

# Kapitoly z neurofyziologie smyslů



# Neurofyziologie

Ambice: Pochopení psychiky  
člověka a jejích poruch.

Bouřlivý rozvoj:

Molekulární neurovědy

Neurofarmakologie

Zobrazovací metody



## Nobel prices related to neuroscience

1901 Wilhelm Conrad Röntgen (Germany) "in recognition of the extraordinary services he has rendered by the discovery of the remarkable rays subsequently named after him"

1904 Ivan Petrovich Pavlov (Russia) "in recognition of his work on the physiology of digestion, through which knowledge on vital aspects of the subject has been transformed and enlarged"

1906 Camillo Golgi (Italy) and Santiago Ramón y Cajal (Spain) "in recognition of their work on the structure of the nervous system"

1909 Emil Theodor Kocher (Switzerland) "for his work on the physiology, pathology and surgery of the thyroid gland"

1914 Robert Bárány (Vienna) "for his work on the physiology and pathology of the vestibular apparatus"

1920 Chemistry: Walther Hermann Nernst (Germany) "in recognition of his work in thermochemistry"

1932 Sir Charles Scott Sherrington (Great Britain) and Edgar Douglas Adrian (Great Britain) "for their discoveries regarding the functions of neurons"

- 1935 Hans Spemann (Germany) "for his discovery of the organizer effect in embryonic development"
- 1936 Sir Henry Hallett Dale (Great Britain) and Otto Loewi (Great Britain) "for their discoveries relating to chemical transmission of nerve impulses"
- 1944 Joseph Erlanger (USA) Herbert Spencer Gasser (USA) "for their discoveries relating to the highly differentiated functions of single nerve fibres"
- 1949 Walter Rudolf Hess "for his discovery of the functional organization of the interbrain as a coordinator of the activities of the internal organs"
- 1949 Antonio Caetano de Abreu Freire Egas Moniz "for his discovery of the therapeutic value of leucotomy in certain psychoses"
- 1952 Physics: Felix Bloch (USA) and Edward Mills Purcell (USA) "for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith"
- 1961 Georg von Békésy (USA/Hungary)"for his discoveries of the physical mechanism of stimulation within the cochlea"

1962 Francis Harry Compton Crick (Great Britain), James Dewey Watson (USA) and Maurice Hugh Frederick Wilkins (Great Britain) "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material"

1963 Sir John Carew Eccles (Australia), Alan Lloyd Hodgkin and Andrew Fielding Huxley (Great Britain) "for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane"

1967 Ragnar Granit (Sweden/Finland), Haldan Keffer Hartline (USA) and George Wald (USA) "for their discoveries concerning the primary physiological and chemical visual processes in the eye"

Robert W. Holley (USA), Har Gobind Khorana (USA) and Marshall W. Nirenberg (USA) "for their interpretation of the genetic code and its function in protein synthesis"

1970 Sir Bernard Katz (Great Britain), Ulf von Euler (Sweden) and Julius Axelrod (USA) "for their discoveries concerning the humoral transmitters in the nerve terminals and the mechanism for their storage, release and inactivation"

1972 Physics: John Bardeen (USA), Leon Neil Cooper (USA) and John Robert Schrieffer (USA) "for their jointly developed theory of superconductivity, usually called the BCS-theory" [Professor Cooper was Director of Brown University's Center for Neural Science.]

1973 Karl von Frisch (Germany), Konrad Lorenz (Austria) and Nikolaas Tinbergen (Great Britain) "for their discoveries concerning organization and elicitation of individual and social behaviour patterns"

1973 Physics: Brian David Josephson (Great Britain) "for his theoretical predictions of the properties of a supercurrent through a barrier, in particular those phenomena which are generally known as the Josephson effects"

1976 Baruch S. Blumberg (USA) and D. Carleton Gajdusek (USA) "for their discoveries concerning new mechanisms for the origin and dissemination of infectious diseases"

1977 Roger Guillemin and Andrew Schally for their discoveries concerning "the peptide hormone production of the brain"

1977 Rosalyn Yalow for "the development of radioimmunoassays of peptid hormones"

- 1979 Allan M Cormack and Godfrey Newbold Hounsfield for the "development of computer assisted tomography"
- 1981 Roger W. Sperry, for his discoveries concerning "the functional specialization of the cerebral hemispheres"
- 1981 David H. Hubel and Torsten N. Wiesel, for their discoveries concerning "visual system".
- 1986 Stanley Cohen (USA) Rita Levi-Montalcini (Italy/USA)"for their discoveries of growth factors"
- 1991 Erwin Neher (Germany) Bert Sakmann (Germany) "for their discoveries concerning the function of single ion channels in cells"
- 1991 Chemistry: Richard R. Ernst (Switzerland) "for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy"
- 1994 Alfred G. Gilman (USA) Martin Rodbell (USA) "for their discovery of G-proteins and the role of these proteins in signal transduction in cells"
- 1997 Stanley B. Prusiner, in Physiology or Medicine for his discovery of "prions - a new biological principle of infection"



- 1997 Chemistry: Paul D. Boyer (USA) and John E. Walker (Great Britain) "for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)"
- 1997 Jens C. Skou (Denmark) "for the first discovery of an ion-transporting enzyme, Na<sup>+</sup>, K<sup>+</sup>-ATPase"
- 1998 Robert F. Furchgott (USA) Louis J. Ignarro (USA) and Ferid Murad (USA) "for their discoveries concerning nitric oxide as a signalling molecule in the cardiovascular system"
- 2000 Arvid Carlsson, Paul Greengard and Eric Kandel for their discoveries concerning "signal transduction in the nervous system"
- 2003 Paul C. Lauterbur Sir Peter Mansfield for their discoveries concerning magnetic resonance imaging
- 2004 Richard Axel, Linda Buck for their discoveries of odorant receptors and the organization of the olfactory system

<http://nobelprize.org/medicine/laureates/1981/>

# Neurofyzologie

Studium nervových a doprovodných buněk, způsobu jak jsou sestaveny do funkčních celků, které vedou, zpracovávají, ukládají informaci a zprostředkují chování.

# Internet a něco z jeho neomezené nabídky:

<http://www.physpharm.fmd.uwo.ca/undergrad/medsweb/>

<http://entochem.tamu.edu/index.html>

<http://web.neurobio.arizona.edu/gronenberg/nrsc581/index.html>

<http://www.biol.sc.edu/~vogt/courses/neuro/neurobehavior.html>

<http://instruct1.cit.cornell.edu/courses/bionb424/links.htm>

<http://nelson.beckman.uiuc.edu/courses/neuroethol/>

<http://www.blackwellpublishing.com/matthews/default.html>

<http://www.hhmi.org/biointeractive/vlabs/neurophysiology/index.html>

# Kapitoly z neurofyzologie smyslů – výběr kapitol

## Fyziologie membrán:

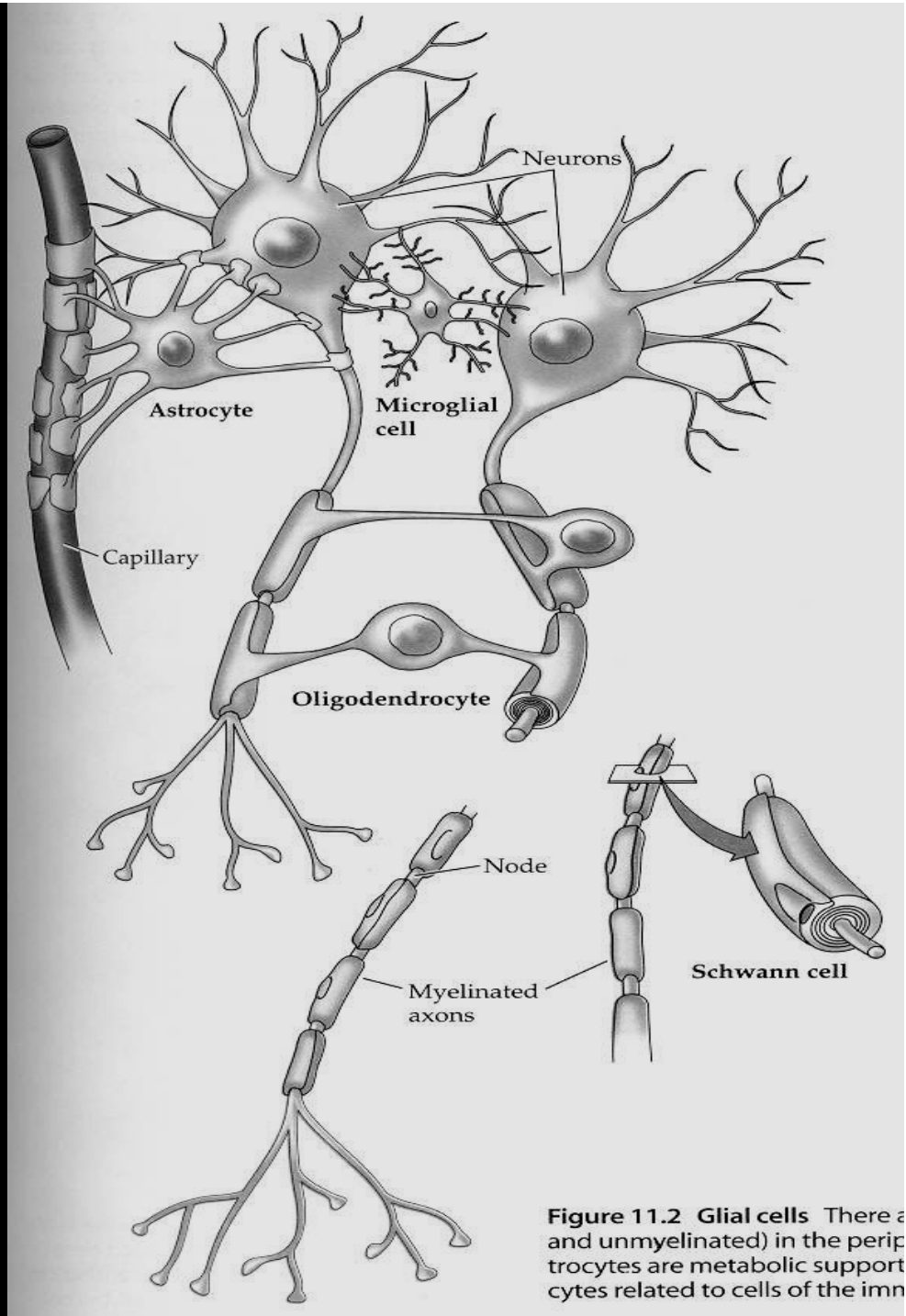
- klidový potenciál
- akční potenciál
- iontové kanály
- šíření signálů a synapse

## Fyziologie smyslů:

- obecné principy
- čich a chuť
- hmat a sluch
- zrak a další smysly

## Psychofyzologie:

- zpracování zrakové informace
- učení a paměť
- chování, neuroetologie

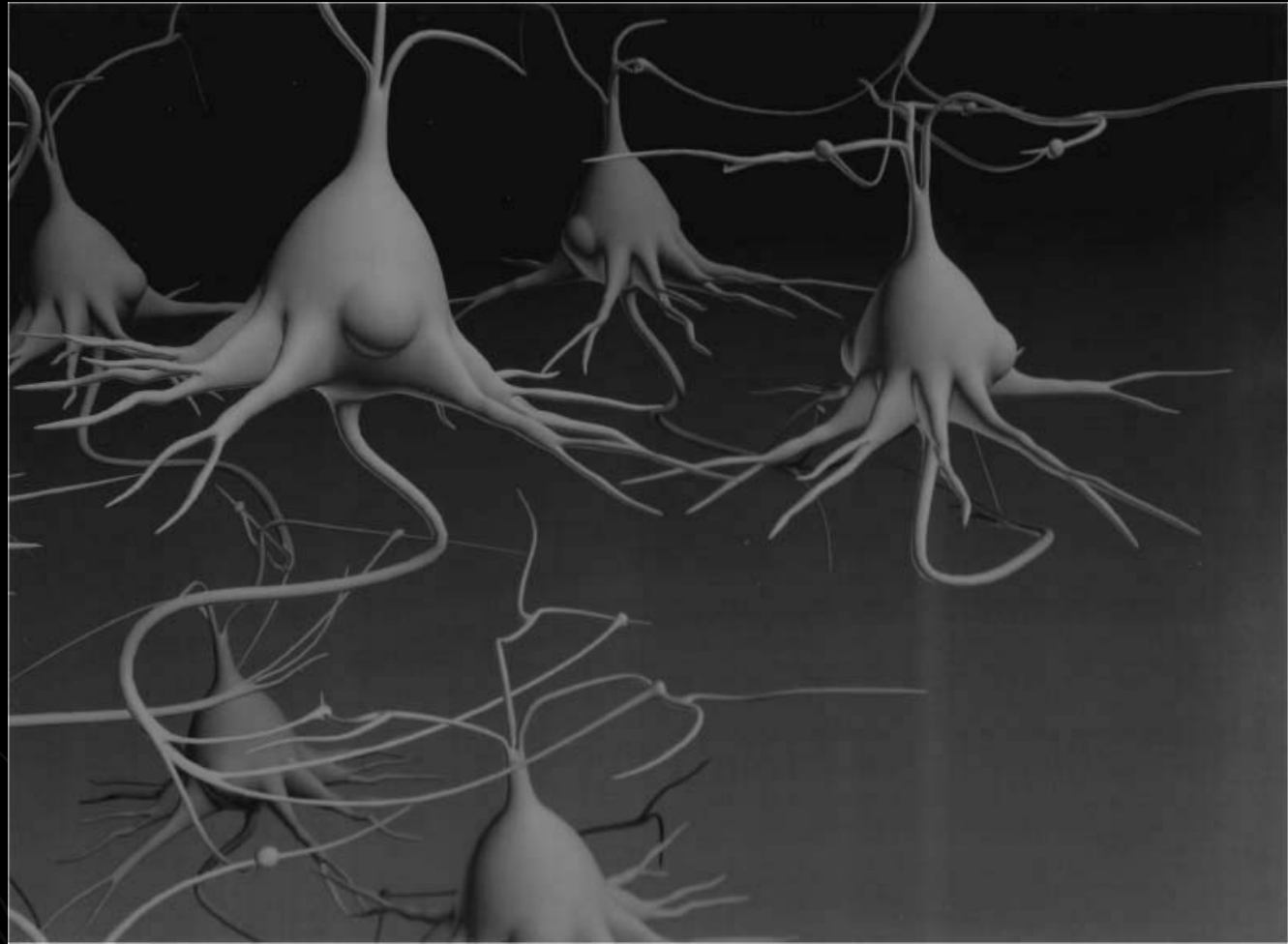


Materiály, prezentace,  
návaznosti na předměty,  
hodnocení.



Předávání a zpracování informací:

elektro - chemická spolupráce

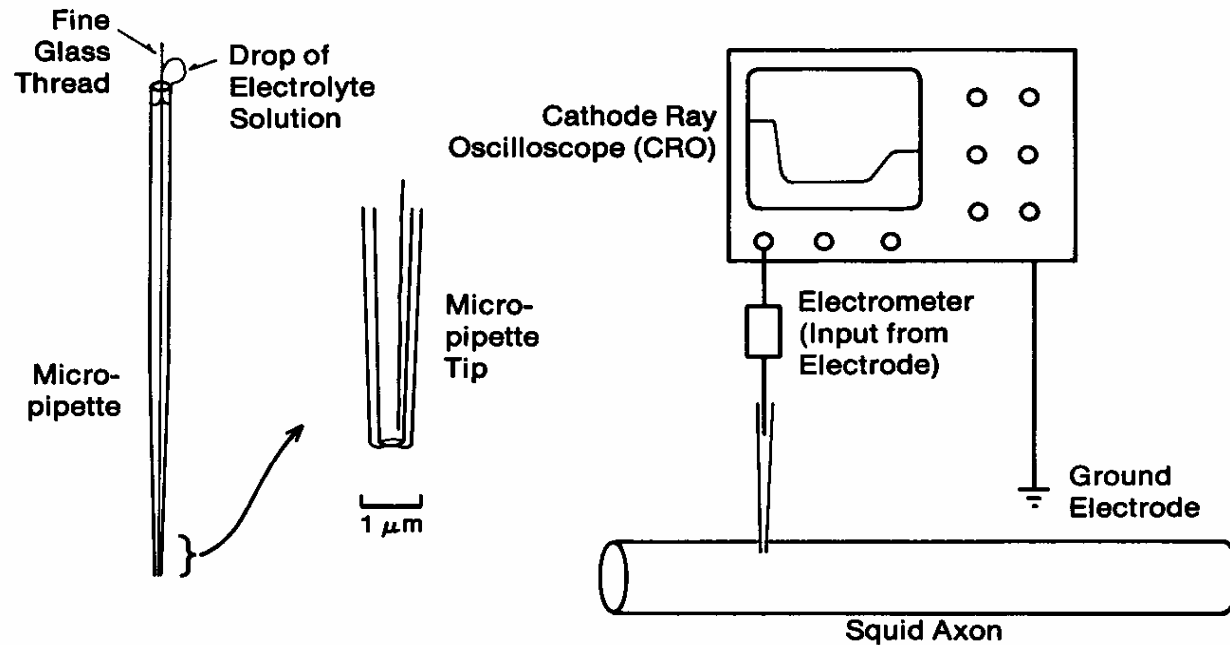


# Fyziologie membrán: potenciál





# Klidový potenciál



**Fig. 4.4** The micropipette is used for electrical recording (extracellular, intracellular, patch), electrical stimulation (current or voltage clamp), or delivery of substances (microionophoresis or pressure ejection). Preparation of an intracellular recording micropipette is shown on the left. The diagram on the right shows the arrangement for recording from a squid axon and observing potentials on a cathode ray oscilloscope (CRO).

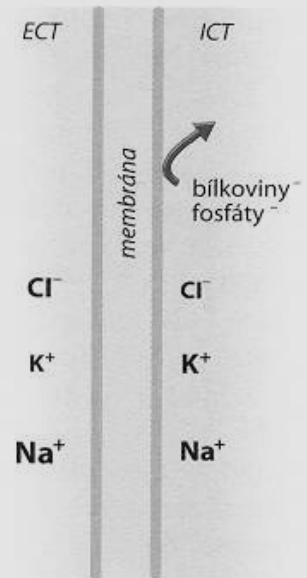
## Gibbs-Donnanova rovnováha

Iont	Koncentrace		Gradient Intra/Extra	Rovnovážný potenciál
	Intracelulární	Extracelulární		
Na <sup>+</sup>	12 mmol/l	145 mmol/l	1:12	+67 mV
K <sup>+</sup>	155 mmol/l	4 mmol/l	39:1	-98 mV
Cl <sup>-</sup>	4 mmol/l	123 mmol/l	1:31	-90 mV
volný Ca <sup>2+</sup>	10 <sup>-4</sup> mmol/l	1,5 mmol/l	1:15.000	+129 mV
fixní anionty	155 mmol/l			

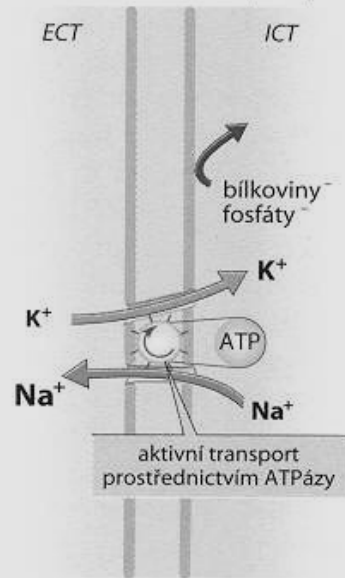
*Tab. 2.2.* Tabulka rozložení iontových koncentrací na buněčné membráně kosterního svalu savce.

