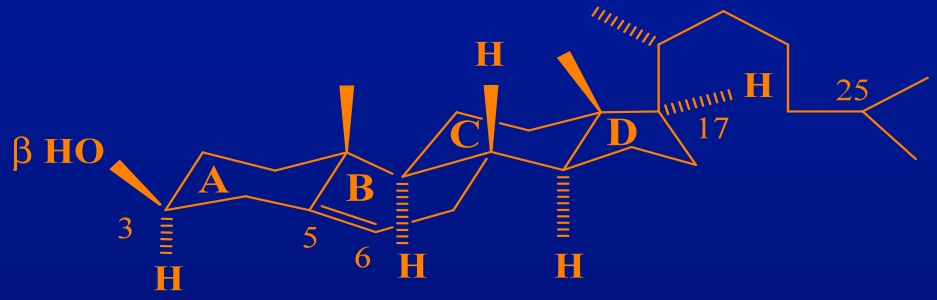
“Cholesterol, the central lipid of mammalian cells”

Maxfield & van Meer, 2010



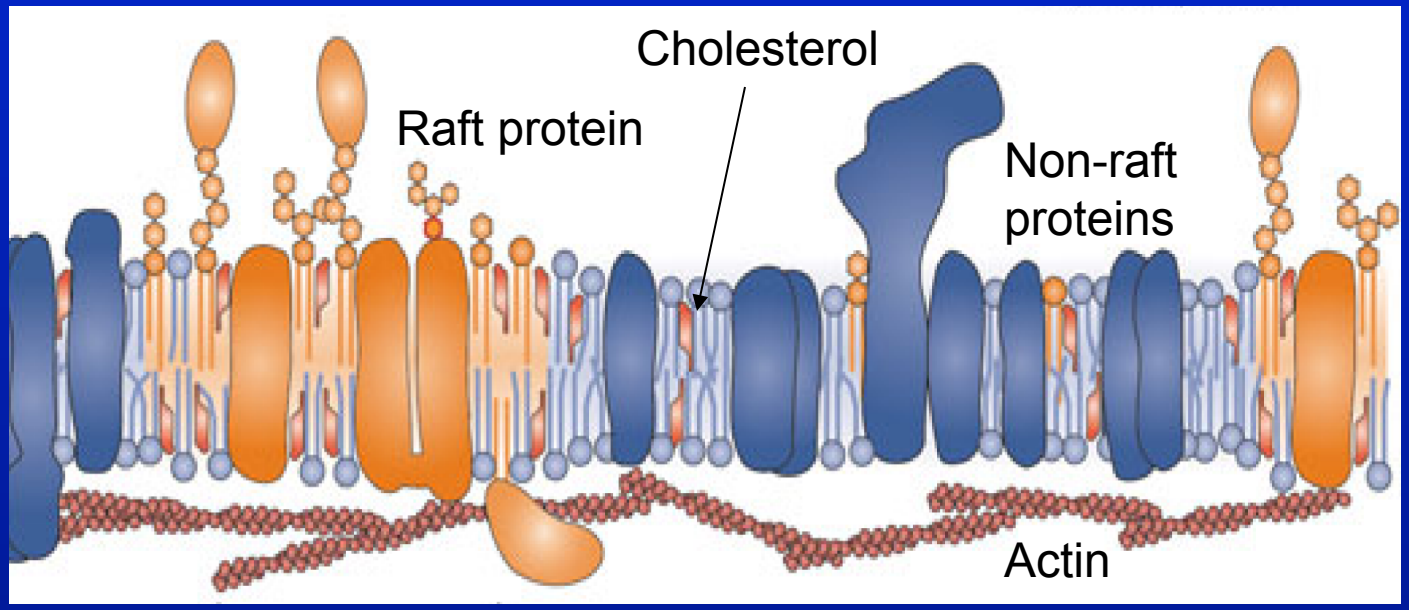


Endogenous synthesis

Dietary intake

1. One of the most abundant lipids
2. Essential for the synthesis of steroid hormones and bile acids
3. Transported by blood, found in cell membranes

Role of plasma membrane cholesterol in cell membrane architecture and function



Concentration varies 5-10 times in different organelles

Highest level is in plasma membrane (30-50mol%)

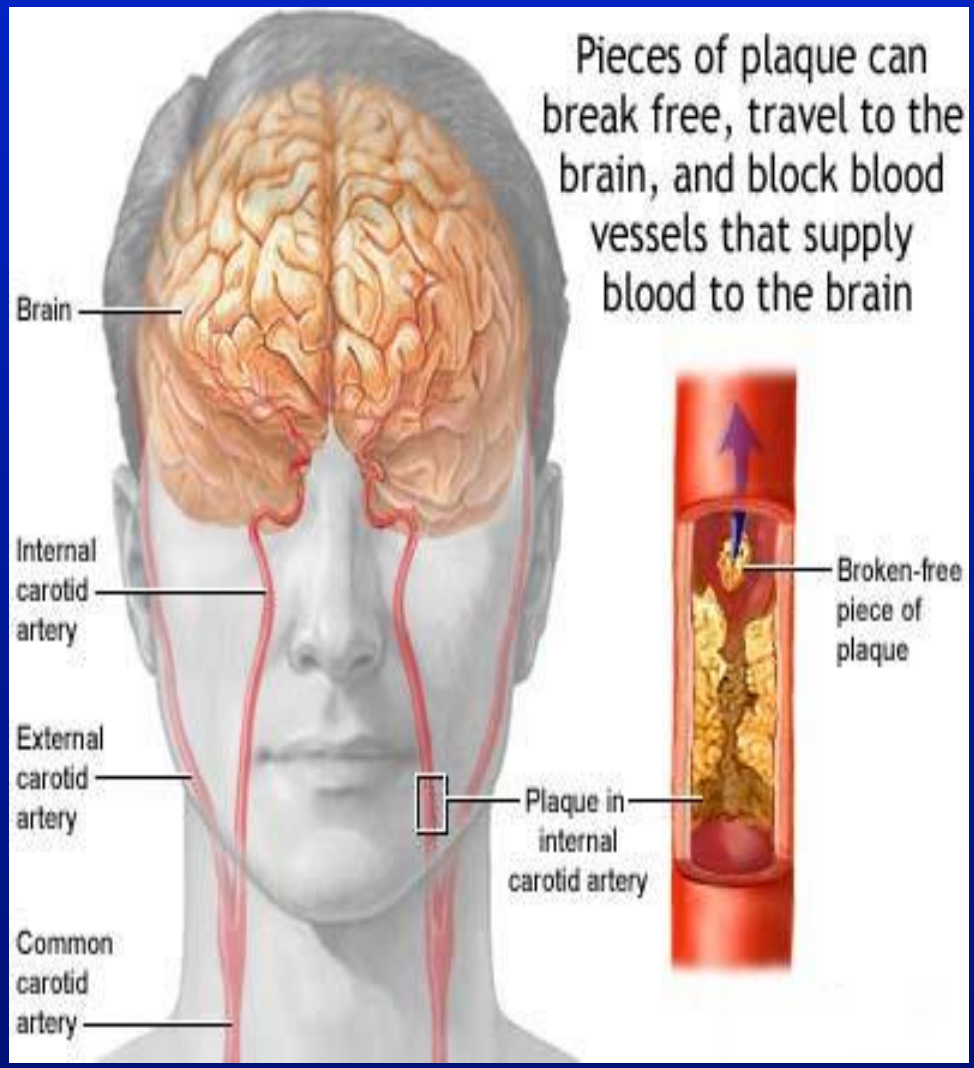
1. Prevents proton leakage across the membrane;
2. Promotes tight lipid packing/condensation;
3. Promotes formation of detergent-resistant domains – lipid rafts;
4. Regulates activity of numerous membrane proteins, including ion channels.

Picture with modifications from Simons & Gerl, 2010

Why is cholesterol considered to be bad?

1. High cholesterol intake causes hypercholesterolemia and atherosclerosis
2. Atherosclerosis – thickening of arterial wall due to build-up of fatty material and cholesterol
3. Pathological results:

   ischemic stroke, ischemic heart disease, peripheral vascular disease

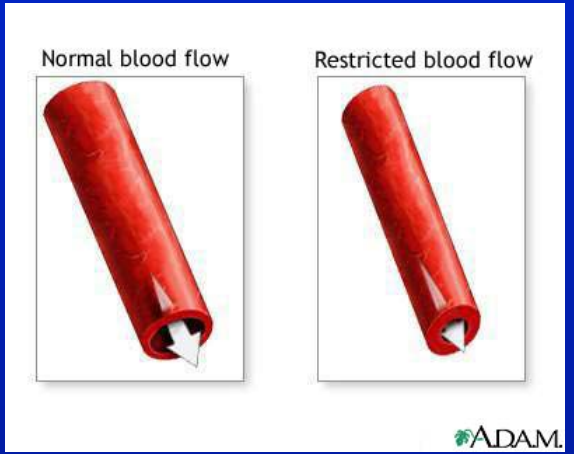


High-cholesterol diet has dual effect on alcohol-induced cerebral artery constriction

Some of the data presented at Faculty for Undergraduate Neuroscience (FUN) meeting, San Diego CA, November 15, 2010 .

Why cholesterol-alcohol research?

1. Binge drinking (moderate-to heavy consumption of alcohol over a short period of time) is a well known cause of cerebral ischemia and stroke.

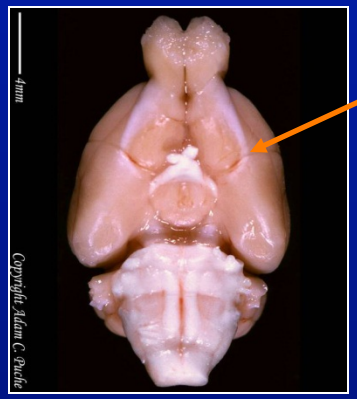


2. High cholesterol intake – is independent risk factor for ischemic stroke and related pathology.

Can cholesterol presence in arterial wall worsen ethanol-induced constriction of cerebral arteries?

Model: rat middle cerebral artery from control animals vs. animals on high cholesterol diet

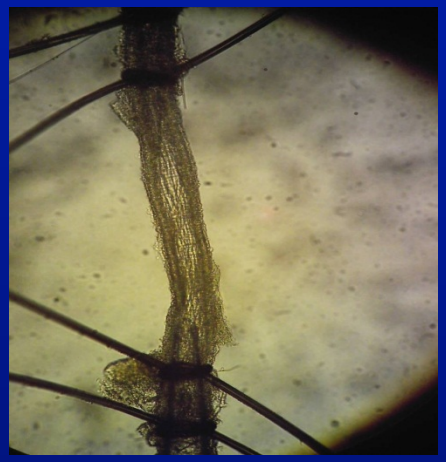
A



Middle cerebral artery

B

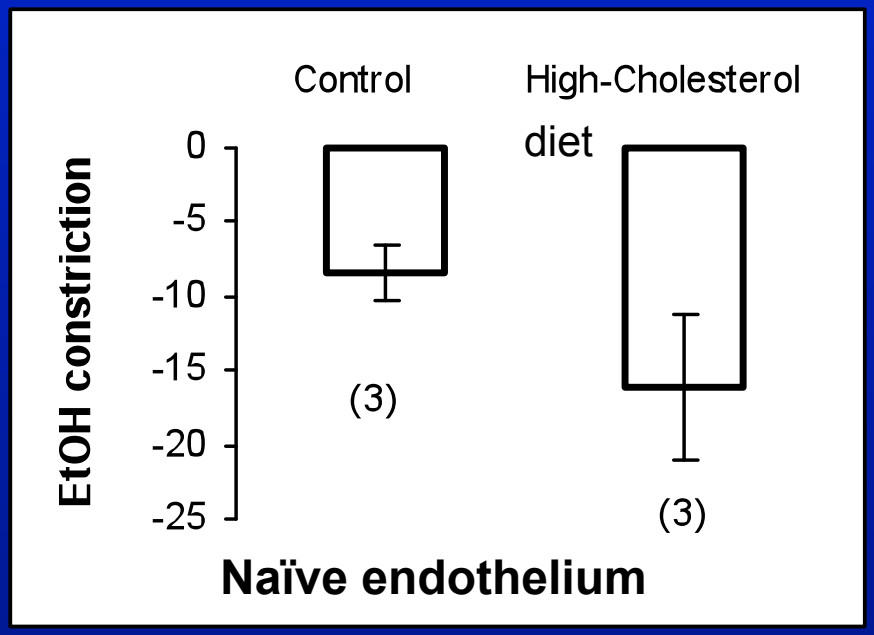
Dissection of the artery, then – pressurization at 60 mmHg

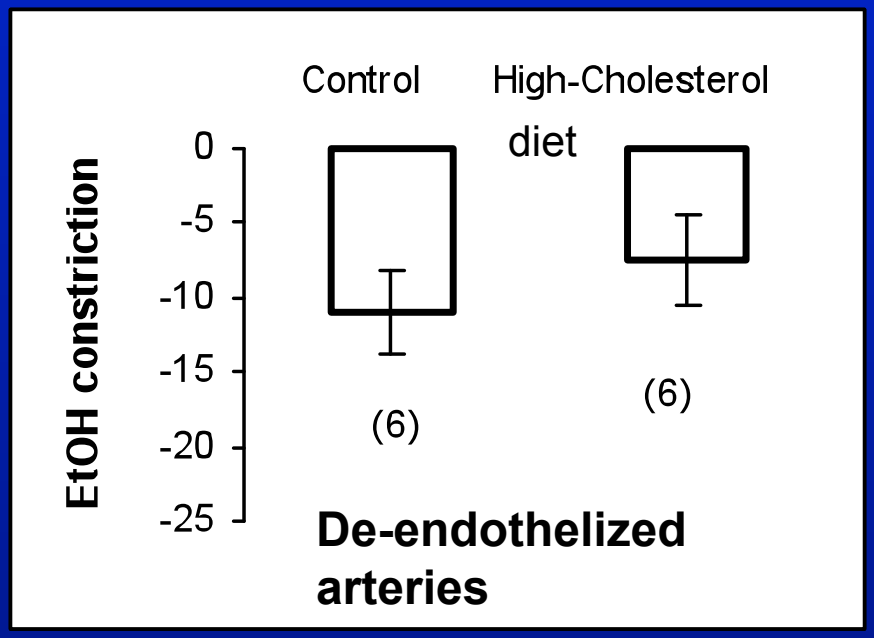


C

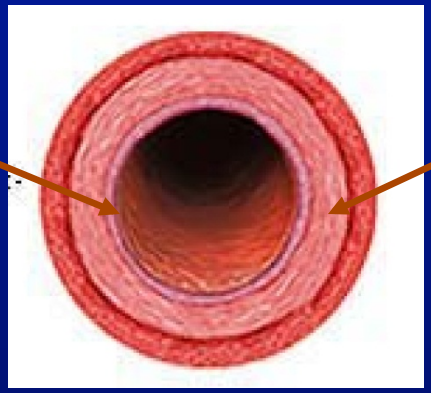
Application of Ethanol (50 mM) and measurement of changes in arterial diameter

High-cholesterol intake differentially affects alcohol-induced constriction of arteries with and without endothelium



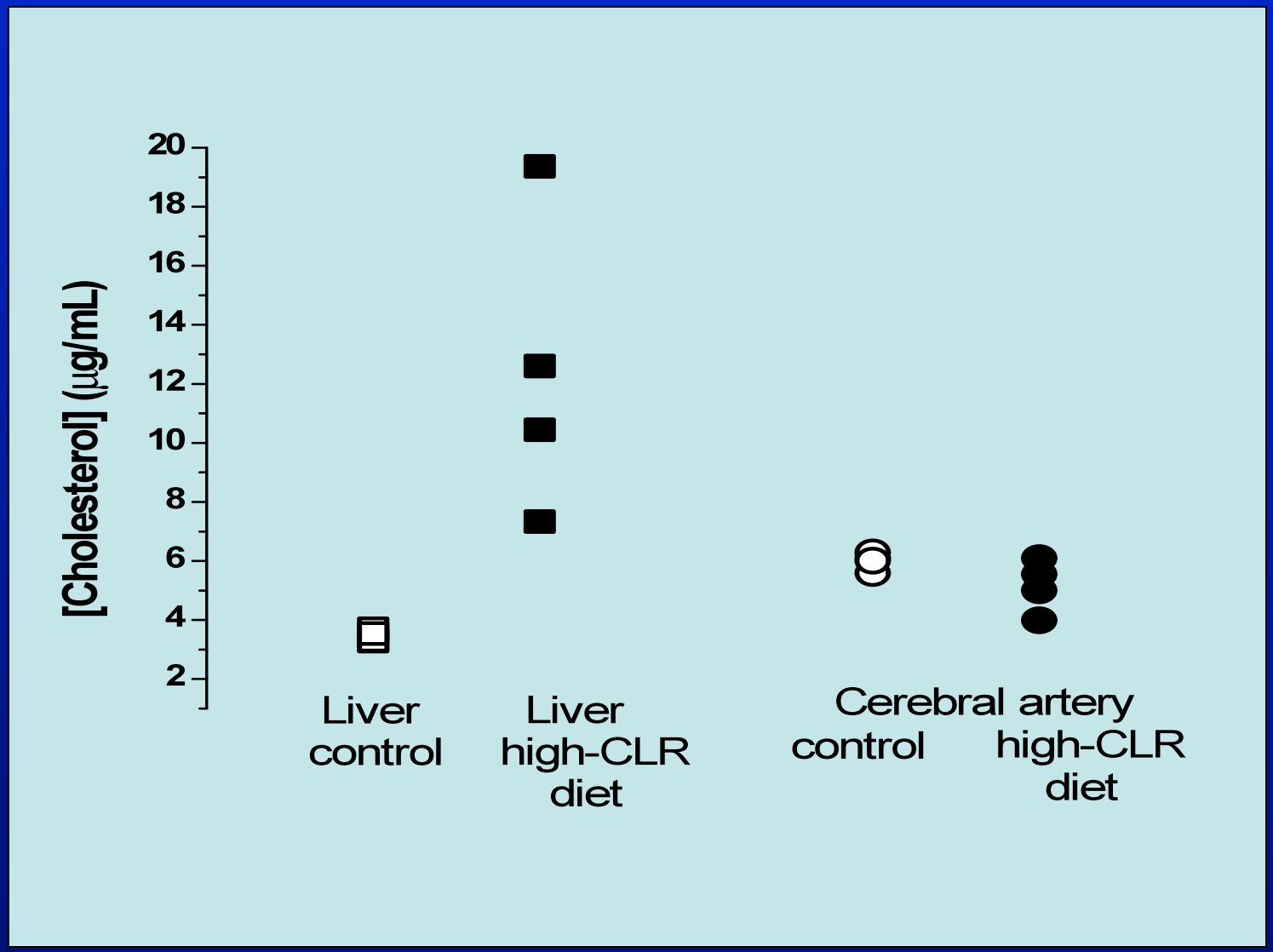


Endothelium



Smooth muscle (myocytes)

High-cholesterol intake causes mild decrease of cholesterol level in cerebral artery myocytes



Conclusions

1. High cholesterol intake poses additional risk for alcohol-induce cerebral artery constriction in healthy vessels

2. High cholesterol intake may have protective effect against alcohol-induced cerebral artery constriction of vessels with impaired endothelium function

3. Protective effect of high cholesterol diet against alcohol-induced cerebral artery is correlated with reduced cholesterol content in cell membranes