## A. Příčiny a důsledky klidového potenciálu membrány

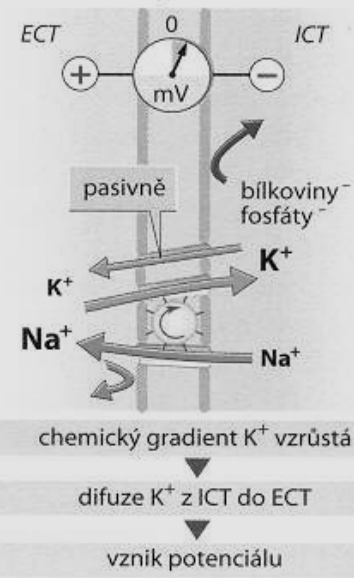
### 1 pasivní rozložení iontů



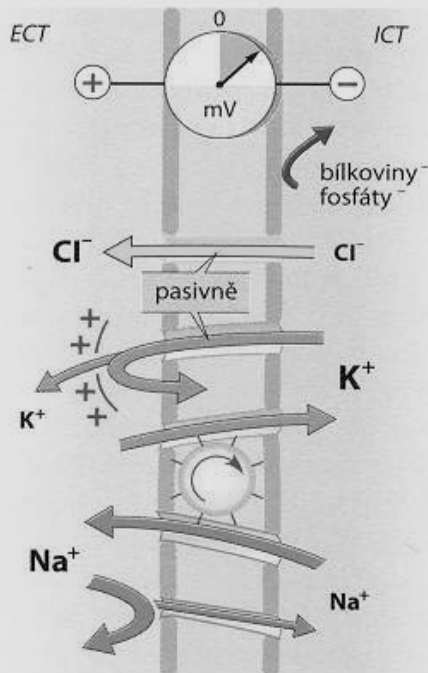
### 2 aktivní Na⁺-K⁺-pumpa



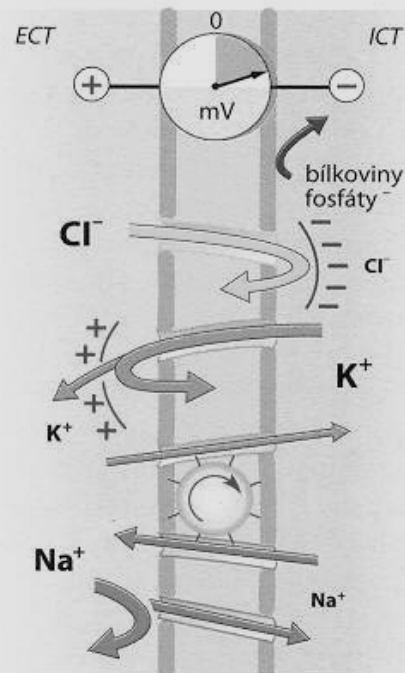
### 3 difuzní potenciál K⁺



### 4 potenciál žene Cl⁻ z ICT do ECT



### 5 konečný stav: klidový membránový potenciál



**K<sup>+</sup>:**

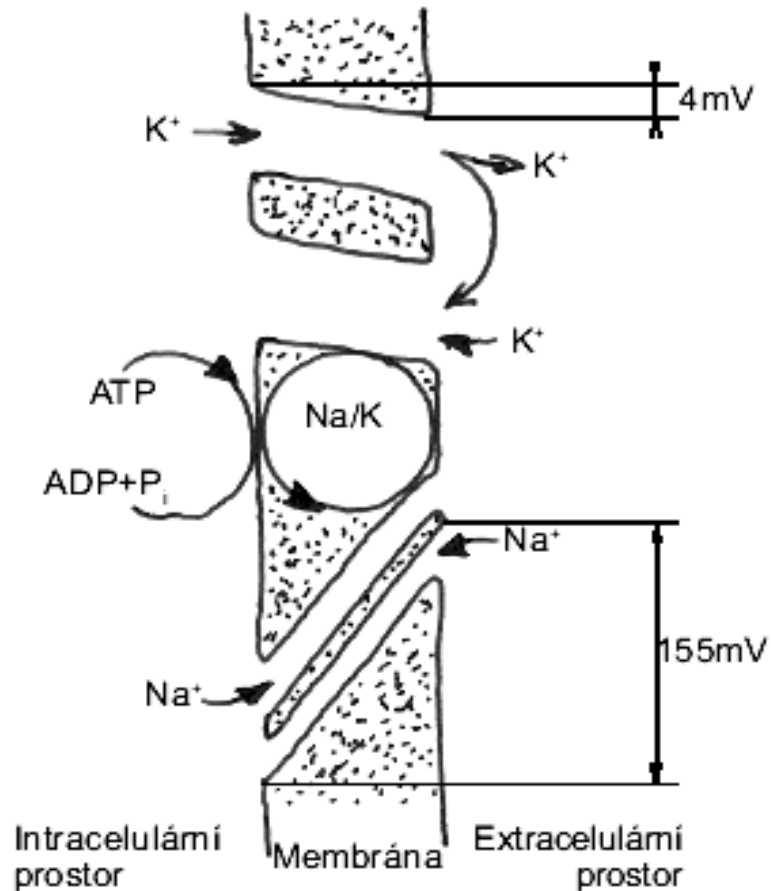


**Na<sup>+</sup>:**



**INTRA**

**EXTRA**



Obr. 2.9. Rozdílné membránové podmínky pro Na<sup>+</sup> a K<sup>+</sup>. Na/K pumpa stále udržuje na membráně gradient Na<sup>+</sup> i K<sup>+</sup>. Zatímco K<sup>+</sup> však může membránou volně procházet, pro Na<sup>+</sup> je téměř nepropustná. Na<sup>+</sup> je čerpáno „do strmého kopce“ – proti velké elektrochemické síle.

Rovnovážný potenciál – pro daný iont  
Nernstova r.

$$E_{ion} = \frac{RT}{zF} \ln \frac{[ion]_o}{[ion]_i}$$

$$E_{ion} = 61mV \log \frac{[ion]_o}{[ion]_i}$$

Goldmanova r.

$$E_r = \frac{RT}{F} \ln \frac{PK[K^+]_o + PNa[Na^+]_o + PCl[Cl^-]_i}{PK[K^+]_i + PNa[Na^+]_i + PCl[Cl^-]_o}$$

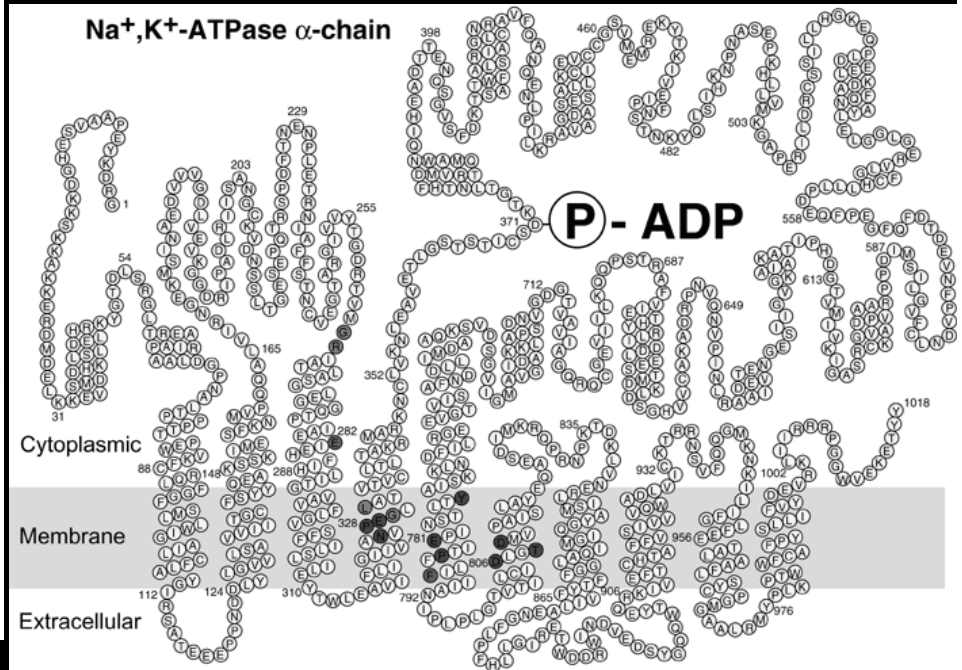
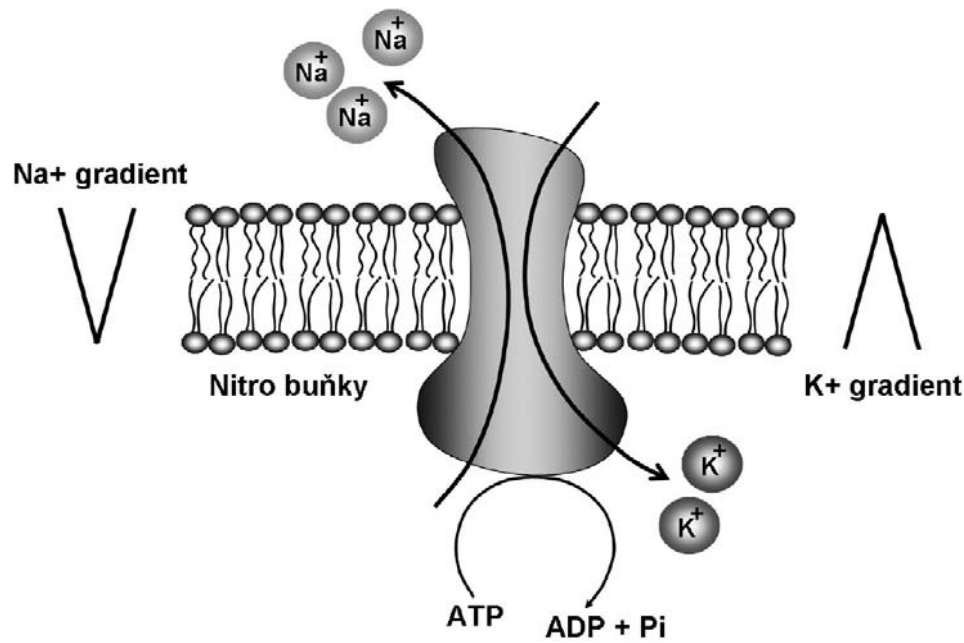
Gradient Intra/Extra	Rovnovážný potenciál
1:12	+67 mV
39:1	-98 mV

Hnací síla = Driving Force  
 -90mV – (Rovnovážný potenciál)

Iont	Koncentrace		Gradient Intra/Extra	Rovnovážný potenciál
	Intracelulární	Extracelulární		
Na <sup>+</sup>	12 mmol/l	145 mmol/l	1:12	+67 mV
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fixní anionty	155 mmol/l			

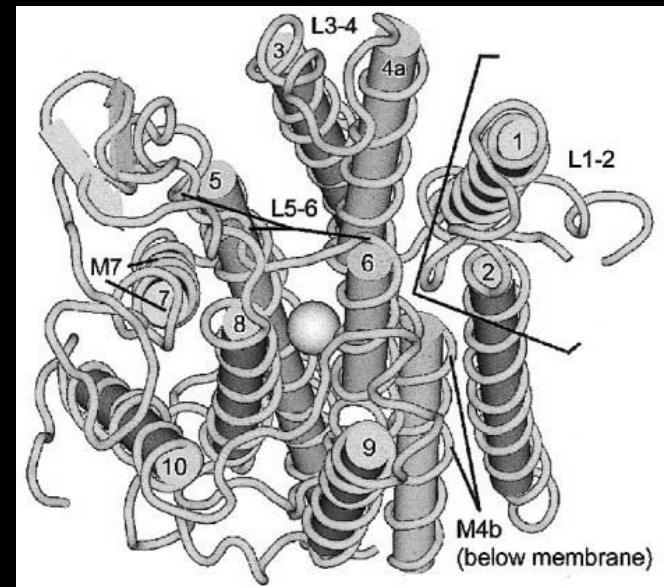
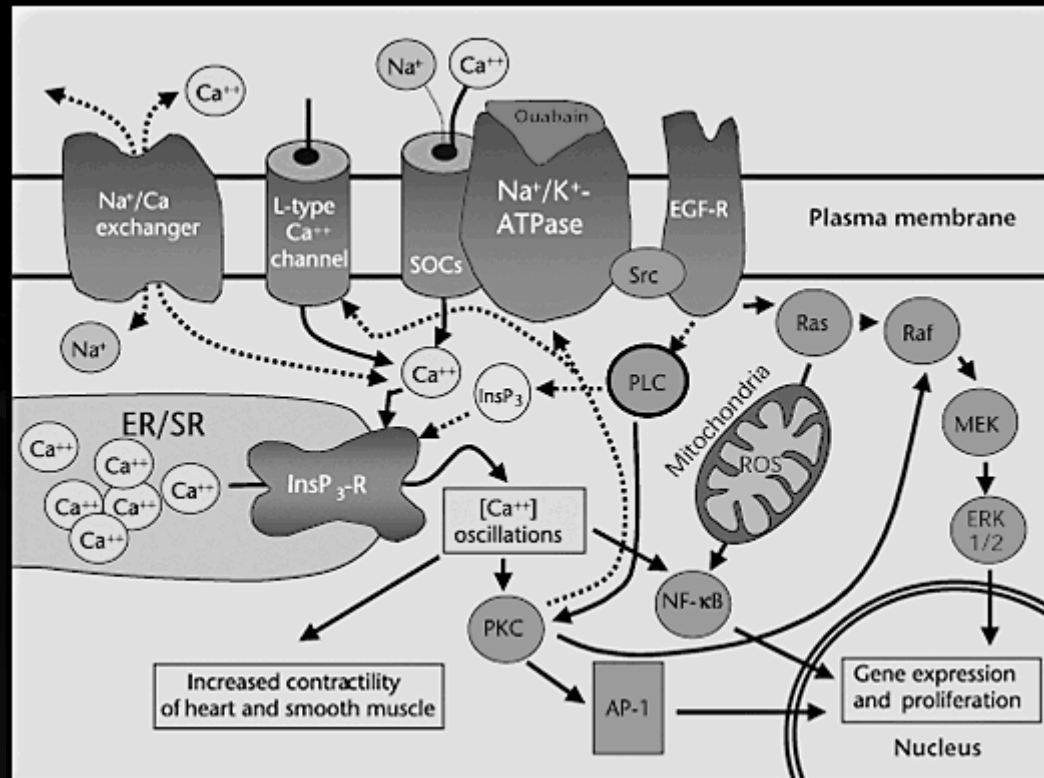
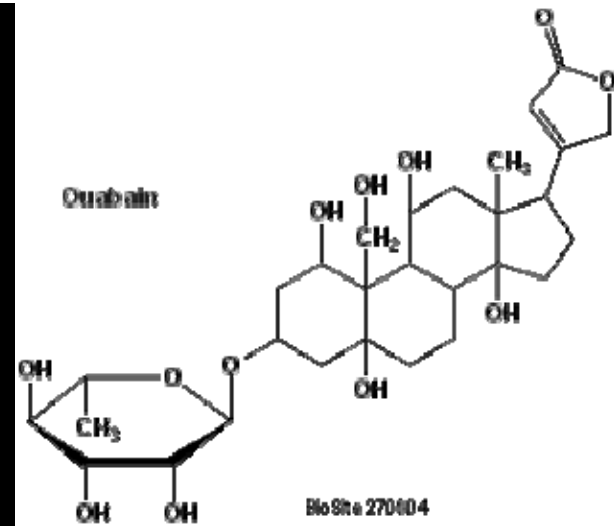
*Tab. 2.2.* Tabulka rozložení iontových koncentrací na buněčné membráně kosterního svalu savce.

# Na/K ATPáza – „nabíjí“ membránu

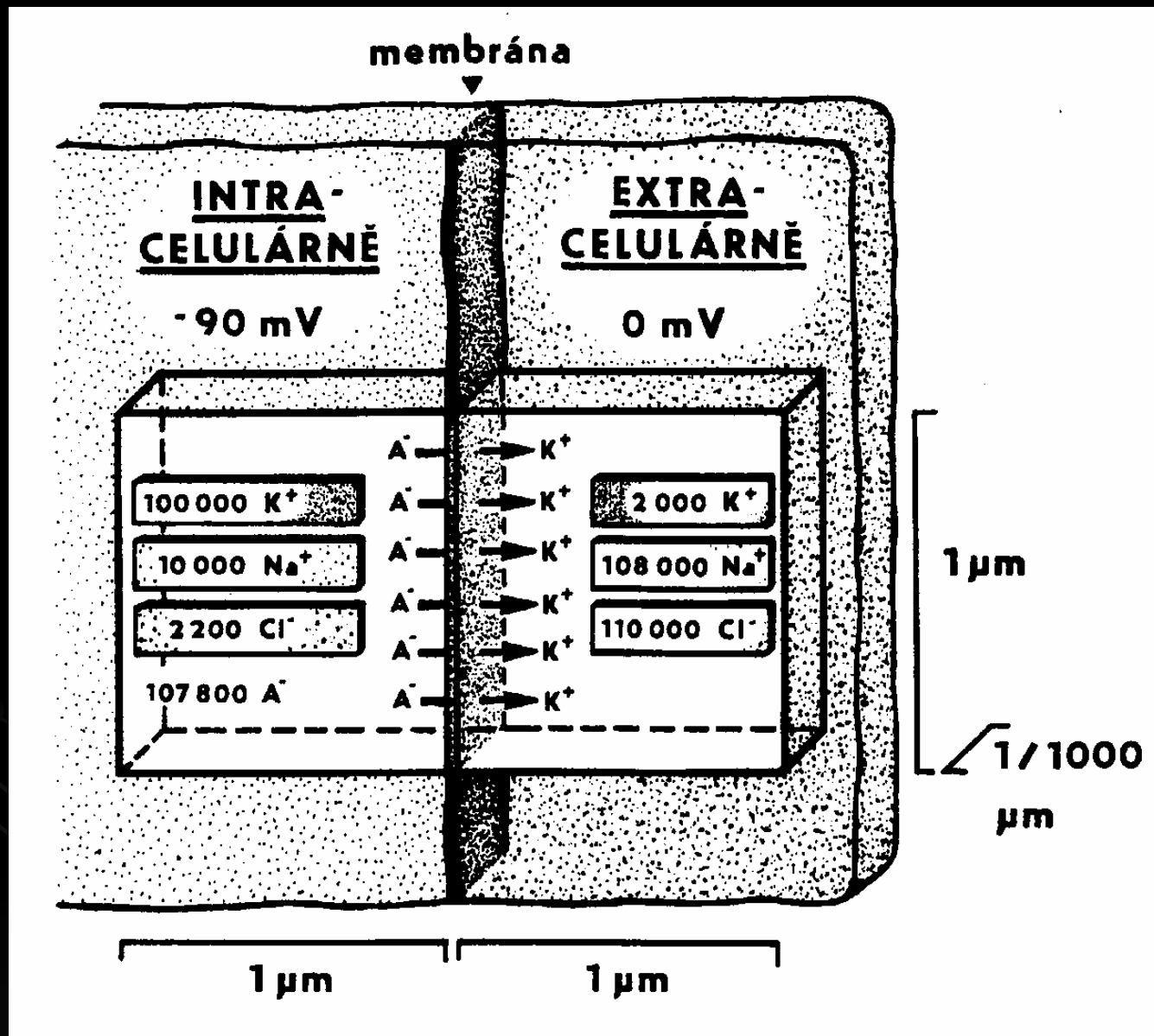


- Cation binding residues
- Residues involved in conformational changes
- Residues affecting Na<sup>+</sup> and K<sup>+</sup> selectivity

# Ouabain





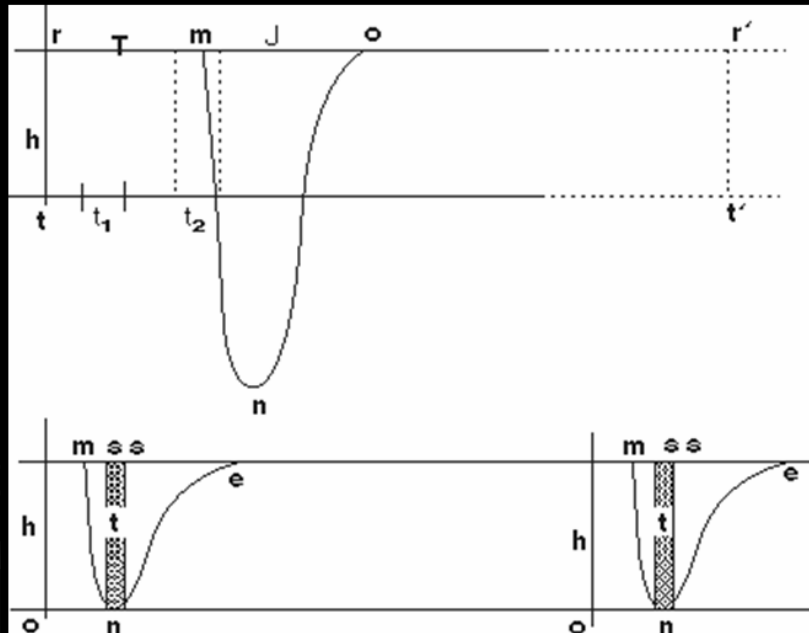


Jen 6 kationtů vně navíc na pozadí 220.000/220.000 nabije membránu

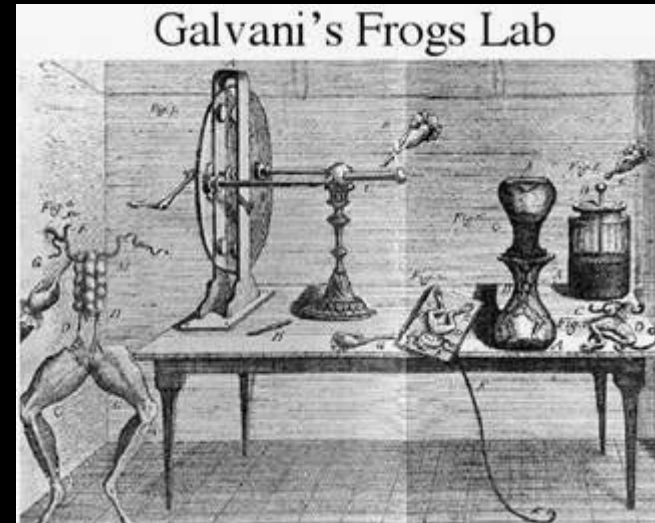
# Klidový potenciál

- Uložená energie pro generování, šíření a zpracování elektrických signálů:
  - Akční potenciál – vhodný pro dálkový, nezkreslený a rychlý přenos signálů
  - Místní potenciál – vhodný pro zpracování, syntézu, modifikaci informací

# Akční potenciál

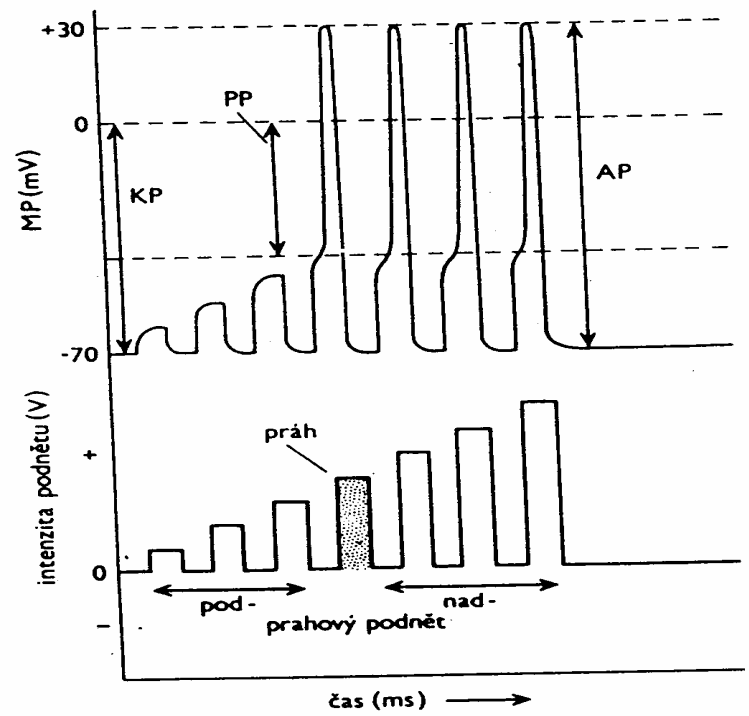
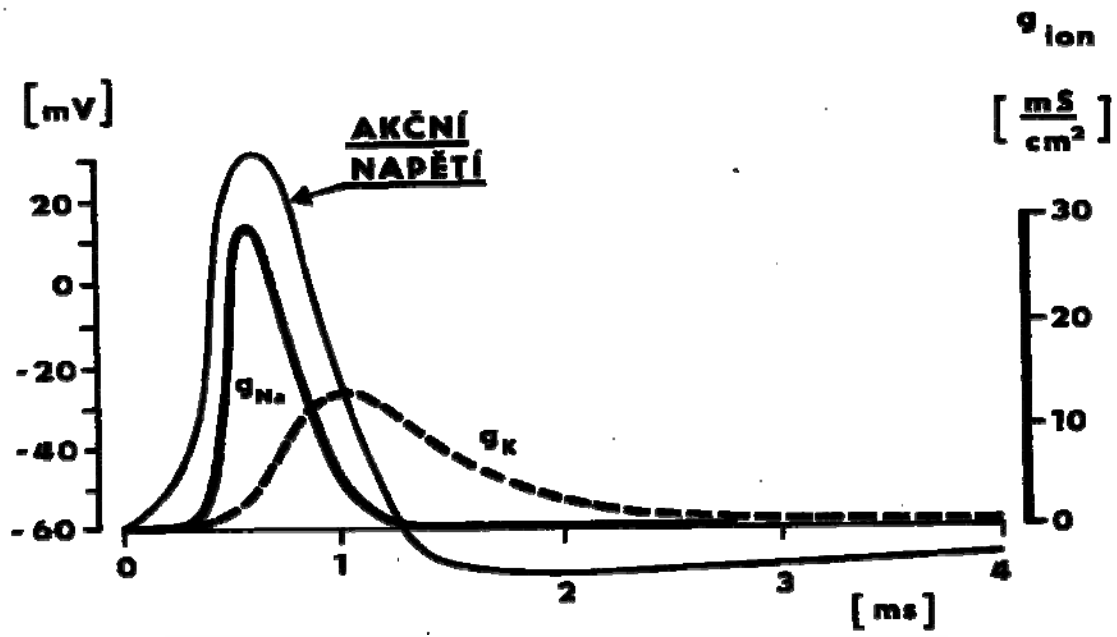


Horní záznam odpovídá průběhu "nervového akčního proudu", tak jak jej Bernstein naměřil r. 1868 a publikoval r. 1871. Na spodním záznamu, který Bernstein publikoval v *Elektrobiologii* r. 1913, chybí překmit "akčního proudu" do kladných hodnot (průběhy jsou zaznamenány s opačnou polaritou, než na jakou jsme dnes zvyklí).



**Jak se dnes měří a jak vypadá?**

**<http://www.hhmi.org/biointeractive/vlabs/neurophysiology/index.html>**



—  
The Nobel Prize in Physiology  
or Medicine 1963



"for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane"



**Sir John  
Carew  
Eccles**

1/3 of the  
prize  
Australia

Australian  
National  
University  
Canberra,  
Australia  
d. 1997



**Alan Lloyd  
Hodgkin**

—  
1/3 of the  
prize  
United  
Kingdom

University of  
Cambridge  
Cambridge,  
United  
Kingdom  
d. 1998



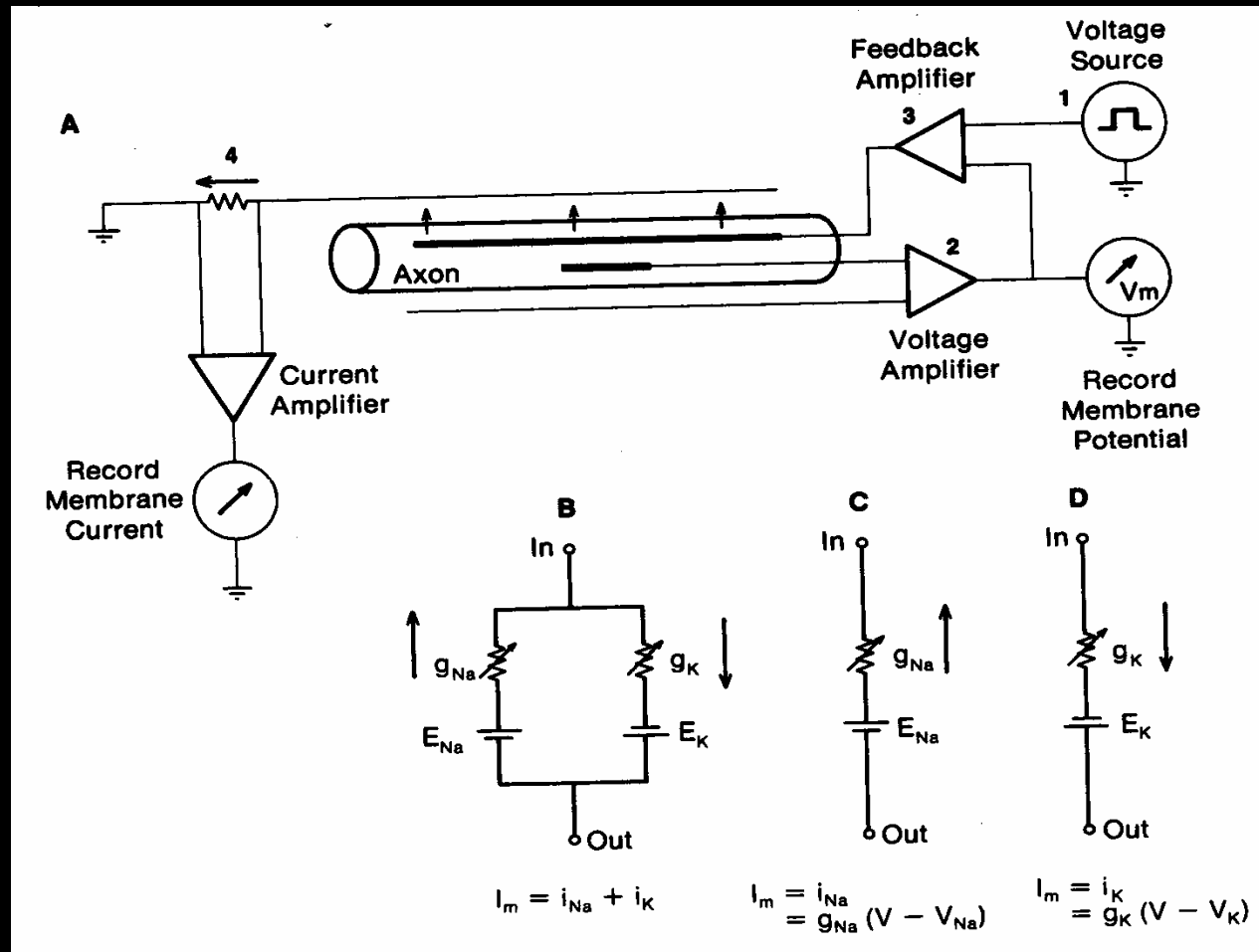
**Andrew  
Fielding  
Huxley**

1/3 of the  
prize  
United  
Kingdom

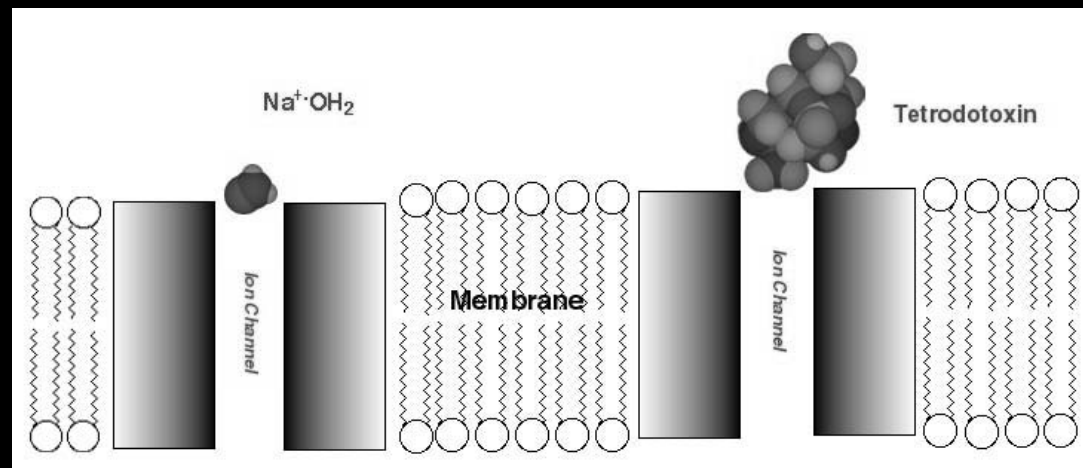
London  
University  
London,  
United  
Kingdom

Hodgkin & Huxley  
napěťový zámek, 1963.

Dodávaný proud kompenzuje  
iontové toky tak, aby napětí  
zůstalo konstantní. Proud  
je registrován.



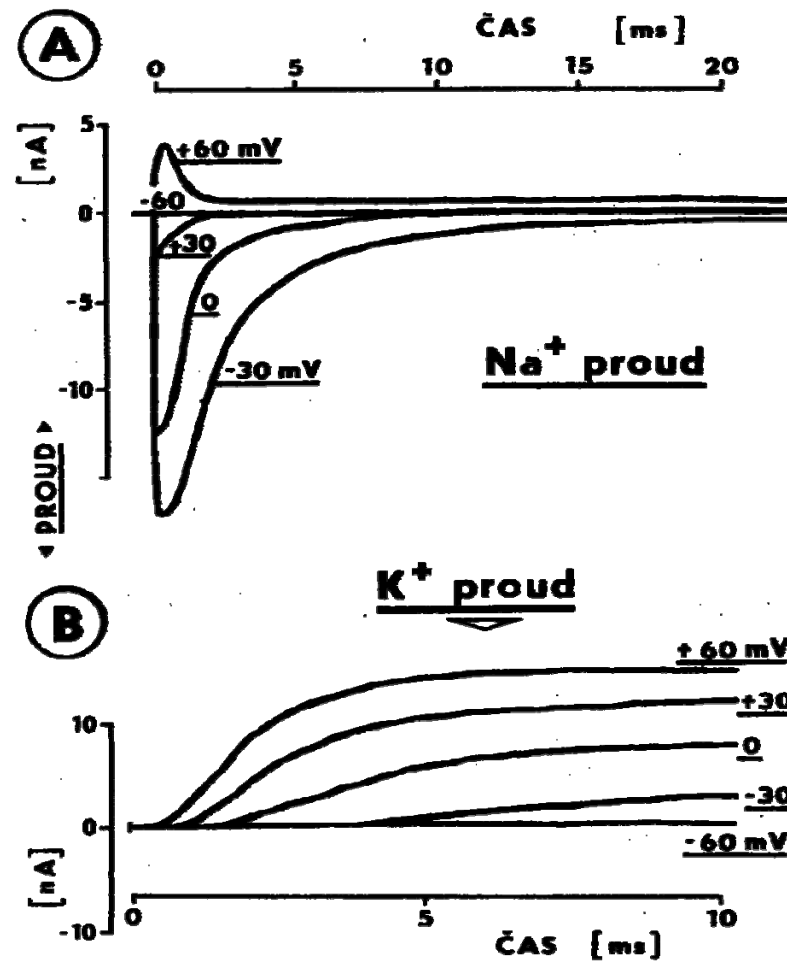
Na – TTX (Tetrodotoxin) – „ucpe“ ústí kanálu  
K – TEA (Tetraethyl amonium)



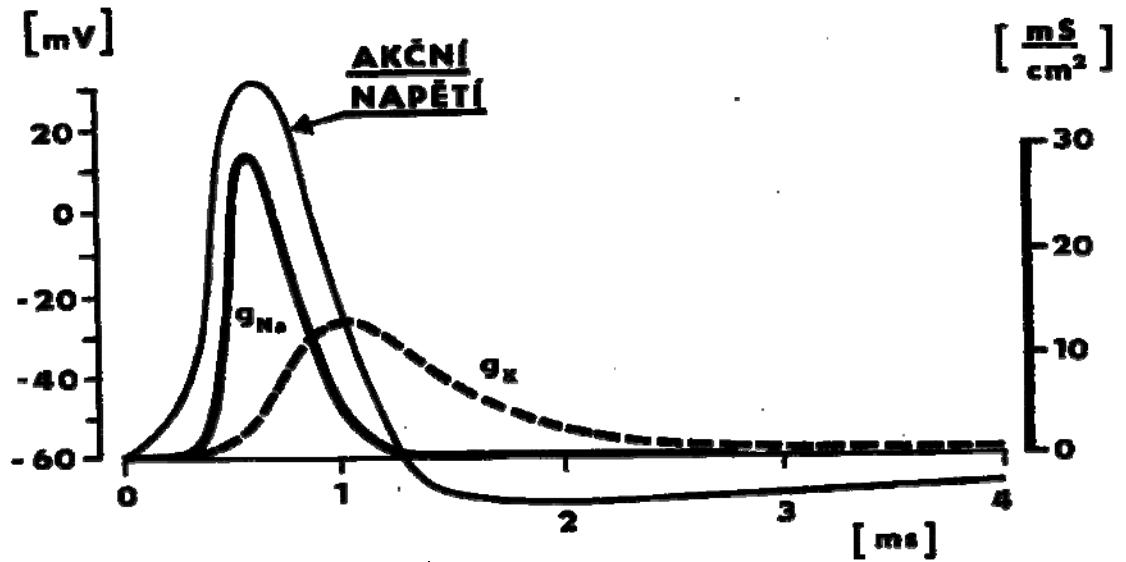
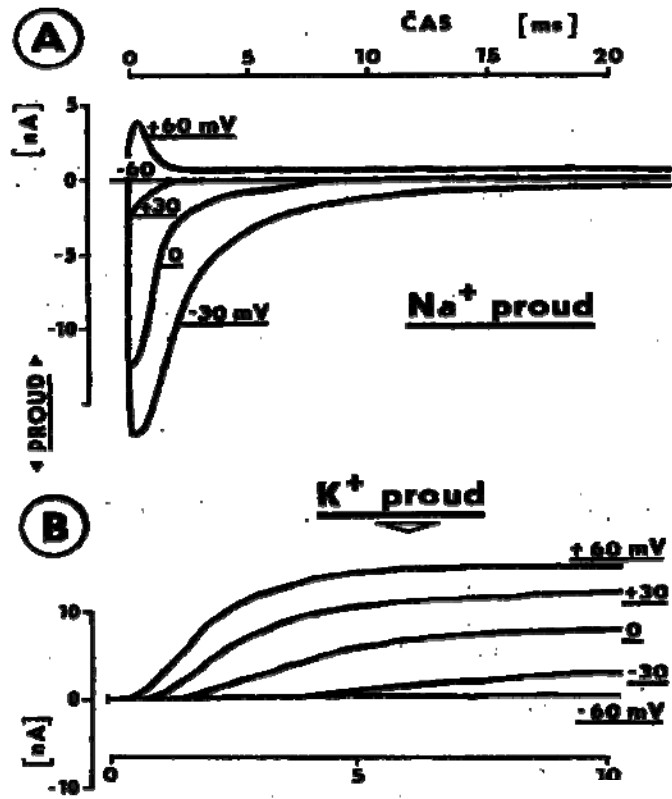
Čtverzubec Tetraodon - fugu