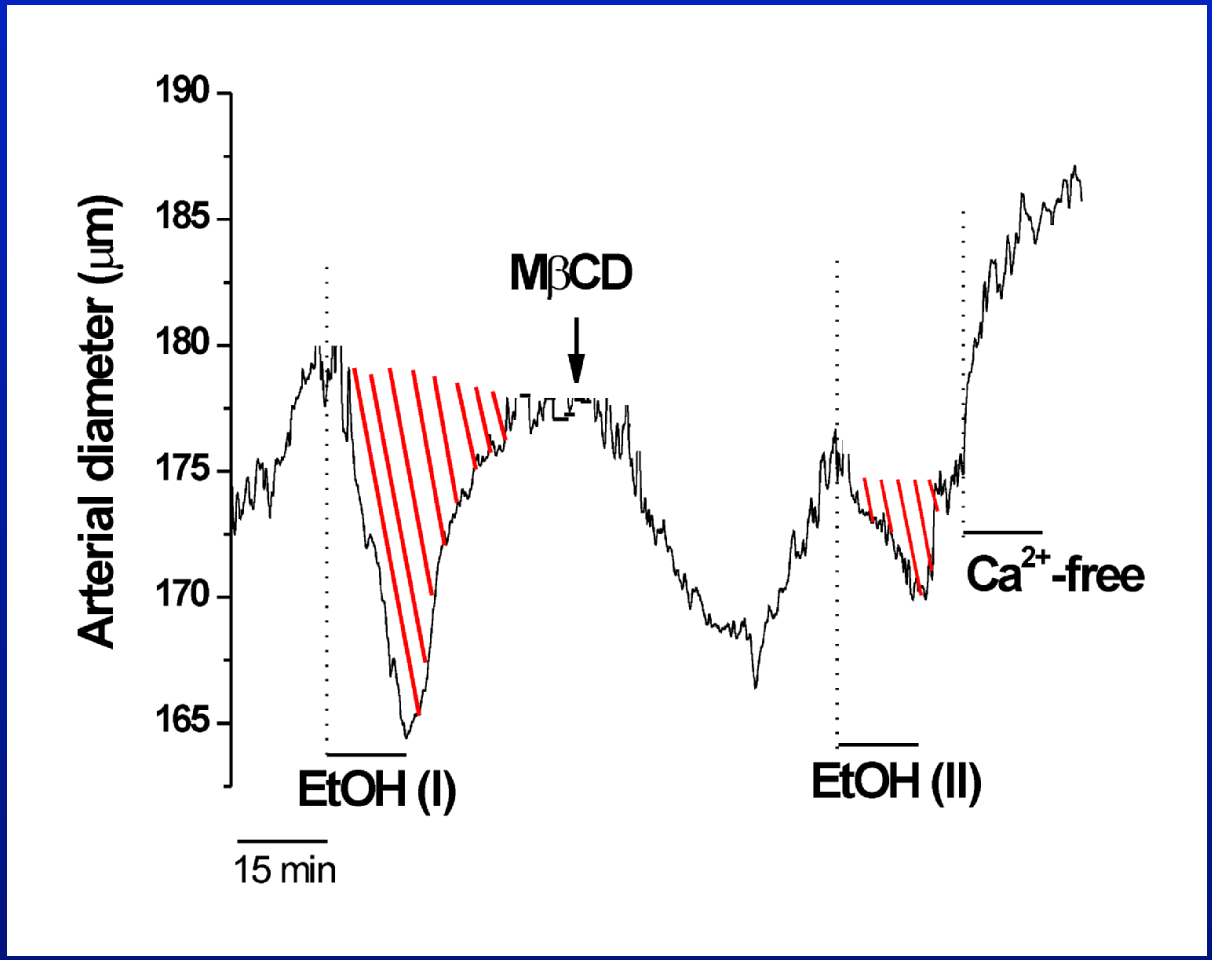
Membrane cholesterol critically controls ethanol-induced cerebral vasoconstriction

1. *Presented at the 2010 World ISBRA Congress, Paris, France, September 13-16, 2010.*
2. *Bukiya et al., Journal of Experimental Medicine in preparation.*

Artery cholesterol depletion or enrichment drastically reduces EtOH-induced vasoconstriction of de-endothelized arteries

A

Methyl-β-cyclodextrin (MβCD): cholesterol carrier



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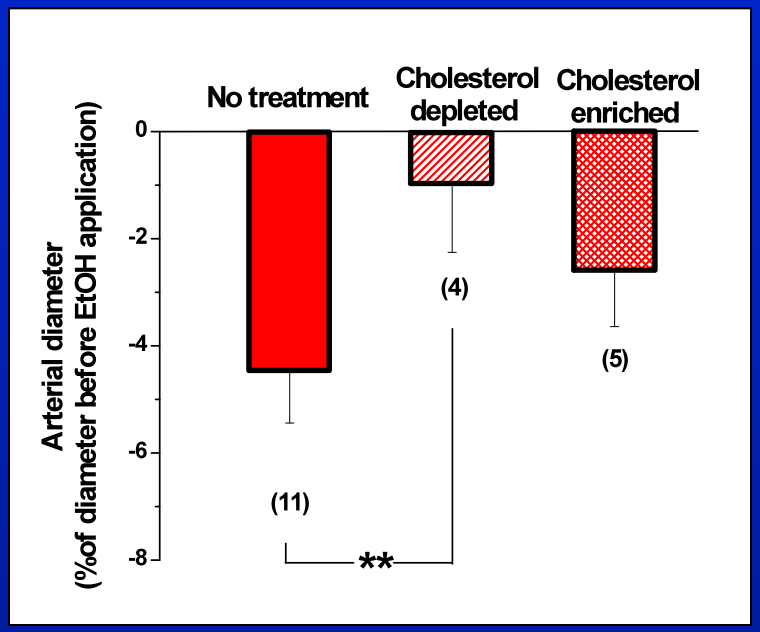

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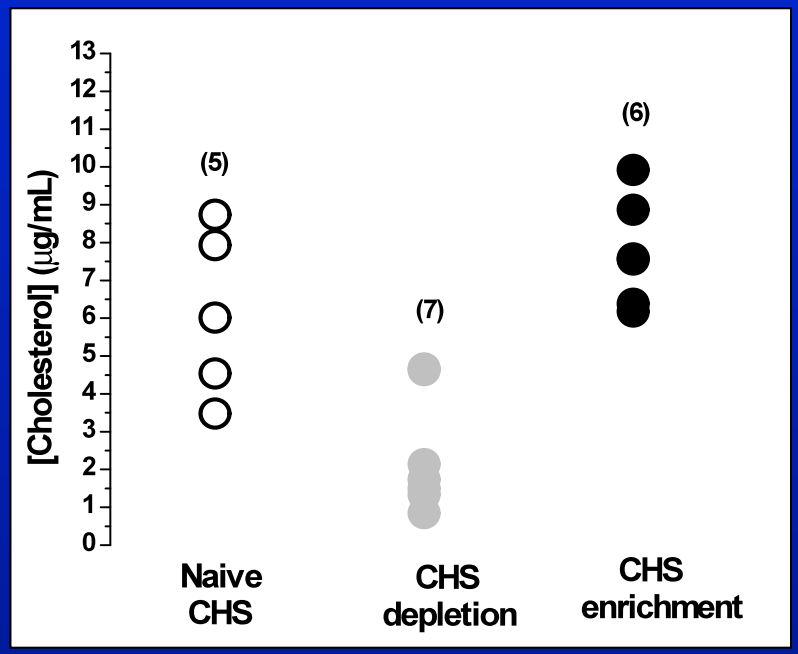


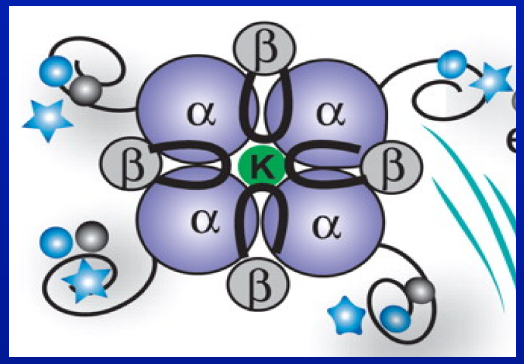
What is molecular target of cholesterol-ethanol interaction?

A

•Ethanol-induced vasoconstriction is due to ethanol inhibition of potassium channels of big conductance (BK type) (Liu et al., 2004).

•BK channels are also inhibited by cholesterol (Bukiya et al., 2009).