<http://www.osel.cz/index.php?clanek=238>





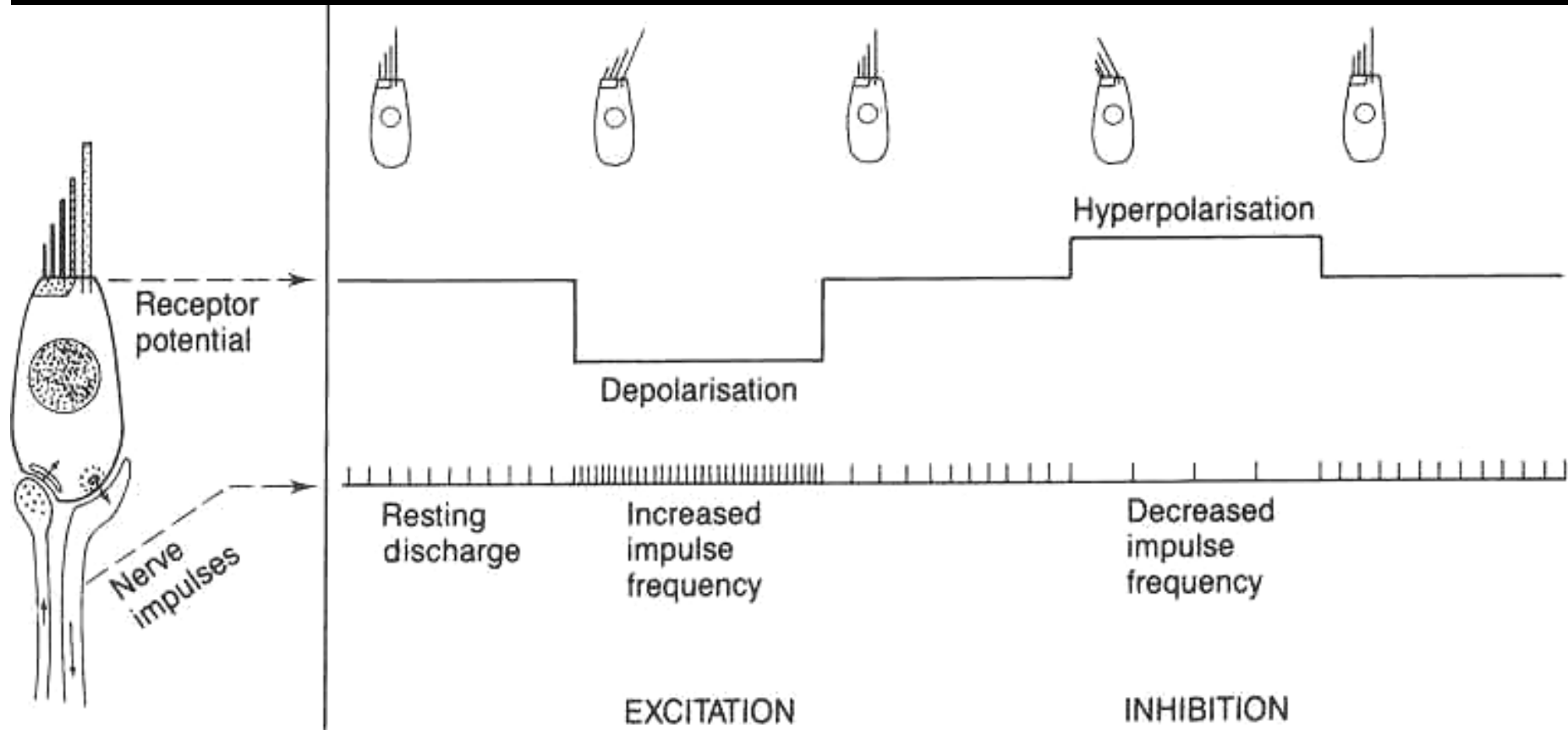
Obr. 14.16 Membránové proudy na myelinisovaných axonech po experimentálně provedené skokové změně membránového napětí. Membránové napětí bylo v čase  $t=0$  skokem změněno z  $-95$  mV na hodnoty udané u jednotlivých křivek. Křivky vyjadřují naměřené proudy iontů, nahoře natria, dole kalia. Svislá osa - intenzita proudu, vodorovná osa - čas. Při skoku na  $-60$  mV je skok ještě podprahový a nevyvolává žádný proud. A:  $\text{Na}^+$  proud; mezi  $+30$  a  $+60$  mV mění  $\text{Na}^+$  proud polaritu z negativního (směrem do buňky, pod vodorovnou přímkou) na pozitivní (ven z buňky, nad přímkou, pro daný preparát leží totiž hodnota rovnovážného napětí pro  $\text{Na}^+$  pod  $+60$  mV) a s přibývajícím depolarisací teče stále kratčeji. B:  $\text{K}^+$  proud; tento proud stoupá po depolarisaci (do pozitivních hodnot, tj. ven z buňky) mnohem pomaleji než proud  $\text{Na}^+$  a drží se na stejné výšce během celé depolarisace. (Z DUDELA 1990b)



## Propagace, Voltage clamp

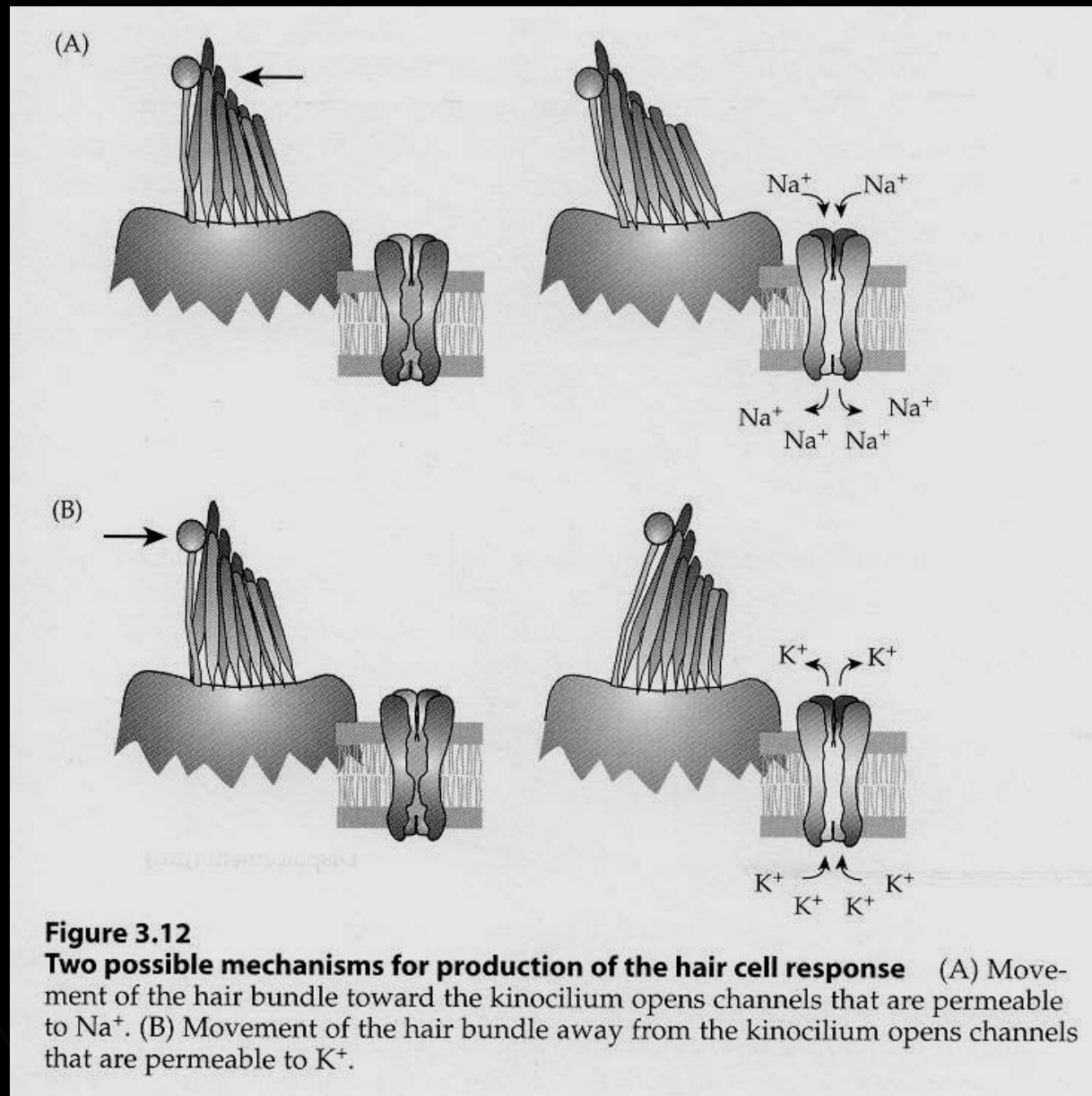
<http://bcs.whfreeman.com/thelifewire/content/chp44/4402s.swf>

# K čemu ještě voltage clamp?



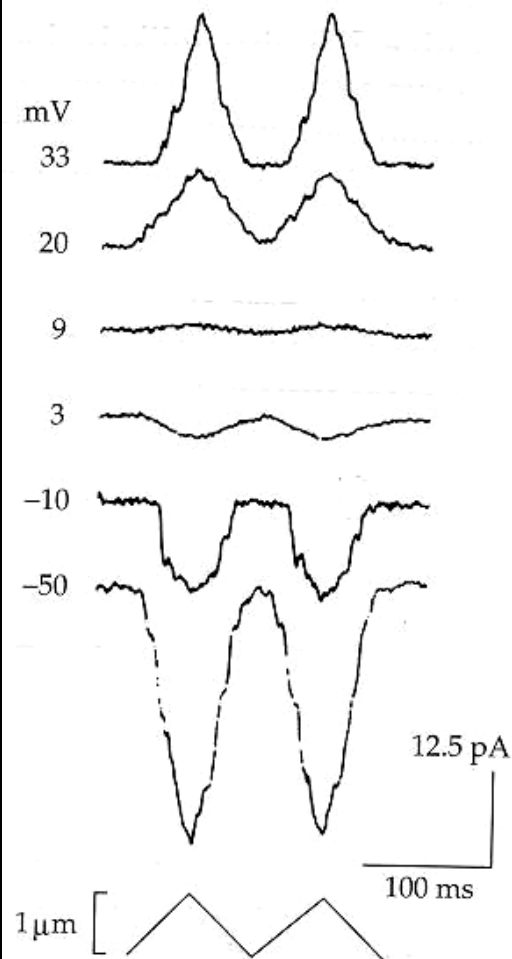
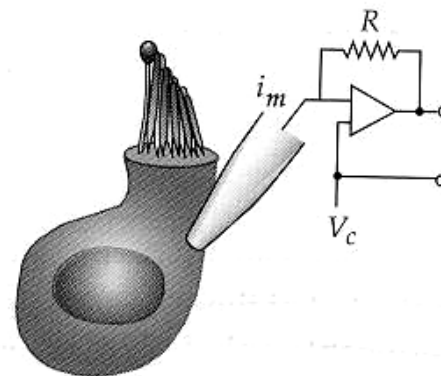
Otevírají se Na nebo zavírají K kanály?

Zamknutí membrány vyšachuje napětově citlivé kanály a iontové toky jsou důsledkem jen mechanické manipulace!

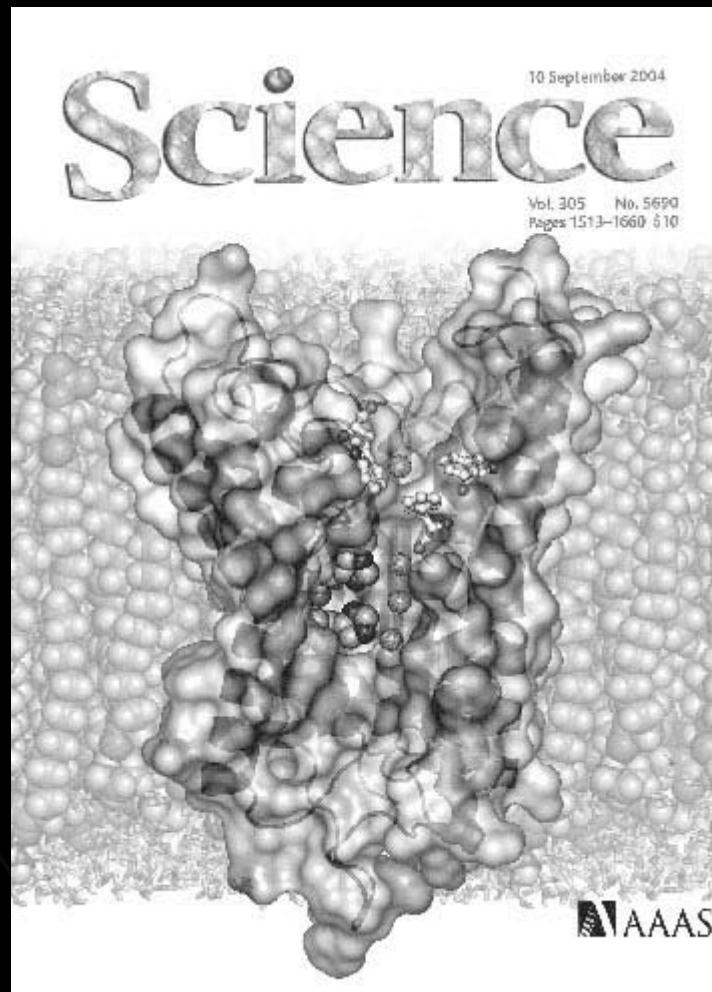


Různé proudy přes membránu po pohy-  
bech cilií u různých hodnot „zamknutého“  
napětí.

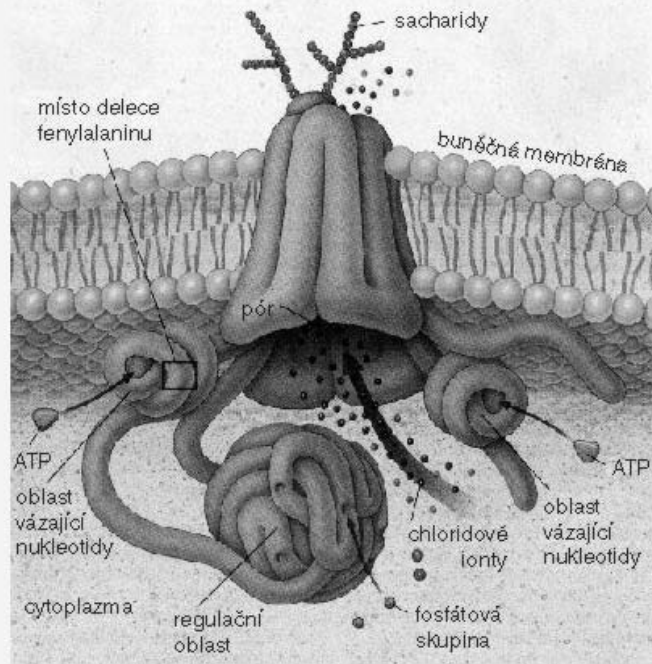
Není to ani K ani jen Na, jsou to neselektivně  
kationty.



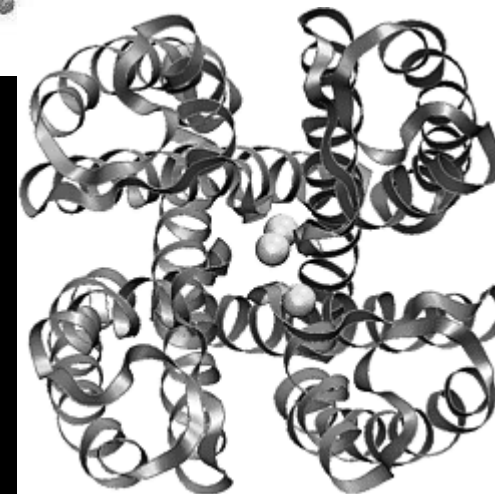
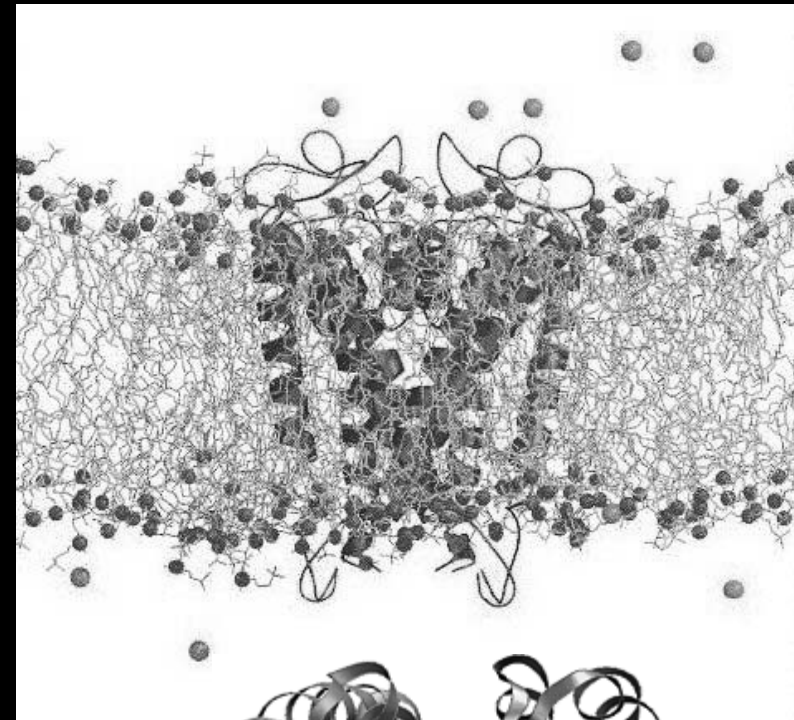
# Kanály a patch clamp



# Kanály – prostředek udržování integrity buňky a komunikace

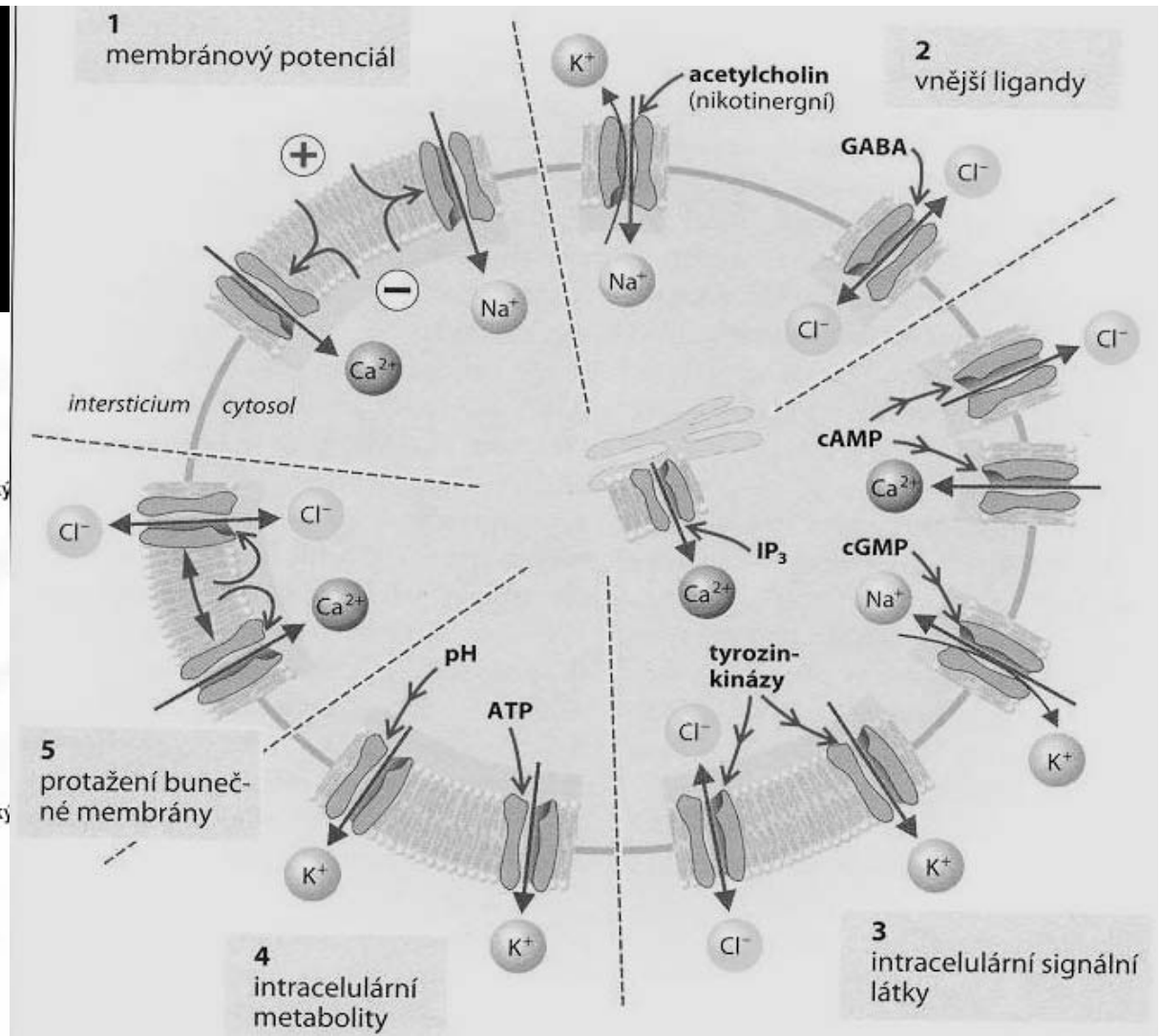
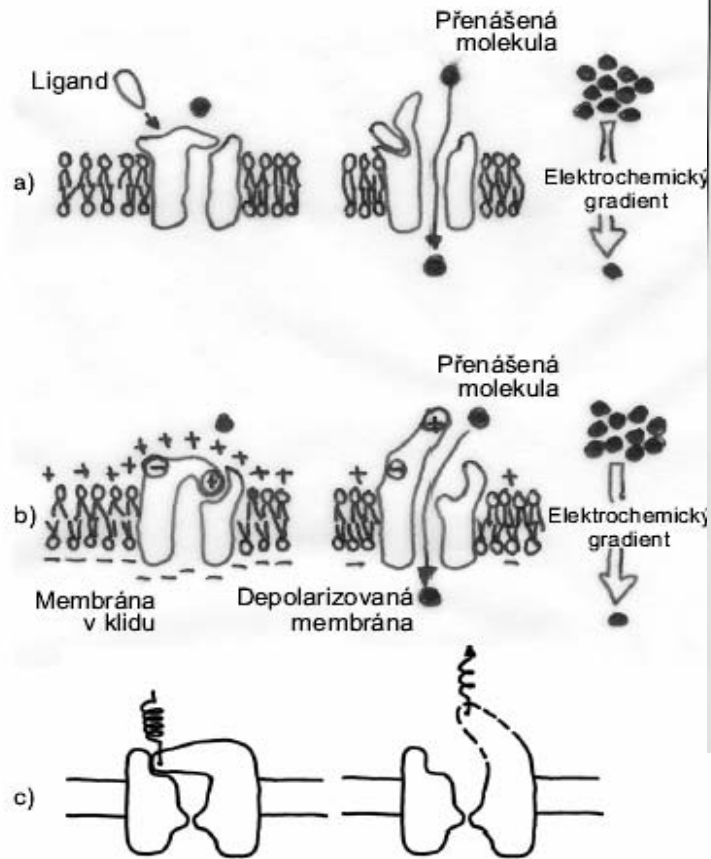


Po ukotvení v membráně se protein CFTR složitě přizpůsobuje – vytváří kanálky, kterými mohou přes membránu proudit chloridové ionty. V cytoplazmatické části proteinu jsou tři regulační oblasti, které se podílejí na uzavírání a otevírání póru. Kanálek se otevře jedině tehdy, když se na CFTR navážou dvě molekuly ATP a zároveň je fosforylována třetí regulační oblast. U mutovaného proteinu je jedna z oblastí vazajících ATP intaktní a membránový kanálek se neotevírá.



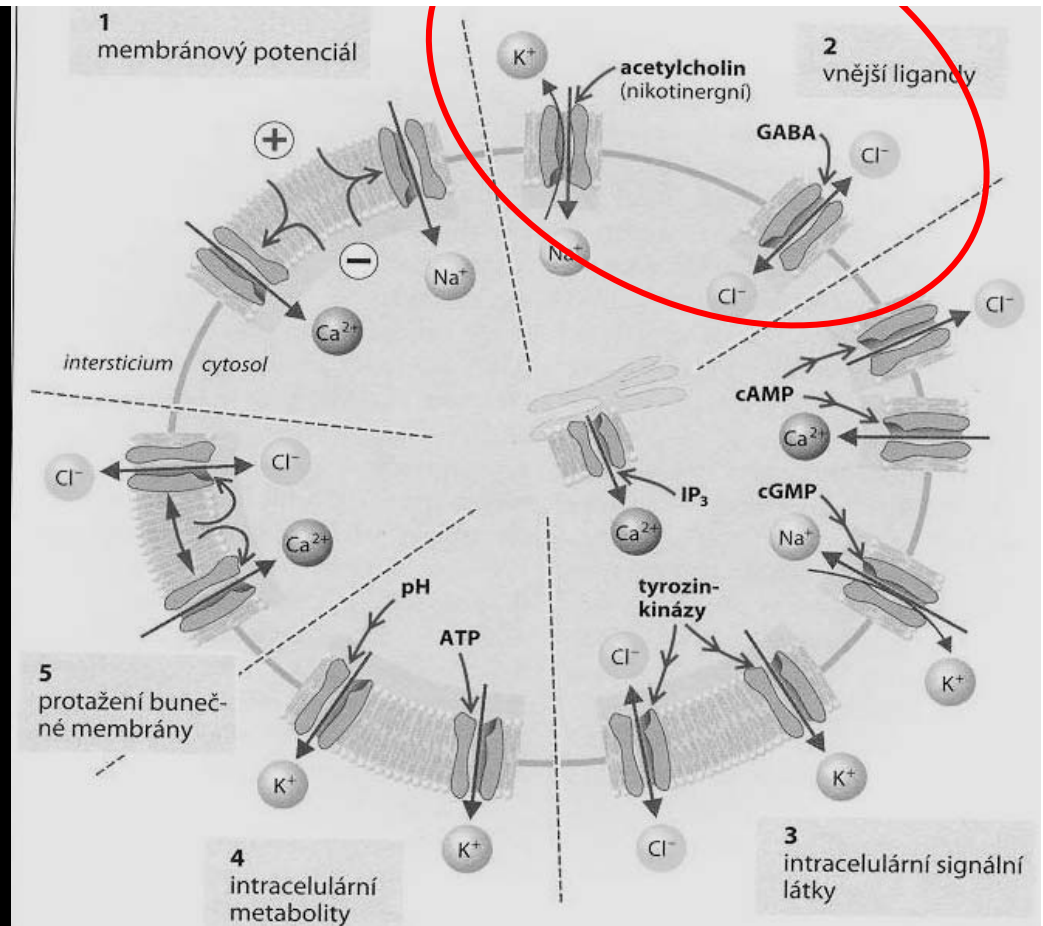


# Kanály – typy vrátkování



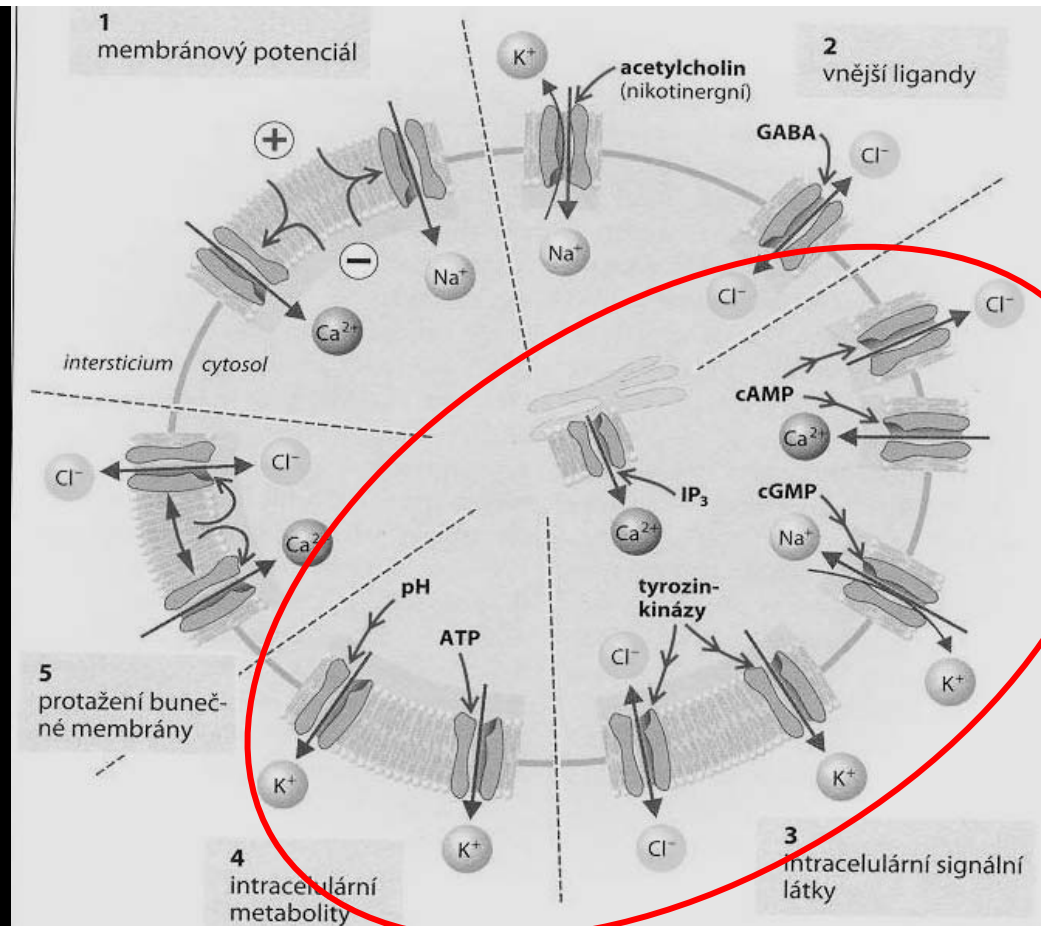
Obr. 2.7. Vrátkované kanály. Pohyblivá uzavírací doména reaguje otevřením kanálu na různé podněty: a) chemicky vrátkované na vazbu ligandu na receptorové místo, b) elektricky vrátkované na změnu membránového napětí, c) mechanicky vrátkované na tah za připojený cytoskeletální filament.

# Ionotropní transdukce – receptor přímo na kanálu



**TABLE 12.3** Ionotropic and metabotropic receptors: Structural, functional, and mechanistic differences

Characteristic	Ionotropic receptors	Metabotropic receptors
Receptor molecule	Ligand-gated channel receptor	G protein-coupled receptor
Molecular structure	Five subunits around an ion channel	Protein with seven transmembrane segments; no channel
Molecular action	Open ion channel	Activate G protein; metabolic cascade
Second messenger	No	Yes (usually)
Gating of ion channels	Direct	Indirect (or none)
Type of synaptic effect	Fast EPSP or IPSP	Slow PSPs; modulatory changes (in channel properties, cell metabolism, or gene expression)



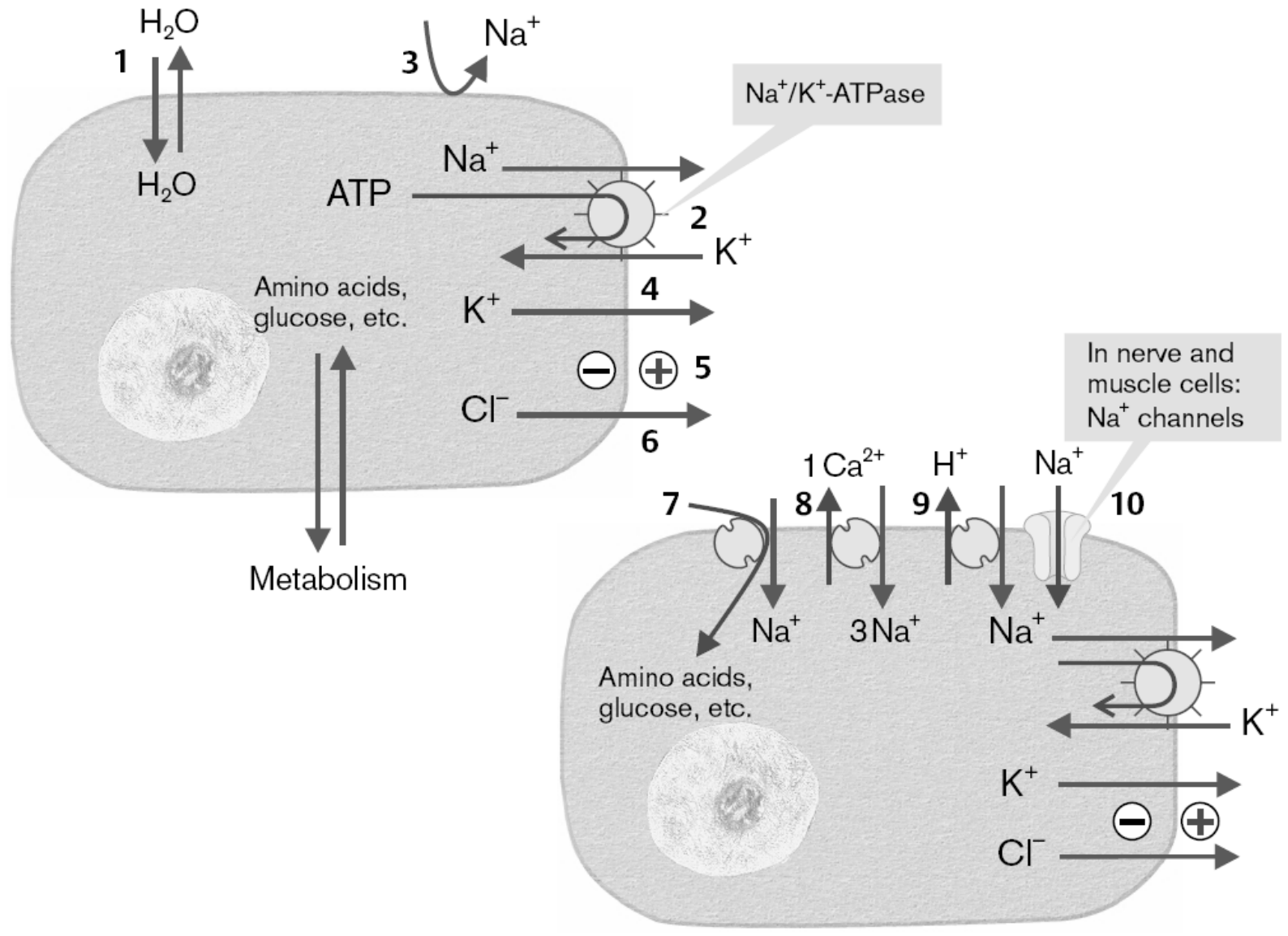
**TABLE 12.3** Ionotropic and metabotropic receptors: Structural, functional, and mechanistic differences

Characteristic	Ionotropic receptors	Metabotropic receptors
Receptor molecule	Ligand-gated channel receptor	G protein-coupled receptor
Molecular structure	Five subunits around an ion channel	Protein with seven transmembrane segments; no channel
Molecular action	Open ion channel	Activate G protein; metabolic cascade
Second messenger	No	Yes (usually)
Gating of ion channels	Direct	Indirect (or none)
Type of synaptic effect	Fast EPSP or IPSP	Slow PSPs; modulatory changes (in channel properties, cell metabolism, or gene expression)

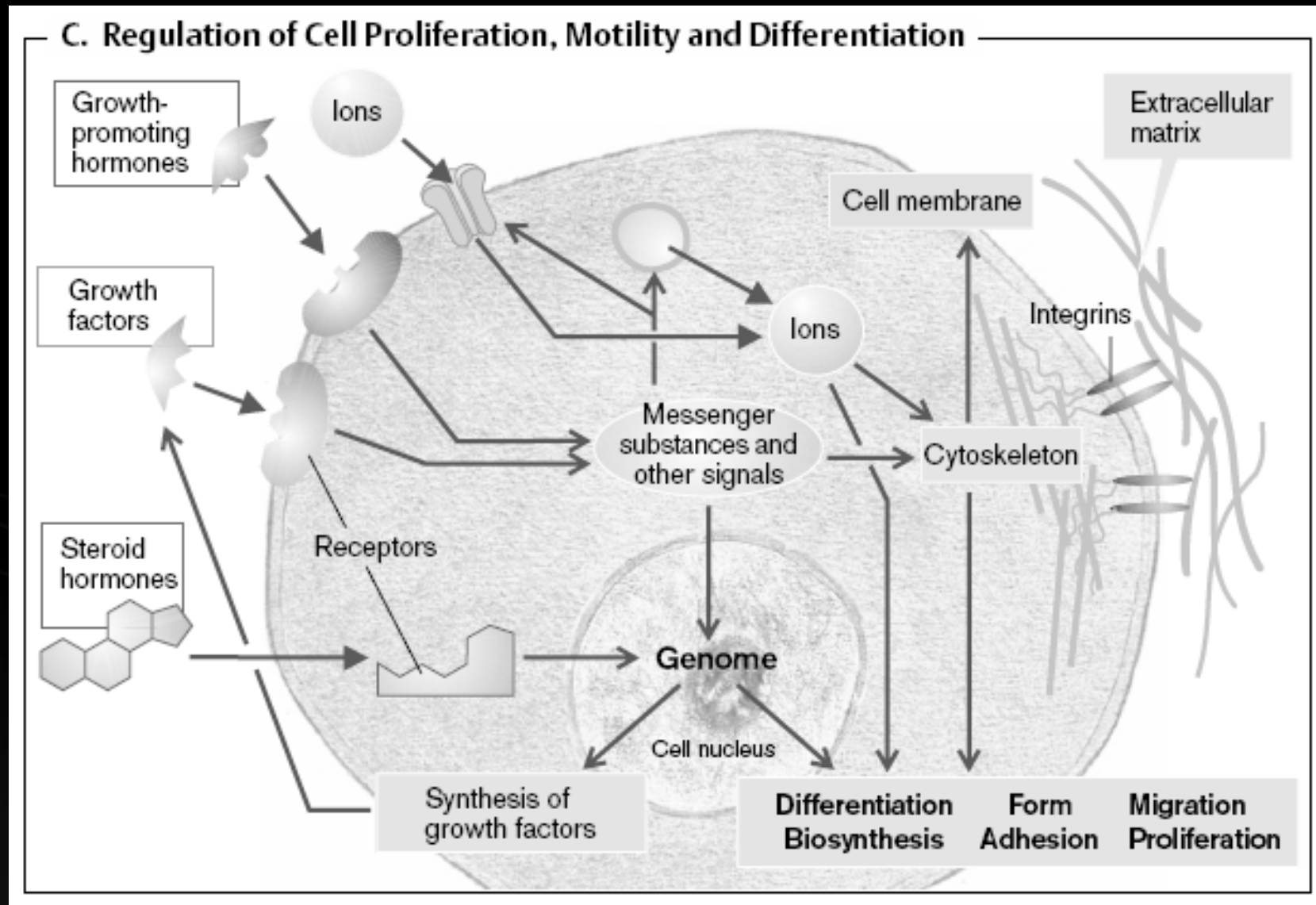
## Metabotropní transdukce

Už jste se potkali s kanály?

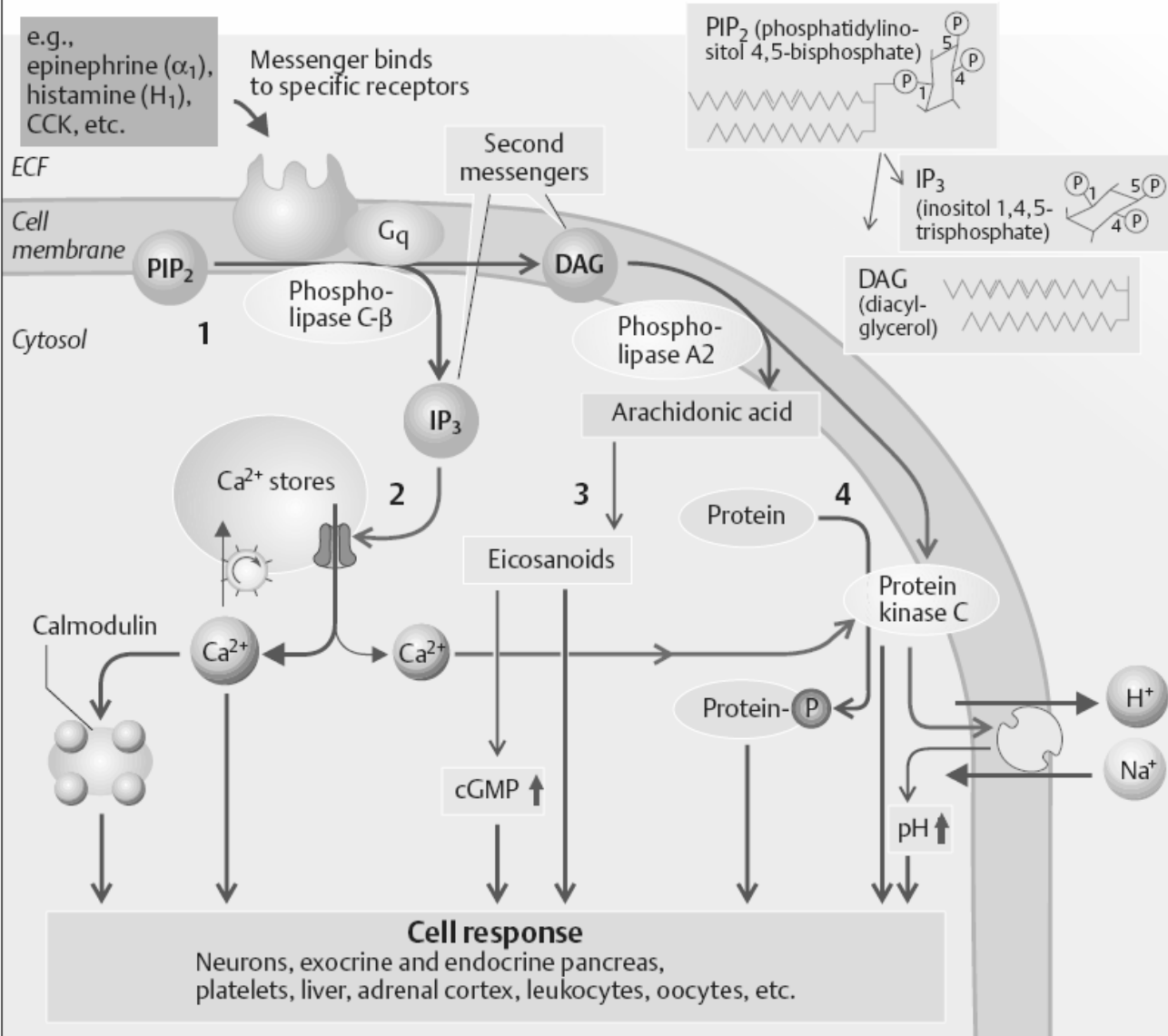
### A. Homeostasis of Volume and Electrolytes in the Cell



Řídí předávání signálů nesených ionty.