B

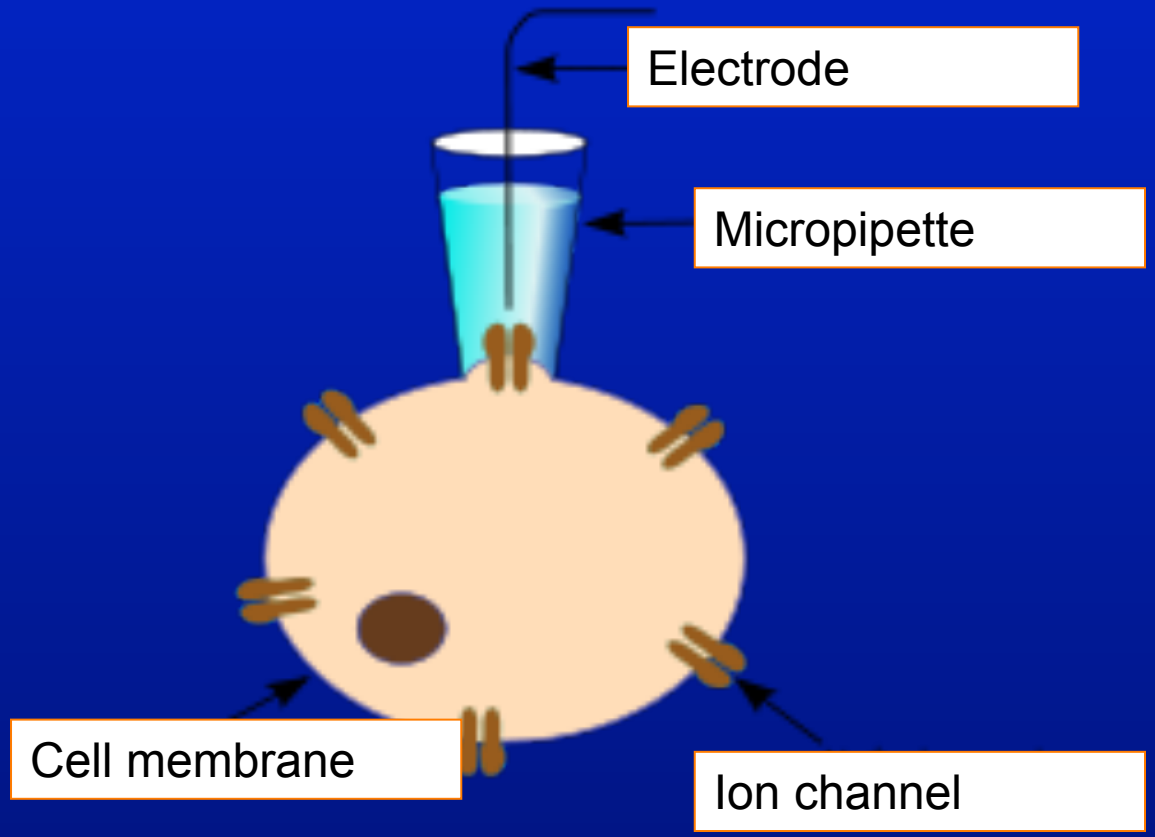


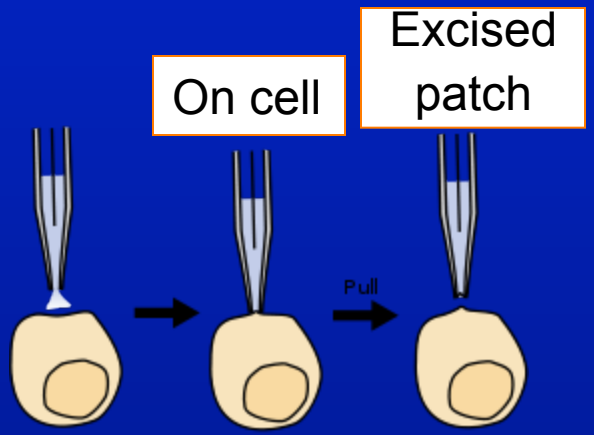
1. BK channels generate outward potassium currents
2. BK channels control numerous physiological functions: neuronal activity, hormone secretion, arterial wall contractility

Will change in cholesterol level affect EtOH induced inhibition of BK channels?

With modifications from Lu et al., 2006

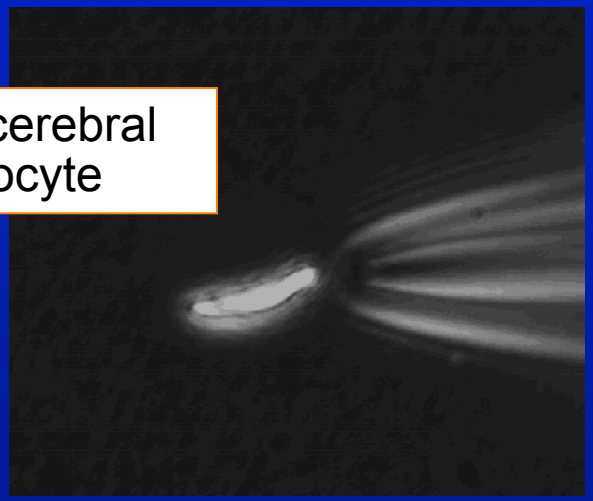
Patch-clamp allows recording of ion channel activity in the native membrane environment



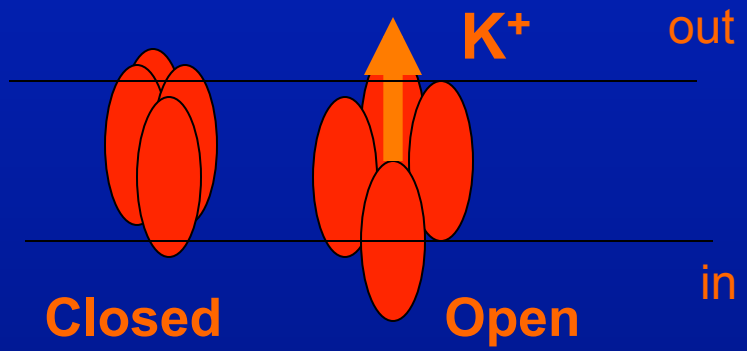


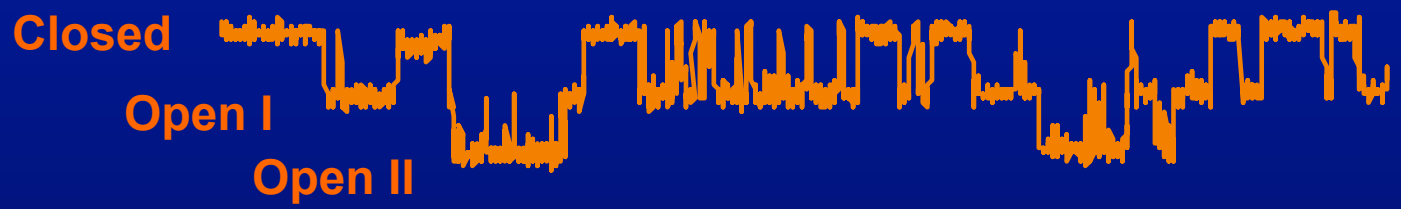
Model: freshly isolated rat cerebral artery myocyte

Isolated rat cerebral artery myocyte



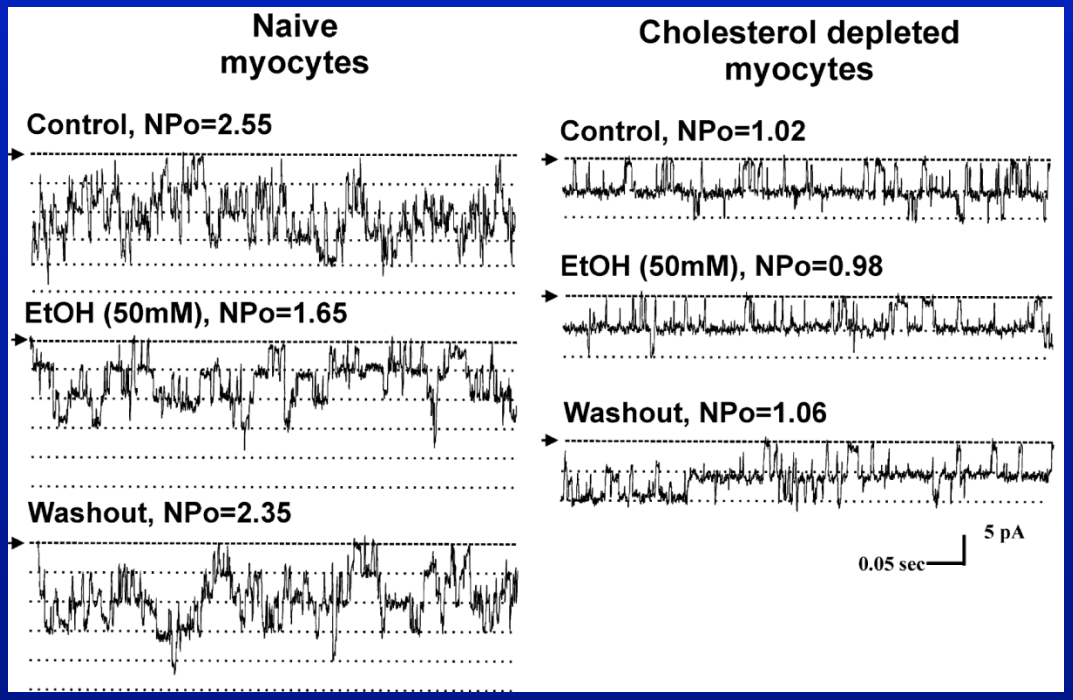
Patch-pipette





Membrane cholesterol depletion or enrichment drastically blunts EtOH-induced inhibition of arterial smooth muscle BK channels.

A



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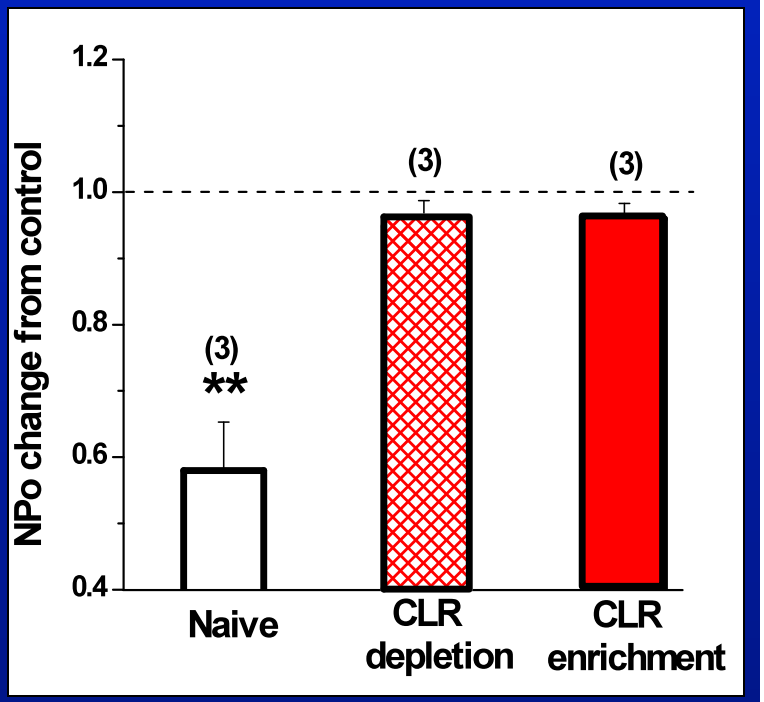


Conclusions

1. Membrane cholesterol critically controls ethanol-induced BK channel inhibition and arterial constriction. In particular, native cholesterol level in the smooth muscle layer is optimal to produce maximal arterial constriction by ethanol.

2. Cholesterol modulation of ethanol effect does not require intracellular environment and likely occurs at BK channel protein.