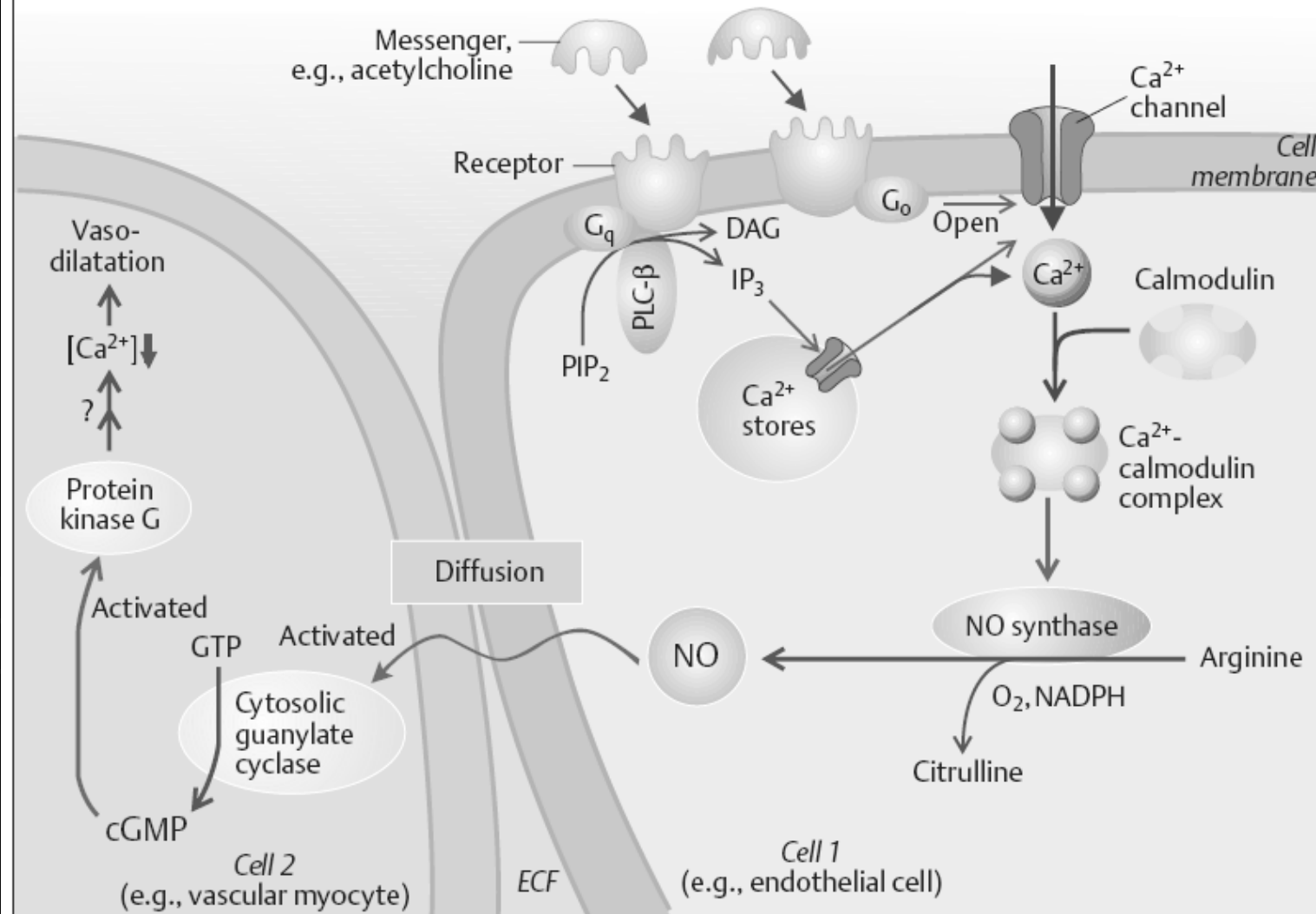


## B. Diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP<sub>3</sub>) as second messengers

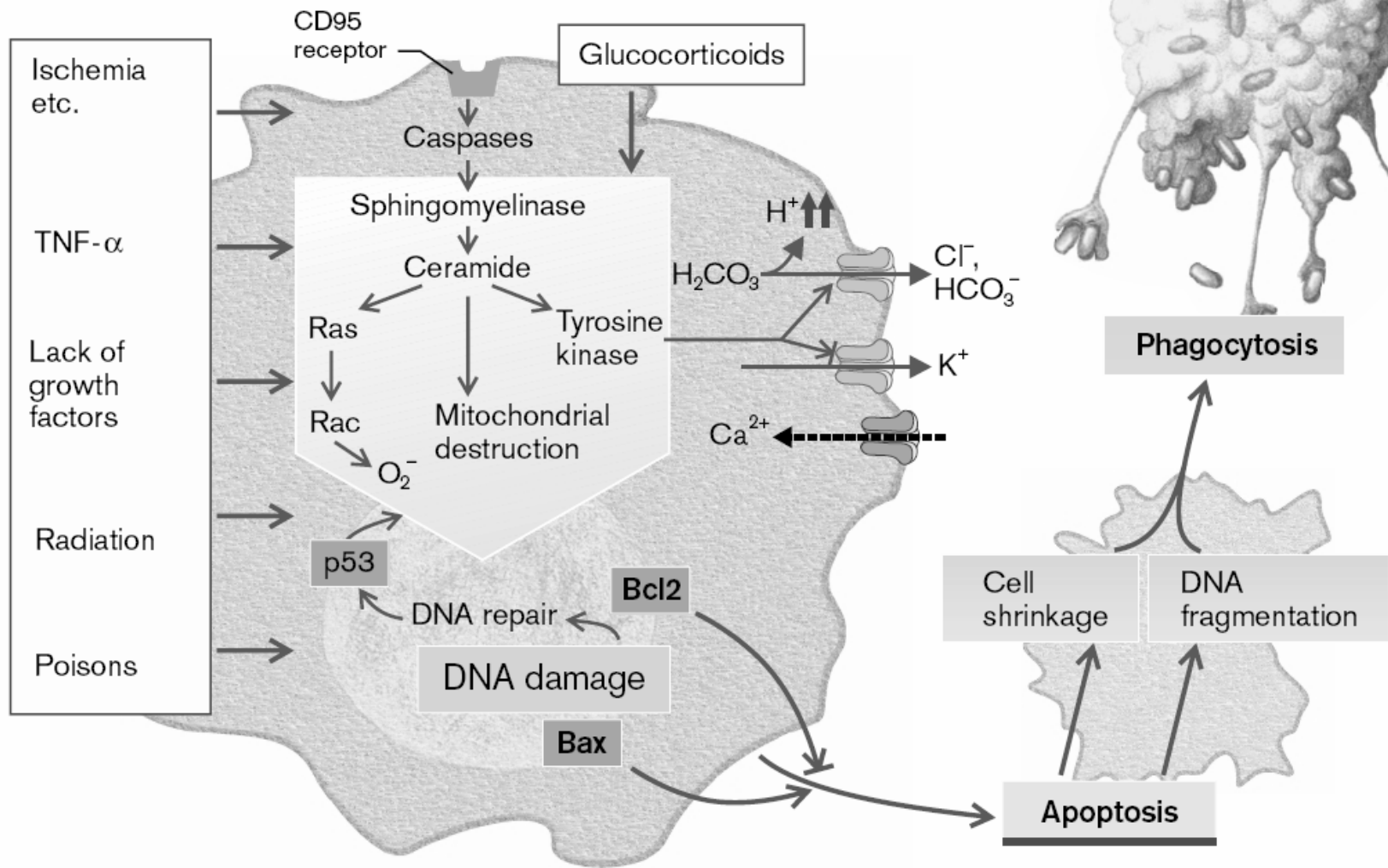




### E. Nitric oxide (NO) as a transmitter substance

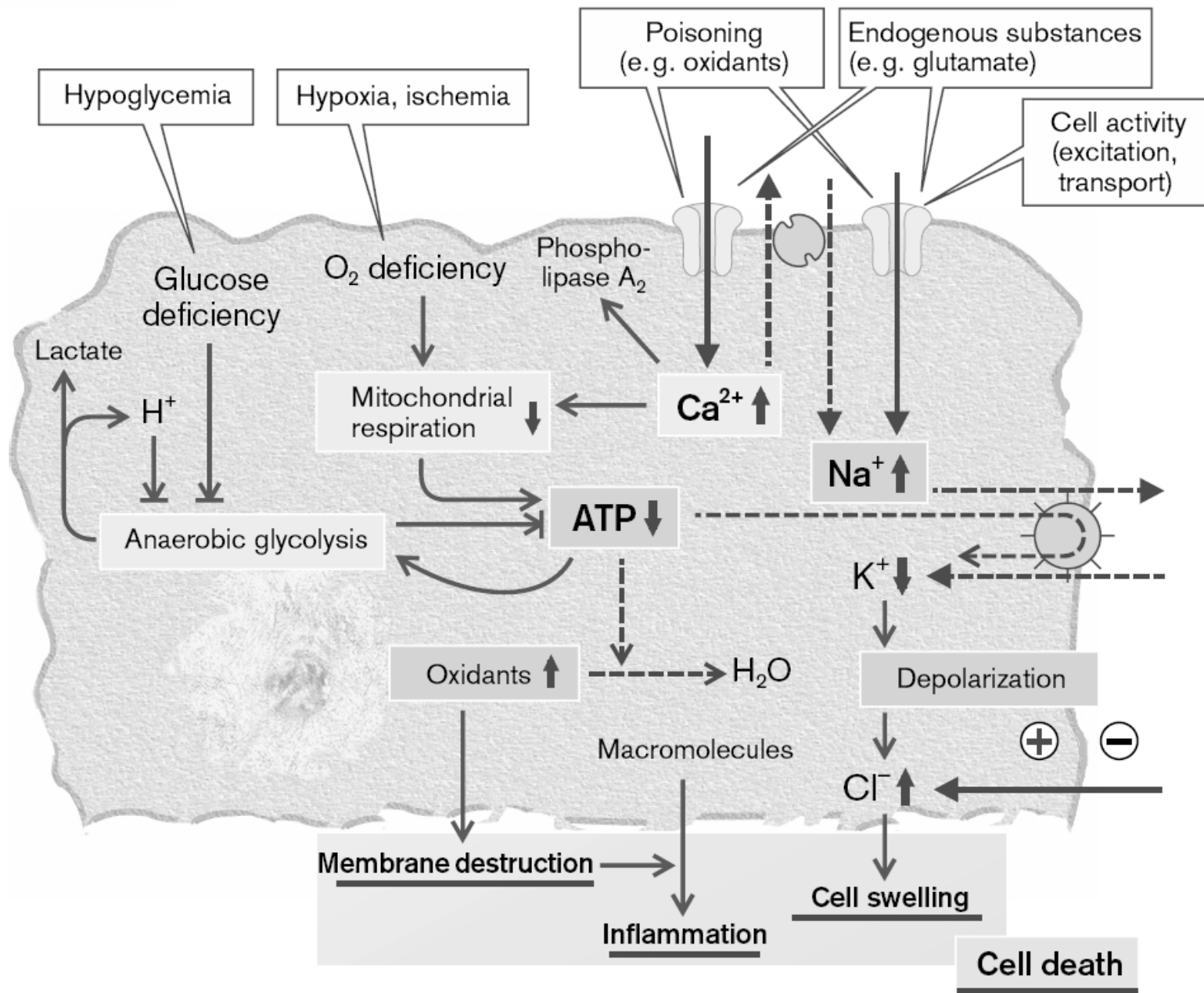


## A. Triggering and Development of Apoptosis

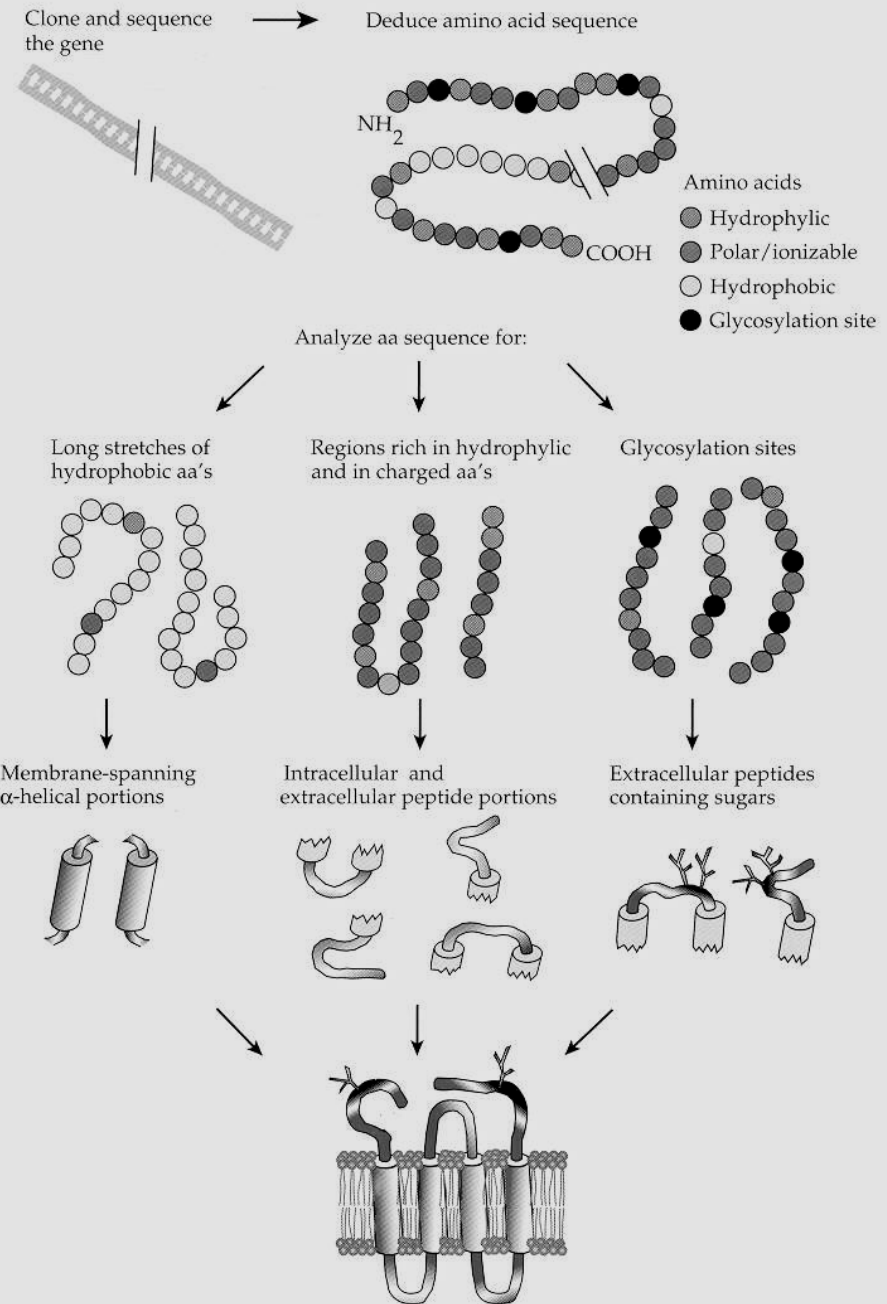
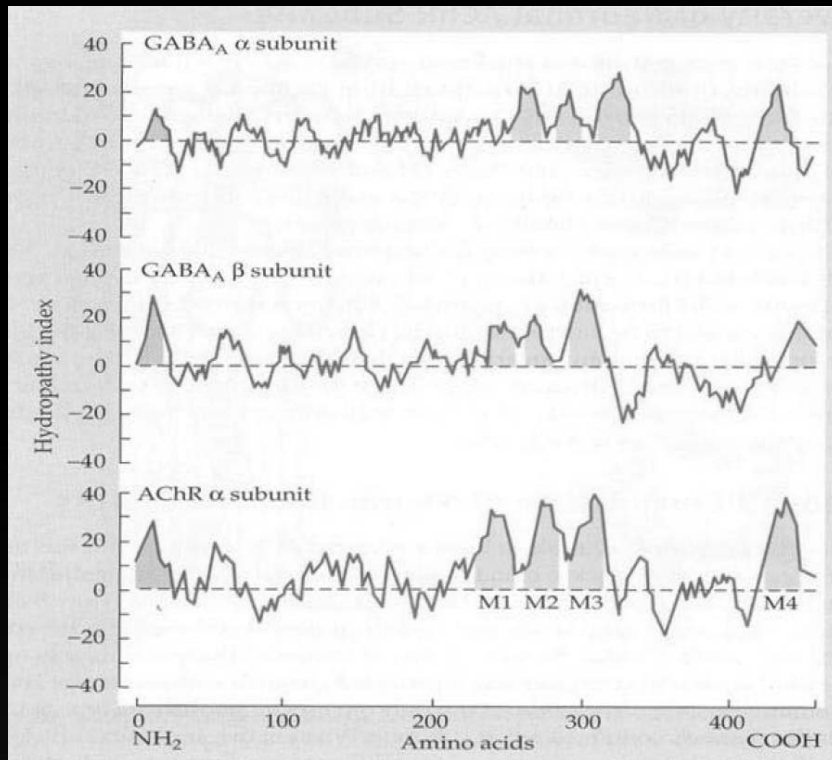




## B. Necrosis

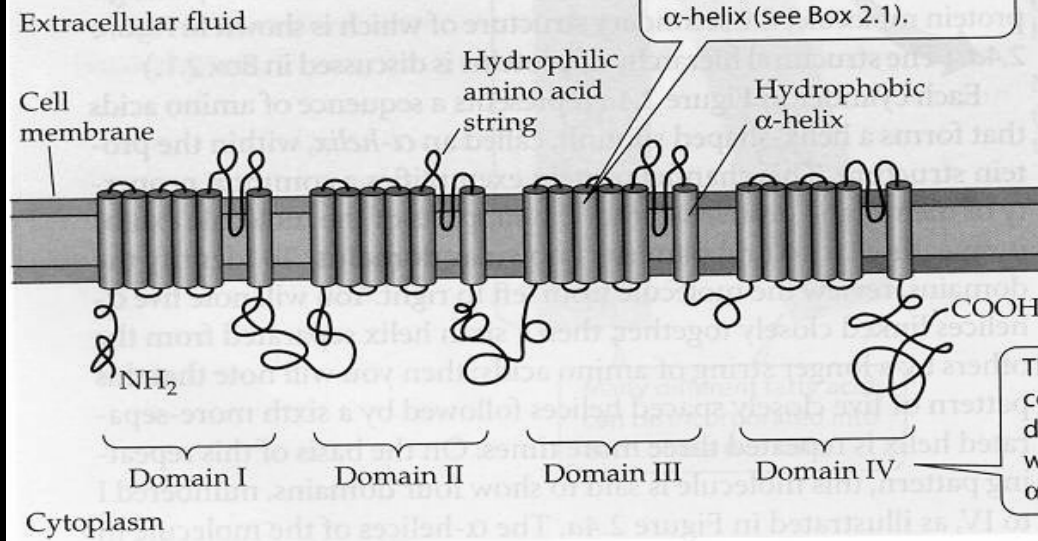


# Struktura – Transmembránové proteiny Rekonstrukce podle vlastností



**Figure 1.10**  
**Analysis of hydropathy and the folding of membrane proteins** The amino acid sequence of a membrane protein can be used to make inferences about protein structure, as described in the text.

(a) Secondary structure (linear presentation)



carbohydrate groups always project from the face, not the inner, cytoplasmic face (see Figure 2.4). Hydrate groups are thought to serve as attachment sites for cellular proteins and as cell recognition sites.