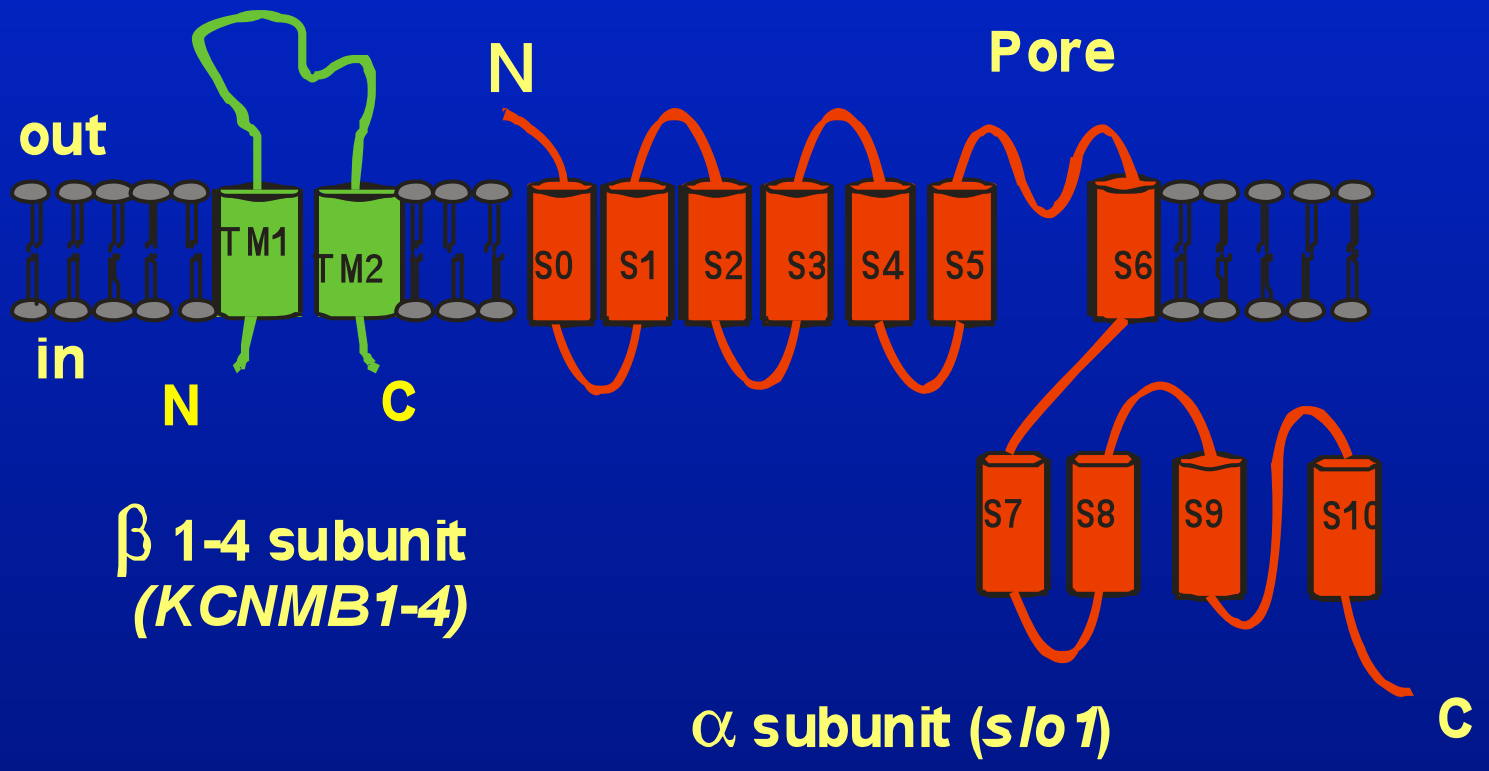
How do cholesterol and ethanol interact?

Specificity of cholesterol and analogs to modulate BK channels points to direct sterol-channel protein interactions

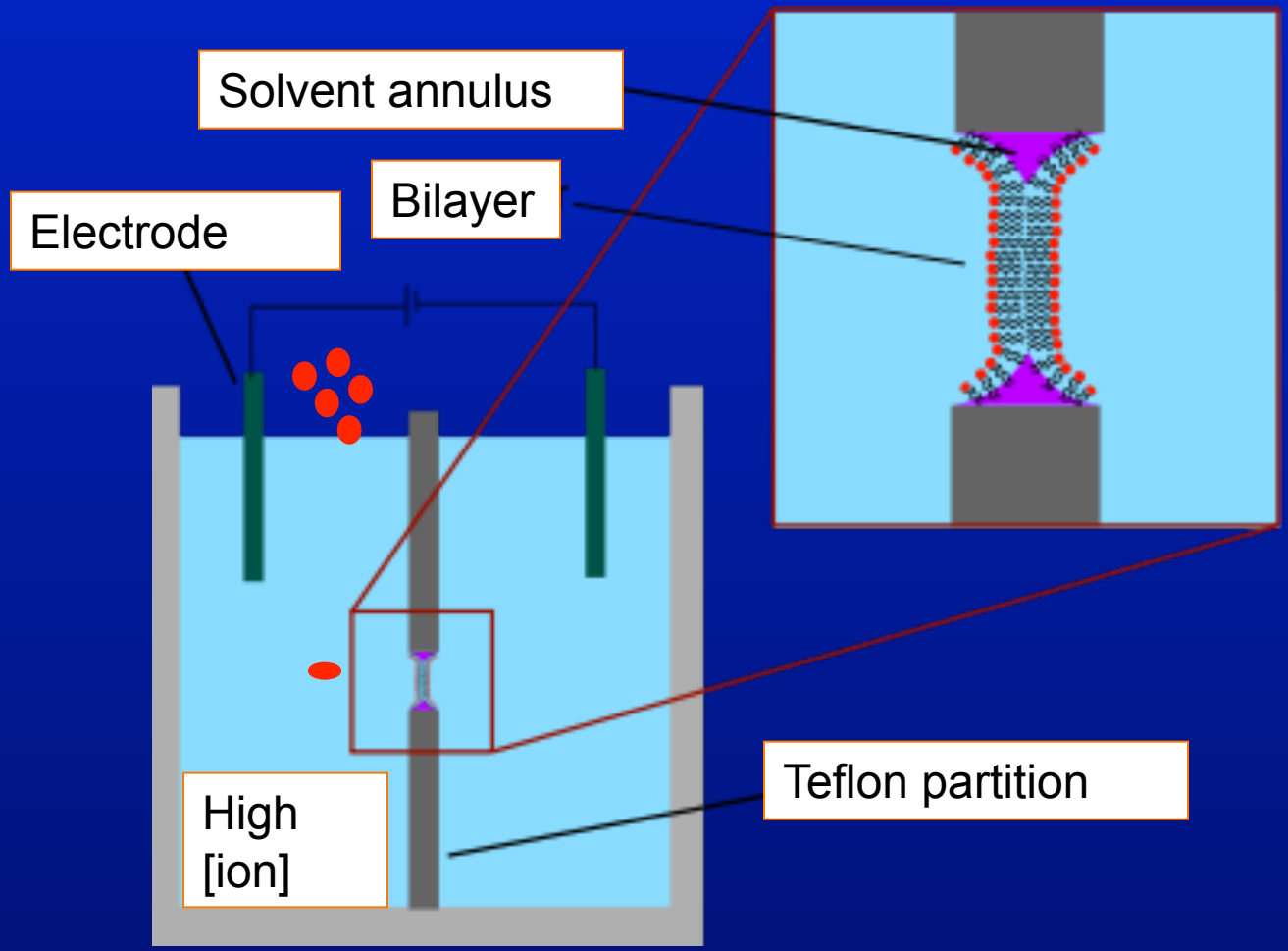
1. Presented at the Biophysical Society 54th Annual Meeting, San Francisco CA, February 20 – 24, 2010.

2. Bukiya et al., Journal of General Physiology, accepted.

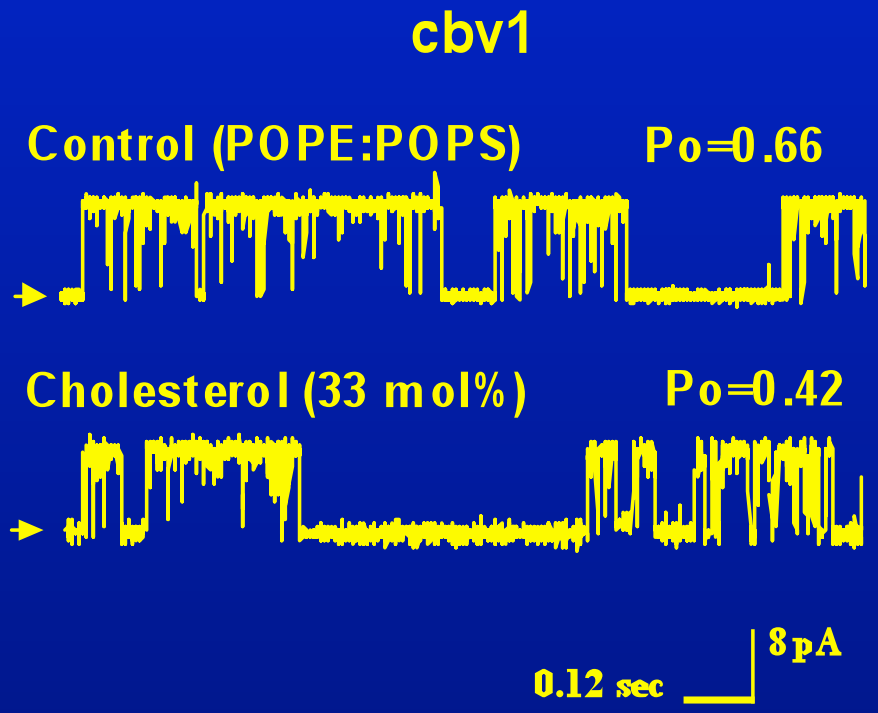
Scheme of a large conductance, voltage- and calcium-gated potassium (BK) channel heterodimer