<sup>1</sup> The word fragment *glyco* refers to carbohydrates (after the

Figure 2.4 The structure of a transmembrane protein—illustrating several modes of

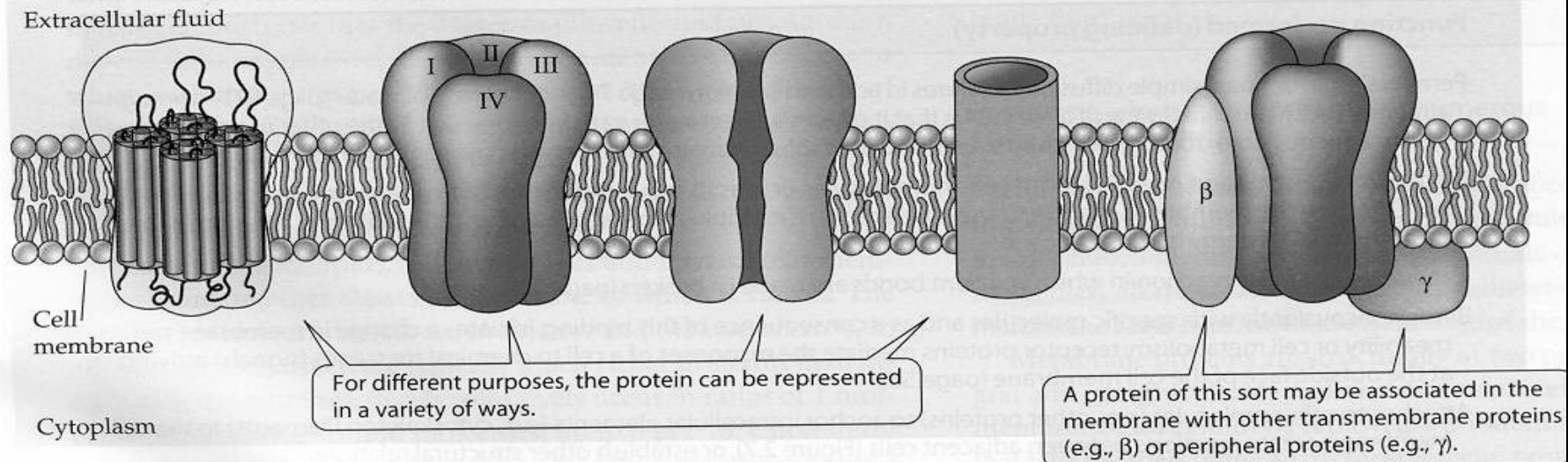
(b) Simplified three-dimensional structure enclosed in a sketch of the envelope of the molecule

(c) Stylized version of chemical structure showing subunits

(d) Semirealistic symbol

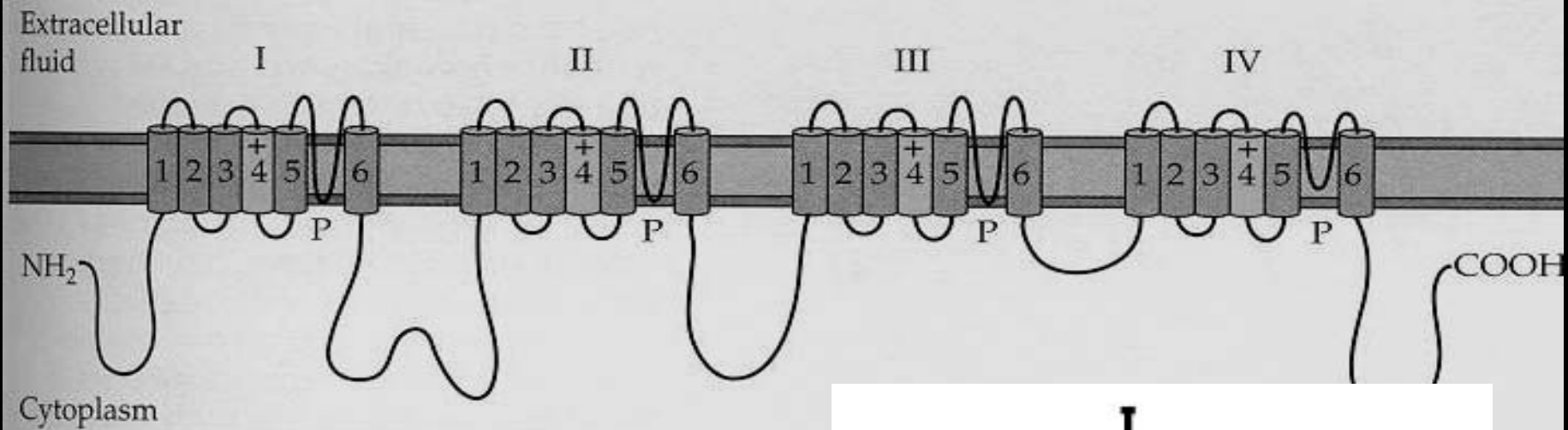
(e) Schematic symbol

(f) Stylized version of chemical structure showing associated protein molecules

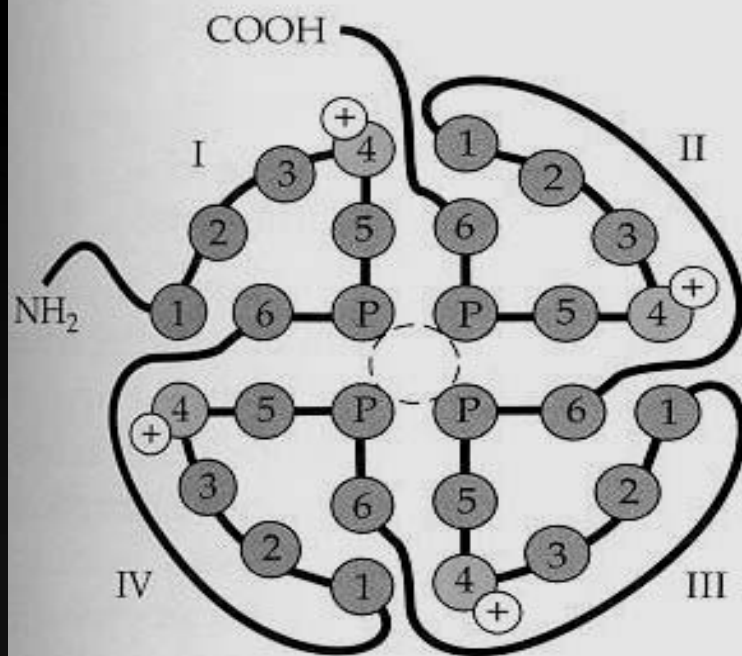


(a) Topology of voltage-gated Na<sup>+</sup> channels

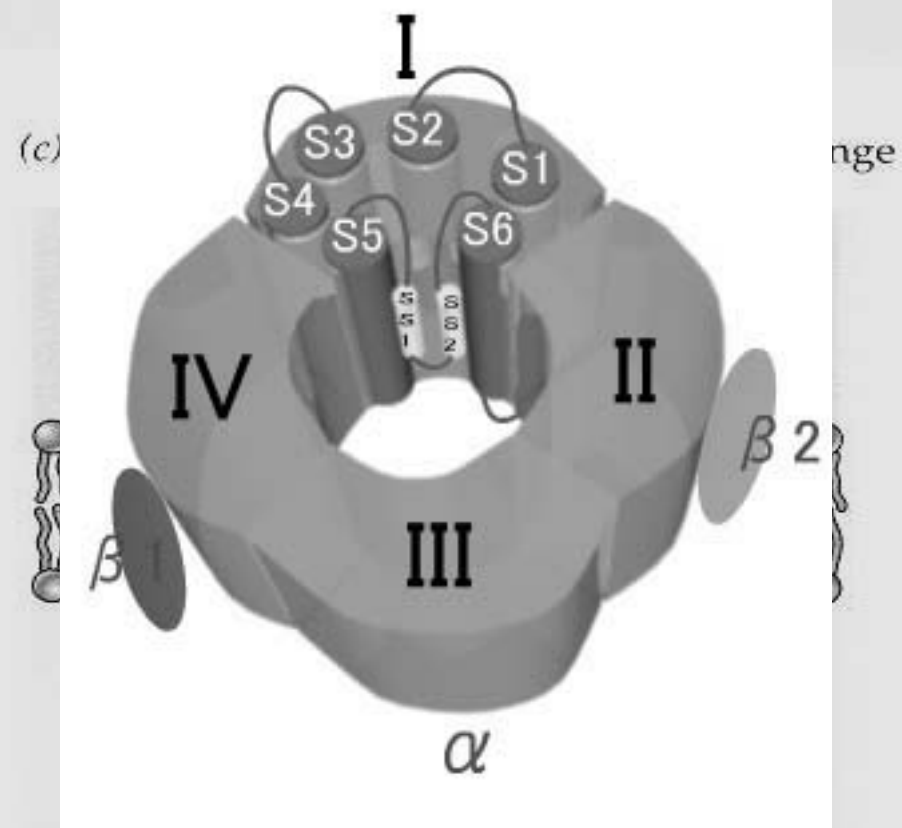
4 domény, 6 segmentů



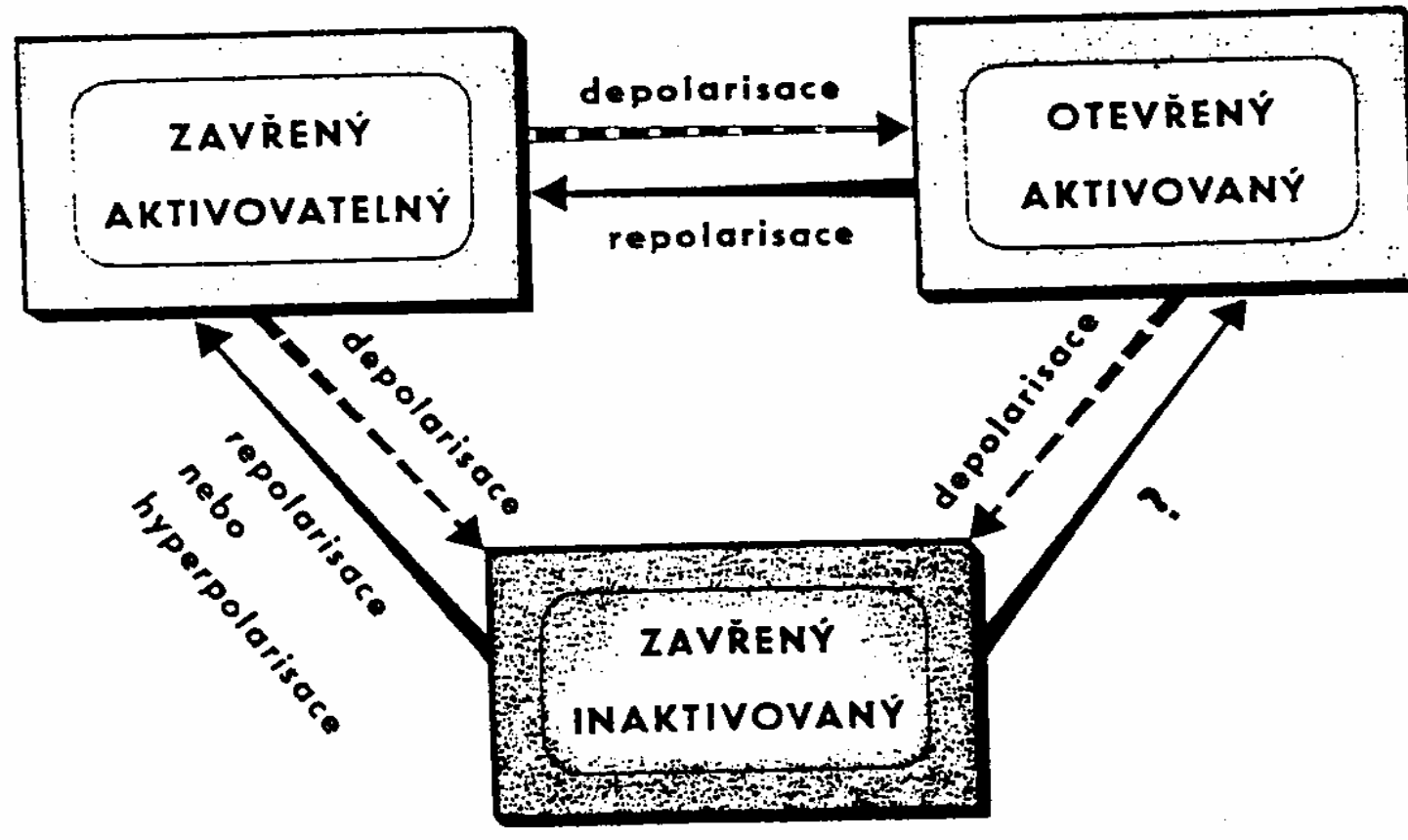
(b) Surface view of a Na<sup>+</sup> channel



(c)



# STAVY KANÁLŮ

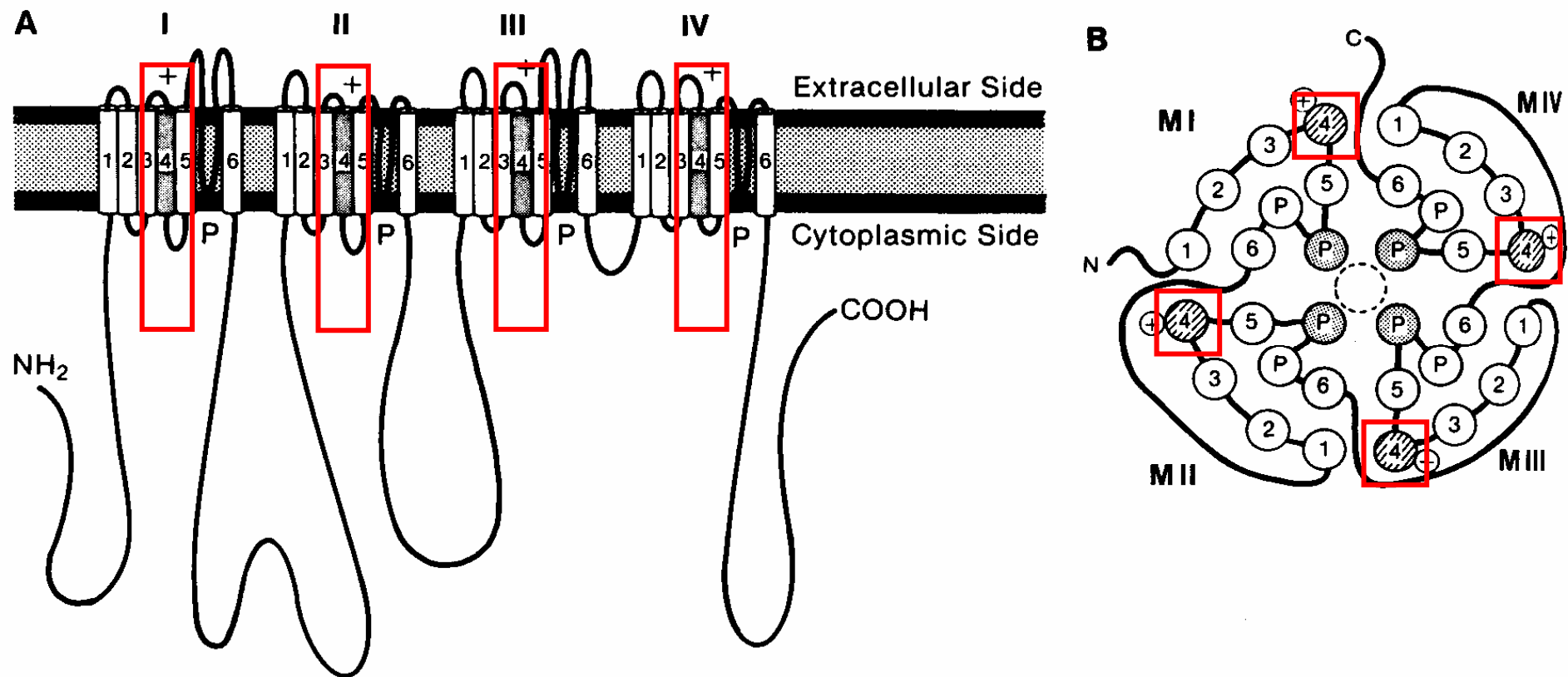


Refrakterní fáze kanálu – omezení frekvence AP

## Na kanál

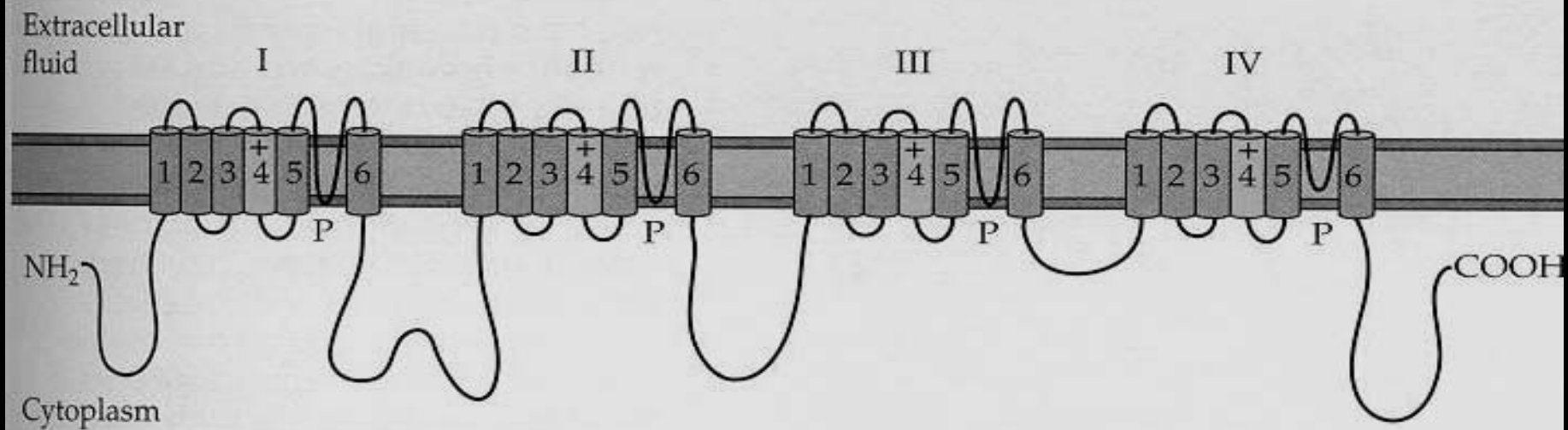
- Citlivý na napětí
- Selektivní
- Schopný inaktivace

## Citlivý na napětí

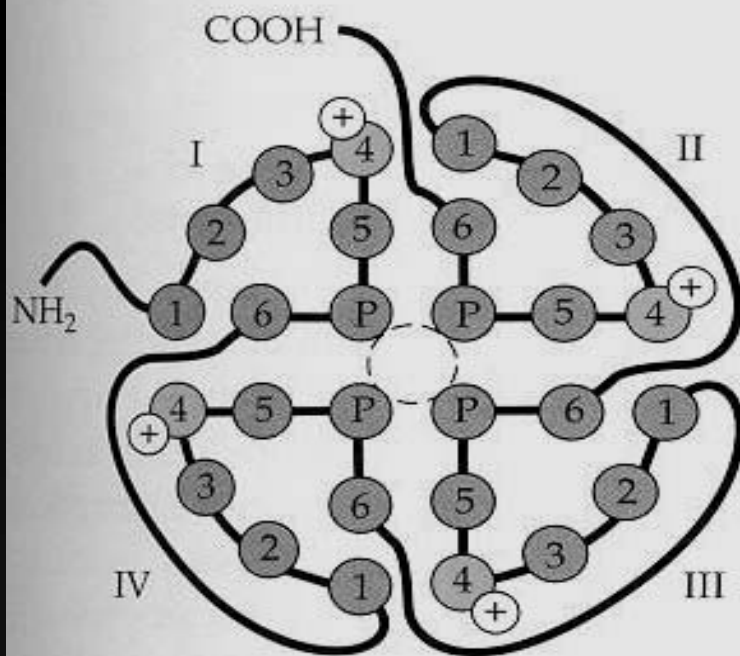


**Fig. 5.3** Presumed tertiary structure of the Na<sup>+</sup> channel protein based on hydropathicity plots of the primary amino acid sequence. **A.** The channel protein consists of four repeating subunits, each containing six presumed transmembrane segments. Segment 4 contains an excess of positively charged residues and is assumed to be the voltage sensor. A long loop between segments 5 and 6 is believed to dip into the membrane and form the face of the pore. A cytoplasmic loop contains the inactivation gate. **B.** View looking down on the membrane to see the arrangement of the four subunits around the central pore. The Ca<sup>2+</sup> channel protein is similar in its construction. (Modified from Catterall, 1988, and Stevens, 1991, in Kandel et al., 1991)