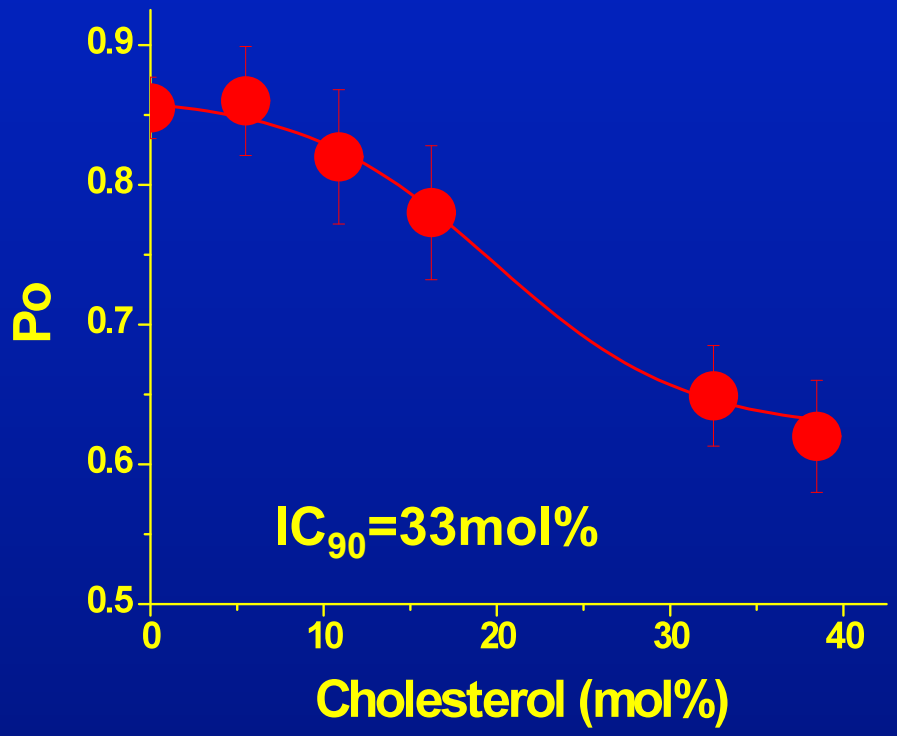


Model (artificial) lipid bilayers allow to study channel function in tightly controlled lipid membrane environment



Cholesterol inhibits arterial myocyte BK channels



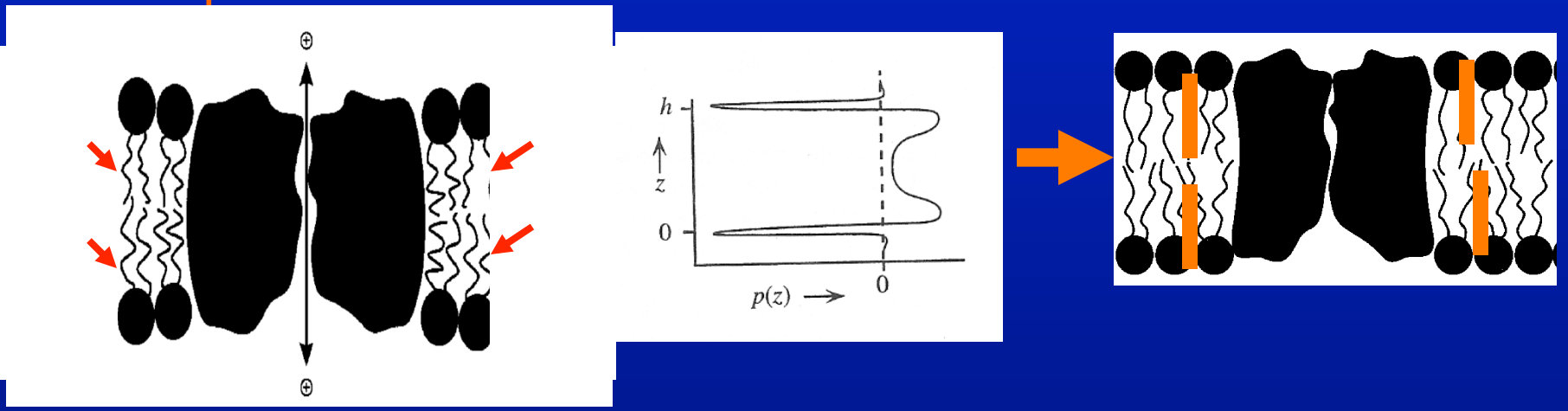


What is the mechanism?

Bukiya et al., FEBS Lttrs, 2008.

Possible mechanisms of cholesterol-induced BK channel inhibition:

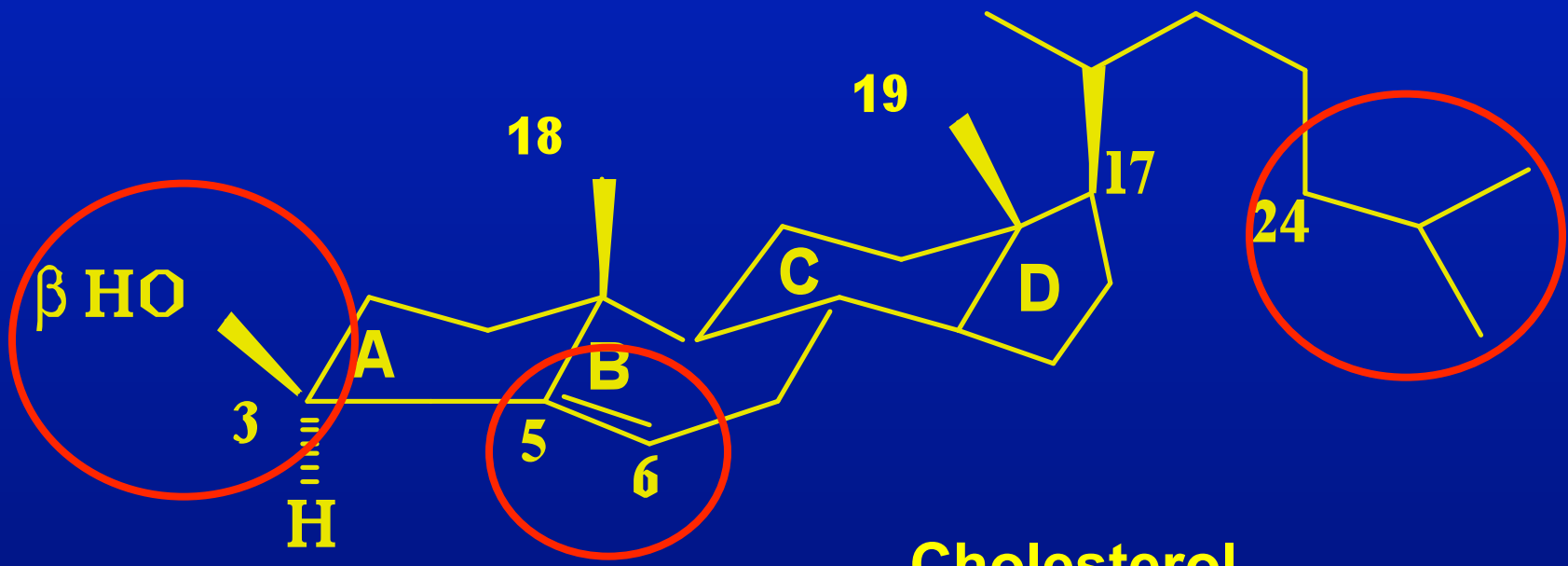
1. Cholesterol increases tight lipid packing, thus, channel Close to Open transition requires higher energy to overcome lateral pressure.



2. Cholesterol has a sensor domain (site) on BK channel protein and promotes conformational changes in BK channel upon binding.

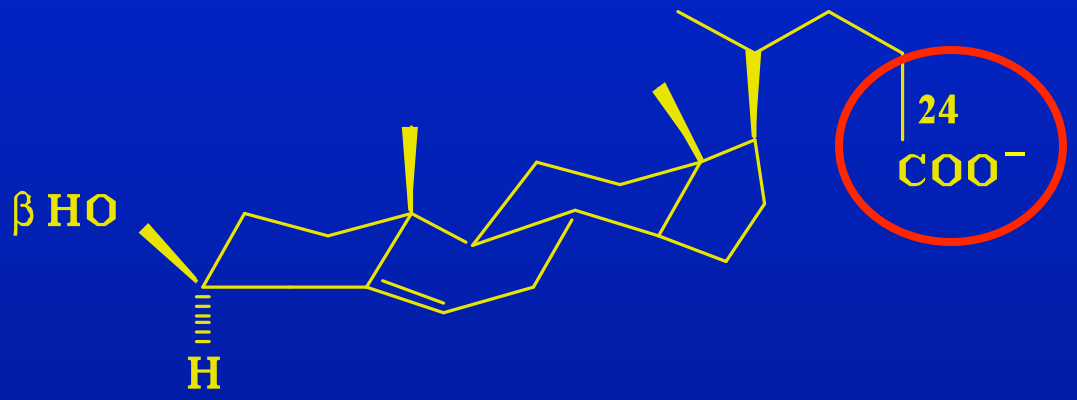
Pictures with modifications from Lundbaek et al., 2004; Cantor, 1997

What are the structural requirements for cholesterol to inhibit BK channel activity?

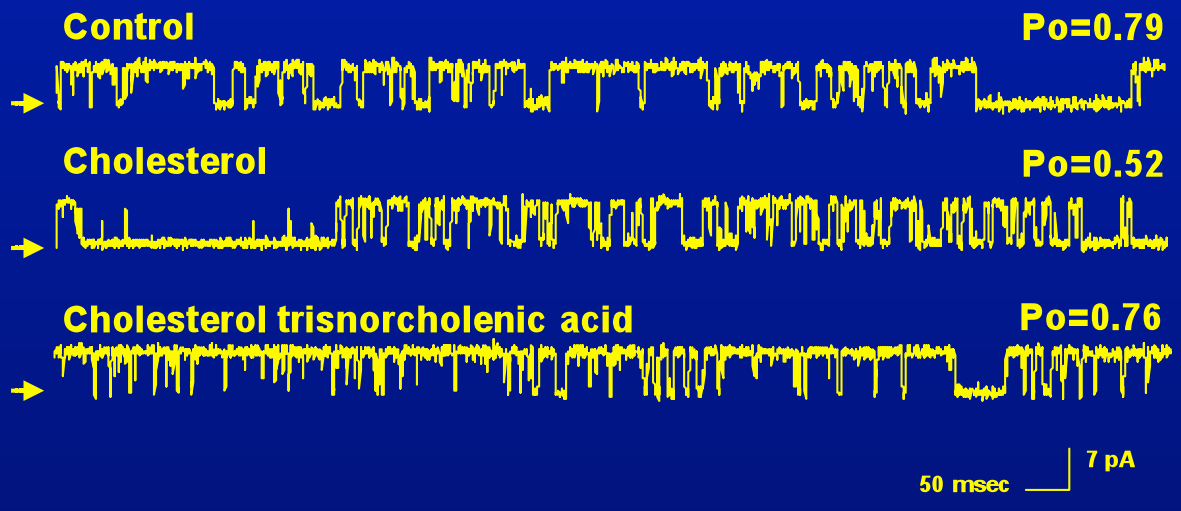


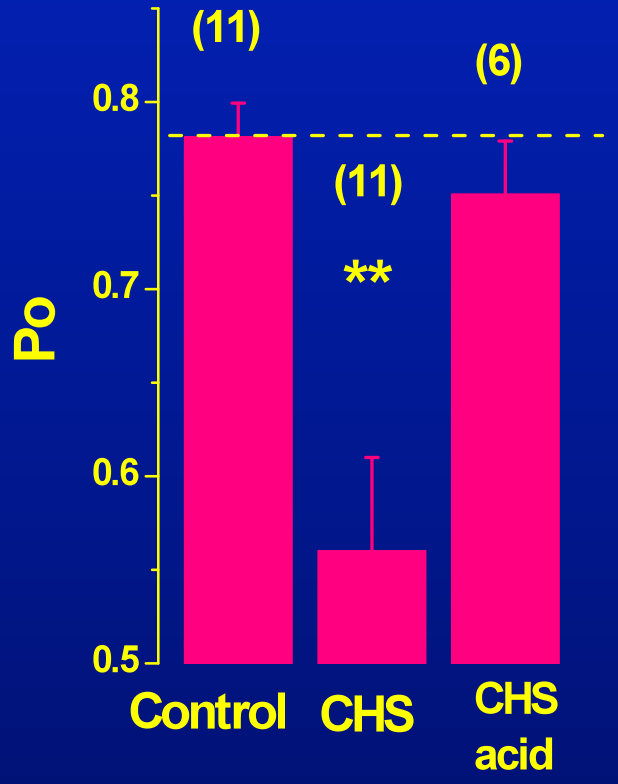
Cholesterol

Cholesterol hydrophobic tail (C24-27) is critical for this sterol to inhibit BK channels

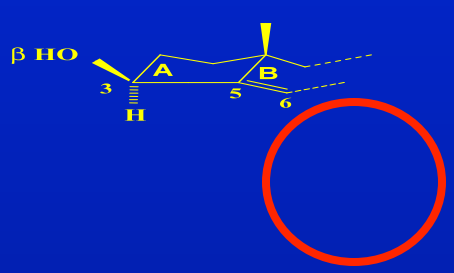


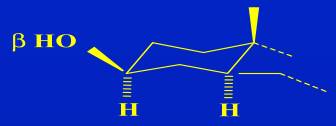
Cholesterol trisnorcholenic acid





Ring A/B junction geometry is not critical for cholesterol-induced BK channel inhibition

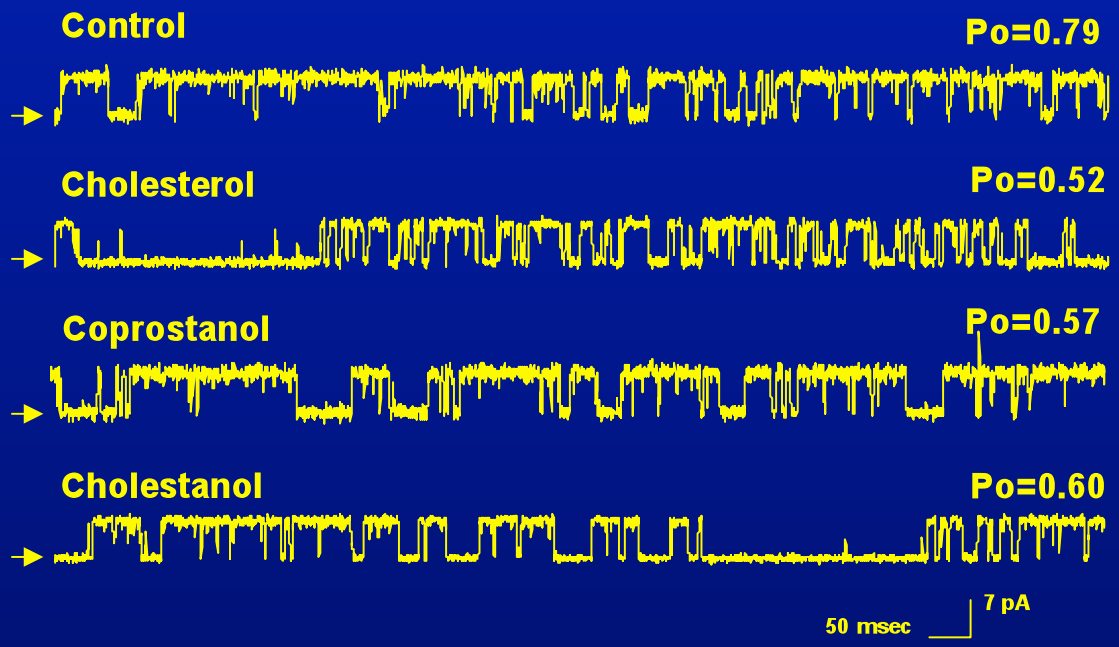


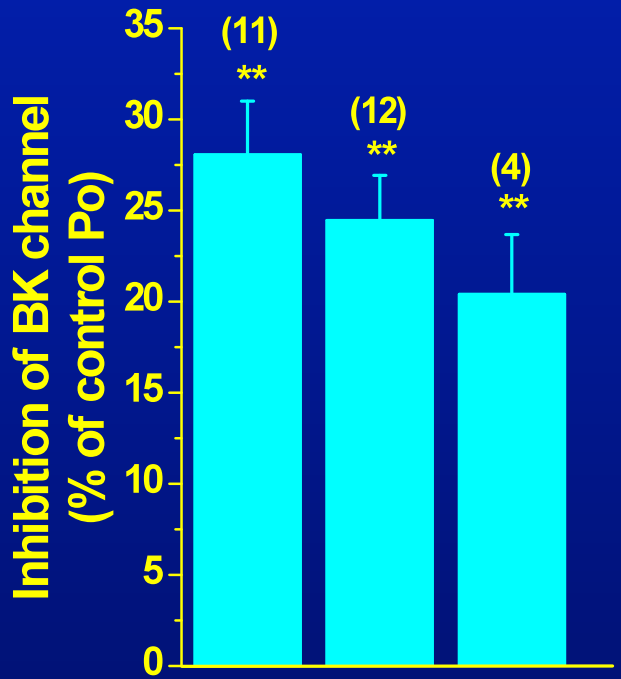


Cholesterol

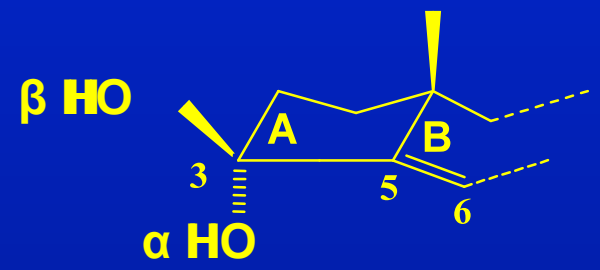
Coprostanol (A/B in *cis*)

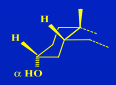
Cholestanol (A/B in *trans*)

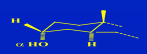




The β-configuration of the C3-hydroxyl is necessary for cholesterol and analogs to inhibit BK channels



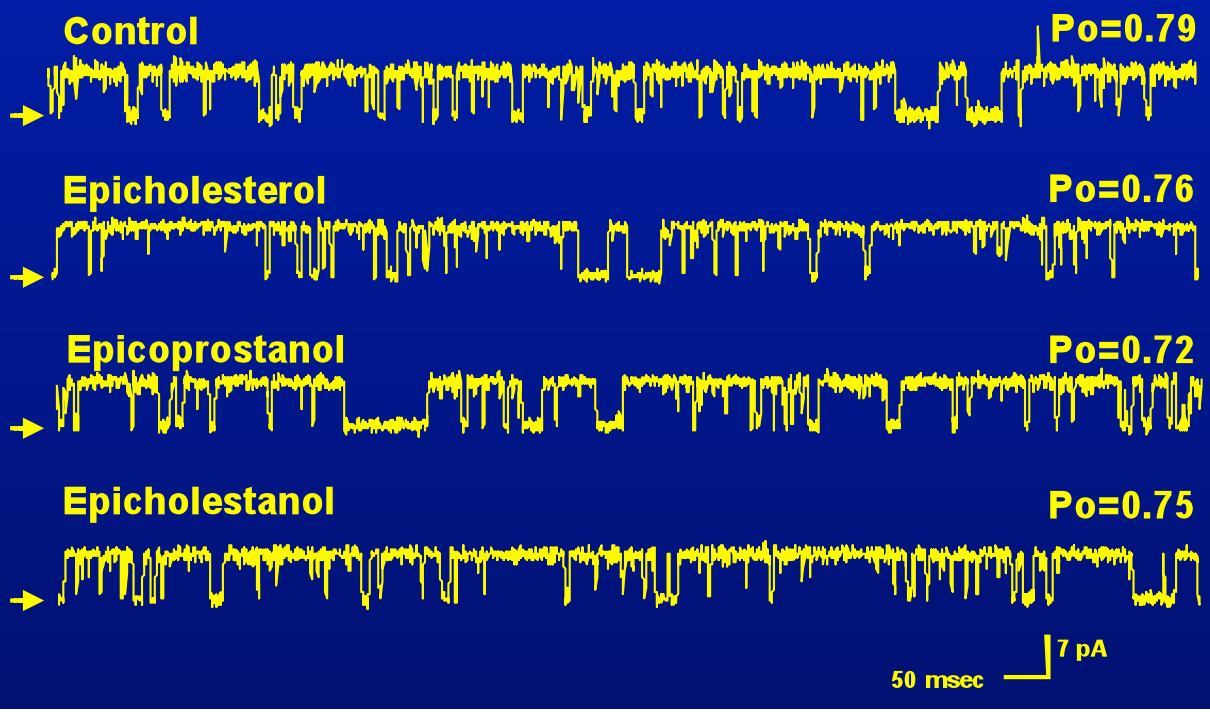


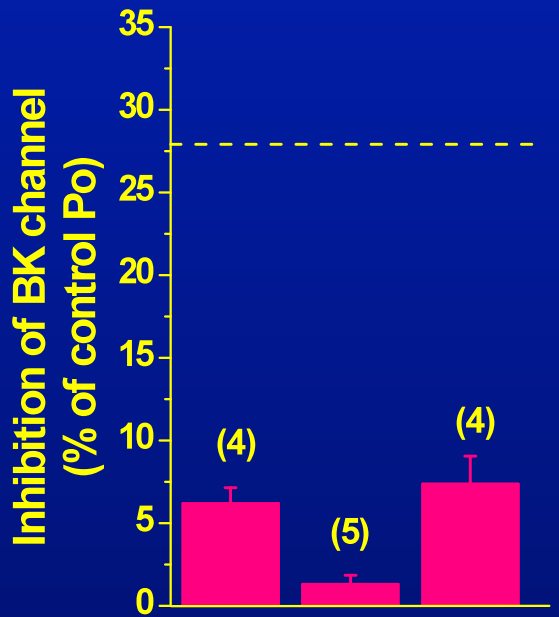


Epi Cholesterol

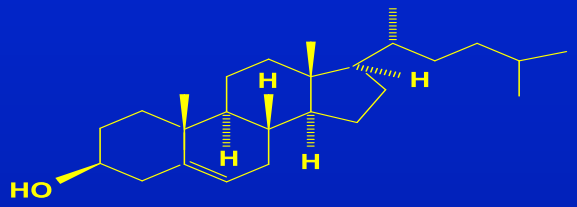
Epicoprostanol

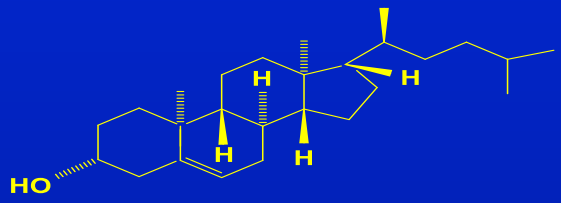
Epicholestanol



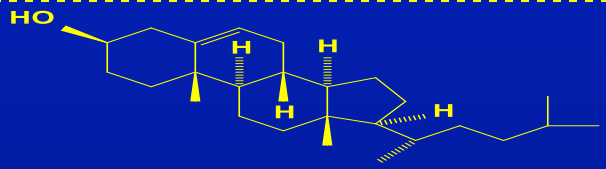


Ent-cholesterol fails to inhibit BK channel

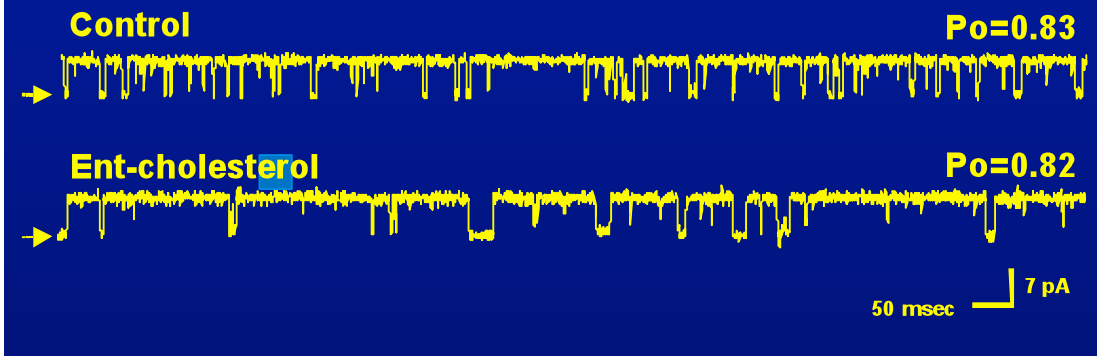


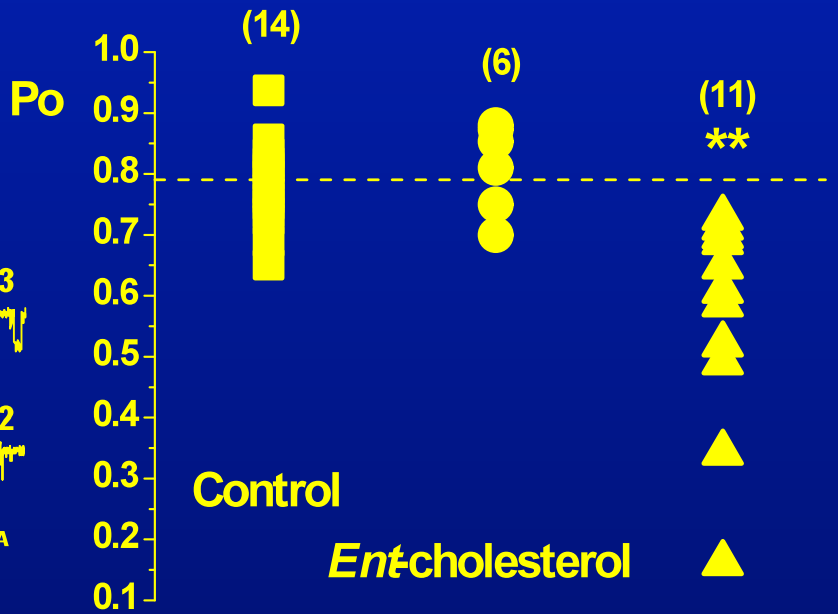


***Nat*-Cholesterol**



***Ent*-Cholesterol**





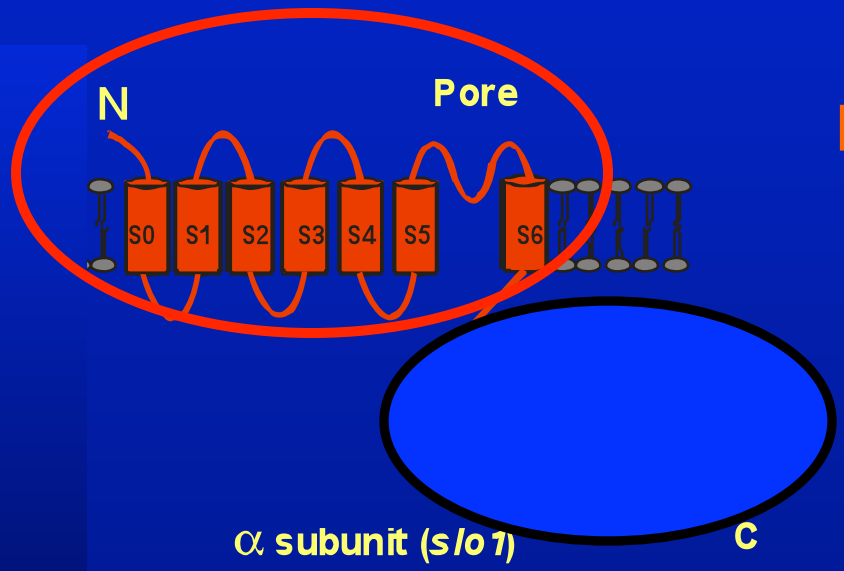
Conclusion

Mechanism of cholesterol action: we propose specific protein-sterol interaction(s) . Justification:

- strict structural requirements for cholesterol molecule to inhibit BK channel (in particular, the hydrophobic tail and a β-configuration in the C3 hydroxyl are necessary)
- enantiospecificity of the whole cholesterol molecule

C-terminus of BK channel confers cholesterol sensitivity to BK protein

A



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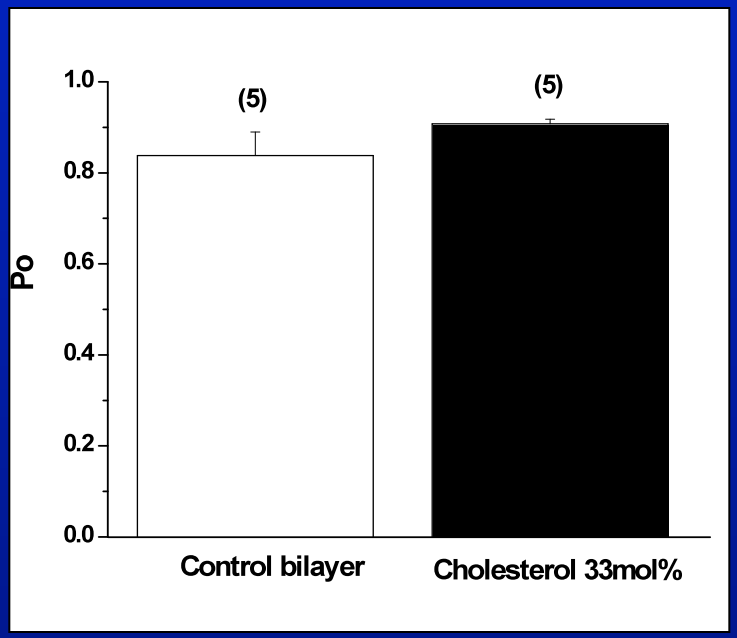

Truncated channel is cholesterol-insensitive

Future directions

- Pinpoint the specific BK channel protein regions and amino acids involved in EtOH and cholesterol sensing
- Define kinetic mechanisms at the single channel level (duration of closed and open times) that underlie EtOH-cholesterol interaction
- Determine relative role of different BK channel subunits (pore-forming α and accessory β1) in EtOH-cholesterol interactions on channel activity and eventual modification of arterial function

Acknowledgements

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