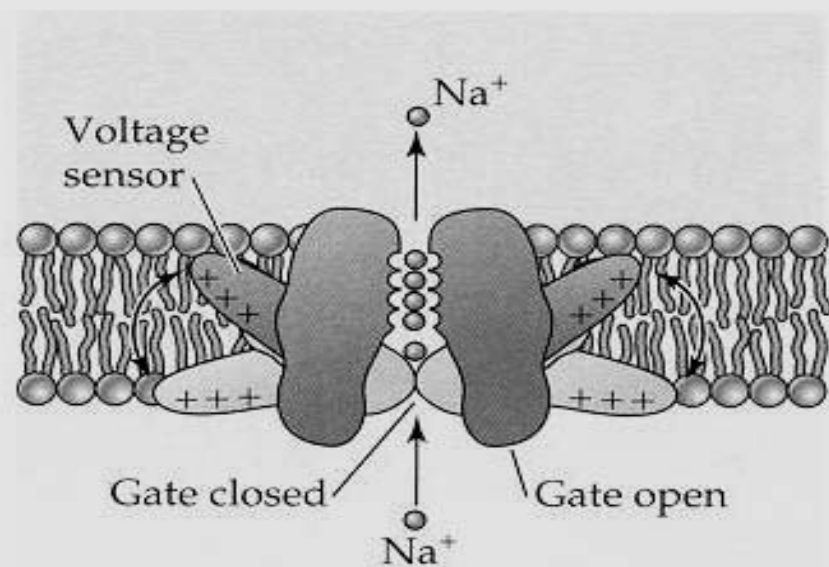
(a) Topology of voltage-gated Na<sup>+</sup> channels



(b) Surface view of a Na<sup>+</sup> channel

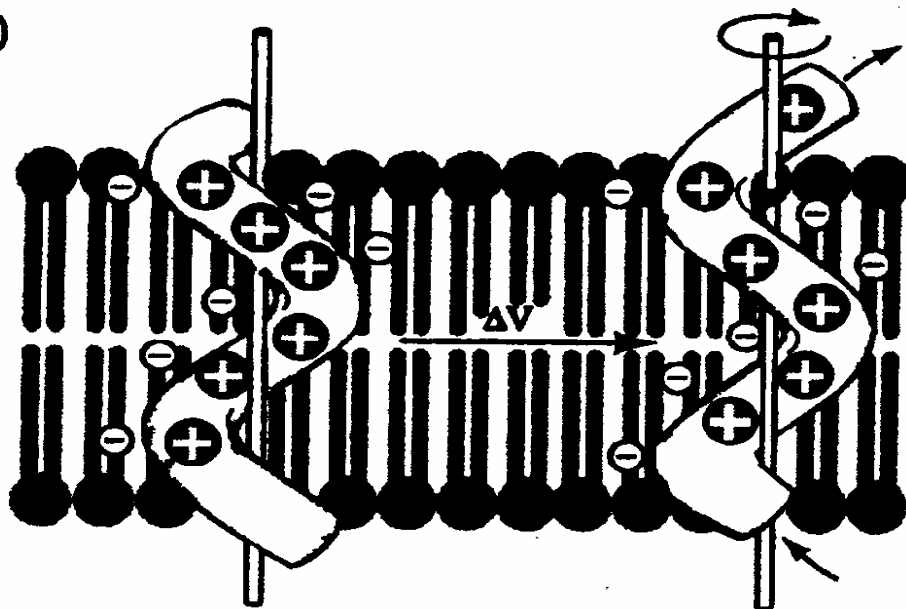


(c) Voltage-dependent conformational change

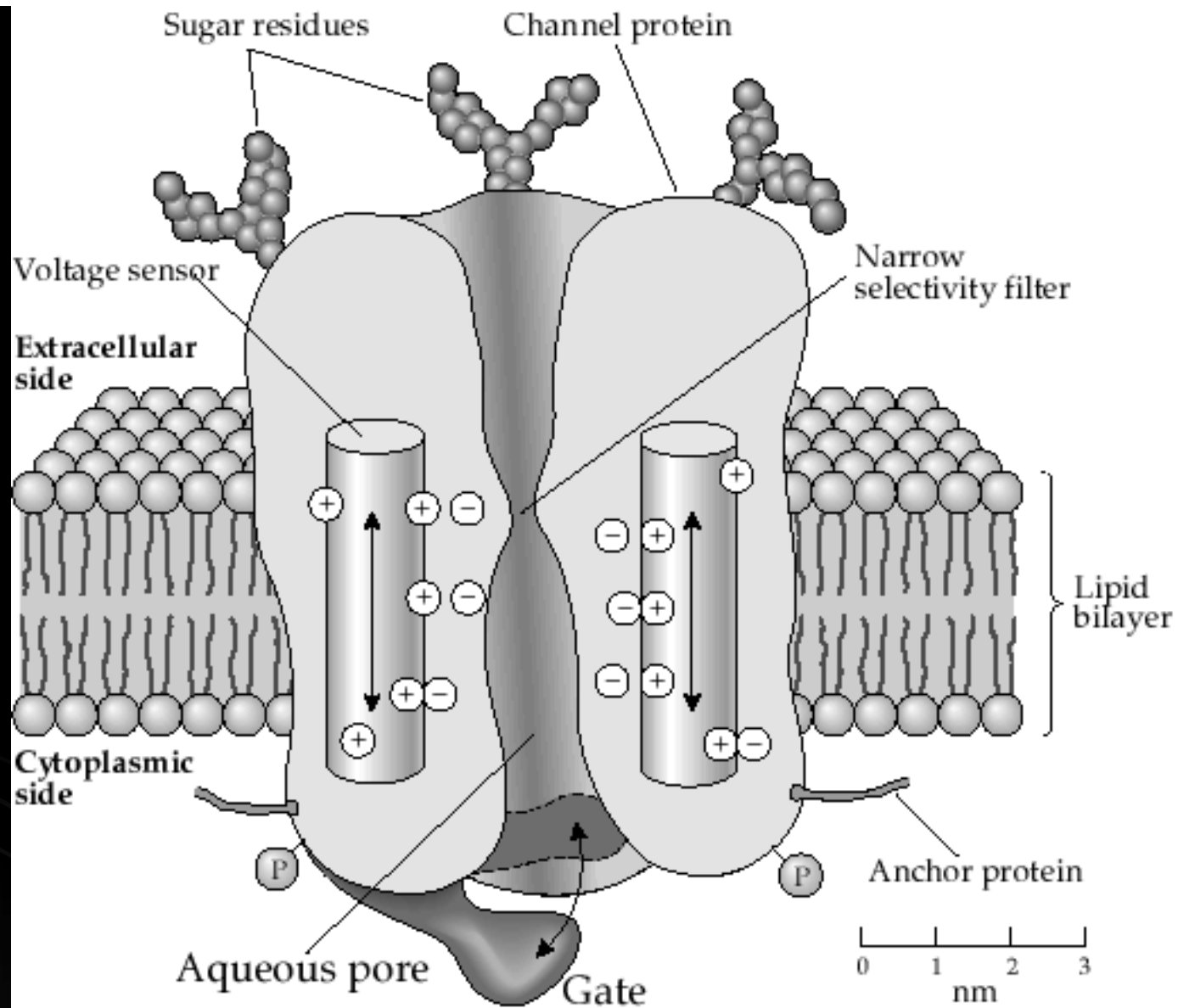




(B)



Obr. 13 Kluzně šroubový model napětově závislého vrátkování podle Catteralla. Segment  $S_4$  je zřejmě senzor napětí. Segment  $S_6$  domény III sodíkového kanálu elektrického orgánu parejnoka Torpedo je znázorněn jako transmembránová  $\alpha$  šroubovice s řadou pozitivních nábojů, tvořených opakujícími se zbytky "zásadité" aminokyseliny argininu. Každý pozitivní náboj je neutralizován negativním nábojem sousedních transmembránových šroubovic (každá třetí v pořadí sekvence). Vytváří se spirála iontových párů prostupujících membránou. Síla membránového elektrického pole stabilizuje tvorbu iontových párů tím, že táhne pozitivní náboje dovnitř a vytlačuje negativní náboje ven podobně jako jádro v elektromagnetu. Při depolarizaci ( $\Delta V$ ) se tato síla uvolní a šroubovice tvořící segmenty  $S_6$  ve všech 4 homologních doménách se vysunou ven jako uvolněné pružiny ve směru spirály přibližně o 5 Å, přičemž se otočí o 60° tak, že se kladné náboje posunou vzhledem k sousední šroubovici o jedno místo ven z buňky. Dojde tím ke snížení kladného náboje na vnitřní straně membrány. Je zajímavé (A), že velmi podobné uspořádání bazických argininových nebo lysinových kladně nabitých zbytků nacházíme v tomto předpokládaném napětovém senzoru  $S_4$  jak u sodíkového kanálu z parejnoka či mozku potkana, tak ve vápníkovém kanálu králičího kosterního svalu a v draslíkovém kanálu mutanta Shaker octomilky Drosophila melanogaster (viz obr. 14). (Podle Catterall 1988)



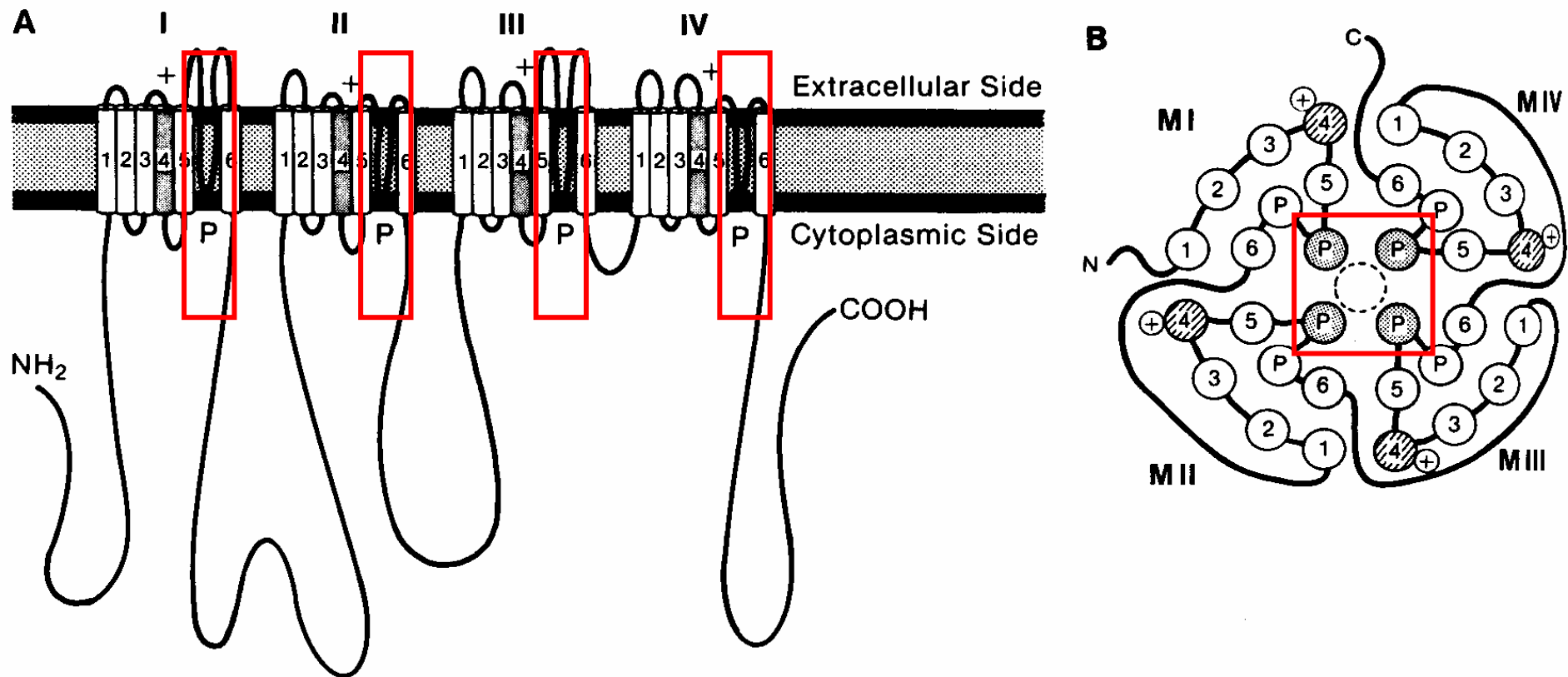
Sinauer Associates, Inc.  
 Feldman  
*Fundamentals of  
 Neuropsychopharmacology*  
 Fig. 5-8

Fig 5.8 Basic Model of a Voltage Gated Ion Channel

Na kanál a citlivost na napětí

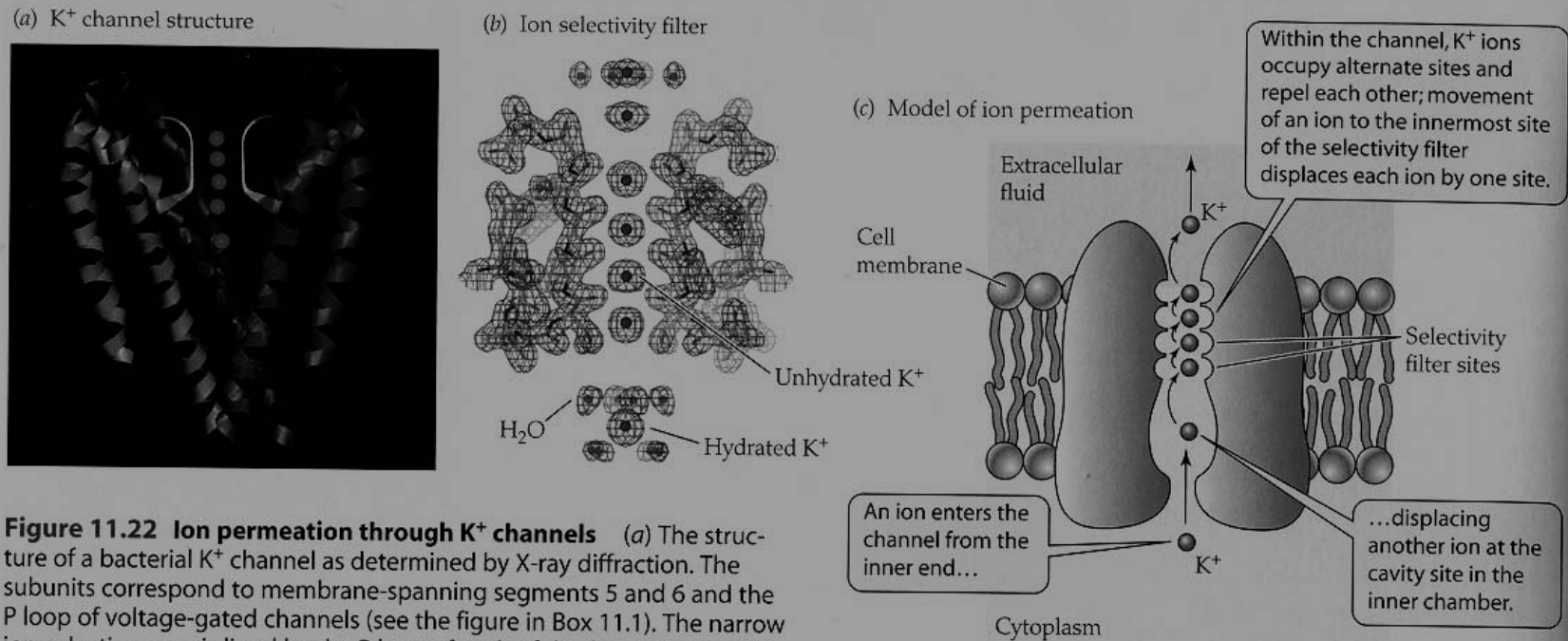


# Selektivní



**Fig. 5.3** Presumed tertiary structure of the Na<sup>+</sup> channel protein based on hydrophaticity plots of the primary amino acid sequence. **A.** The channel protein consists of four repeating subunits, each containing six presumed transmembrane segments. Segment 4 contains an excess of positively charged residues and is assumed to be the voltage sensor. A long loop between segments 5 and 6 is believed to dip into the membrane and form the face of the pore. A cytoplasmic loop contains the inactivation gate. **B.** View looking down on the membrane to see the arrangement of the four subunits around the central pore. The Ca<sup>2+</sup> channel protein is similar in its construction. (Modified from Catterall, 1988, and Stevens, 1991, in Kandel et al., 1991)

## Selektivita K<sup>+</sup> kanálu *Streptomyces*

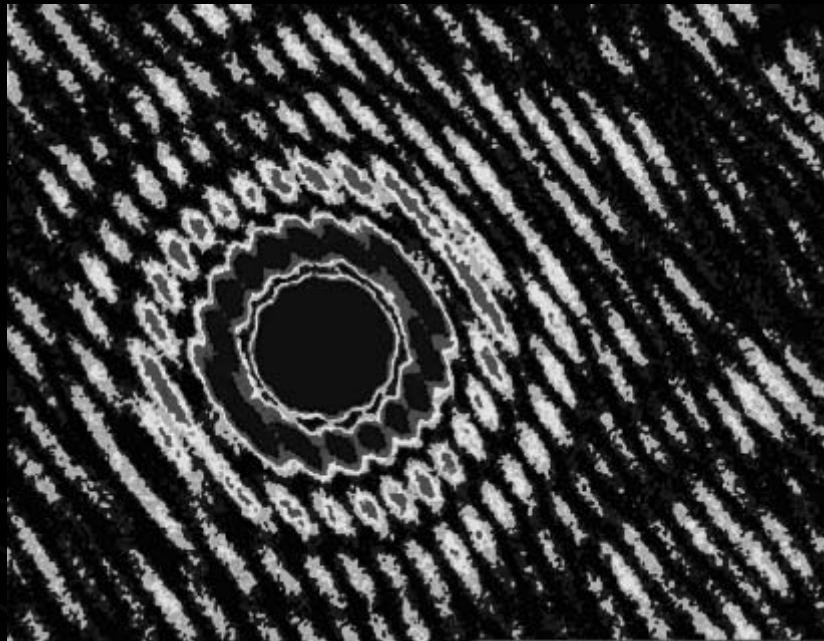


**Figure 11.22 Ion permeation through K<sup>+</sup> channels** (a) The structure of a bacterial K<sup>+</sup> channel as determined by X-ray diffraction. The subunits correspond to membrane-spanning segments 5 and 6 and the P loop of voltage-gated channels (see the figure in Box 11.1). The narrow ion-selective pore is lined by the P loop of each of the four subunits (yellow; only two of the four are visible here), and it has four sites that can be occupied by K<sup>+</sup> ions (green spheres). An additional K<sup>+</sup> ion can occupy an inner cavity below the selectivity filter. (b) Sites occupied by K<sup>+</sup> ions in and near the selectivity filter. K<sup>+</sup> ions are normally surrounded by polar water molecules, but in the pore of a potassium channel, oxygen atoms lining the pore compete with water molecules to attract the cation. The image shows a K<sup>+</sup> ion in the inner chamber with eight water molecules around it (bottom), four unhydrated K<sup>+</sup> ions at the selectivity filter sites, a K<sup>+</sup> ion at the outer face of the pore, and a K<sup>+</sup> ion with a partial shell of four water molecules (top). (c) A model of K<sup>+</sup> ion permeation. This chain reaction allows the channel to be both highly selective and highly permeable to K<sup>+</sup>. (a from Morais-Cabral et al. 2001; b from Zhou et al. 2001; c after Miller 2001.)

with short axons or no axons, so a graded potential change at one of the cell can spread passively (electrotonically) to the terminal without major decrement.

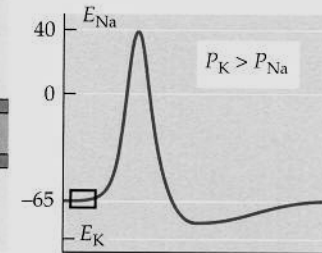
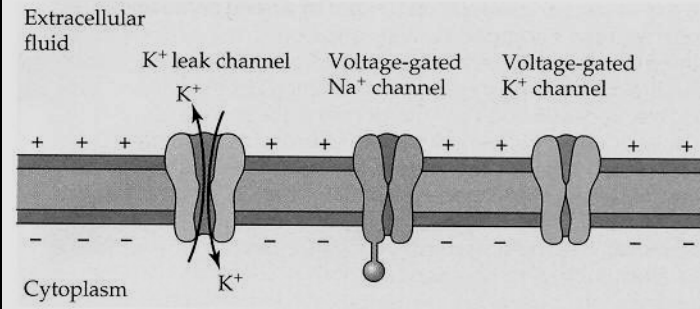
The inputs and outputs of spiking and nonspiking neurons are same, but the short-axon nonspiking neuron does not require spiking encoding to carry the signal over large distances. Examples of nonspiking neurons include the photoreceptors, bipolar cells, and horizontal cells of the vertebrate retina (see Chapter 13), granule cells of the olfactory bulb, and many arthropod interneurons.

# X-ray crystallography

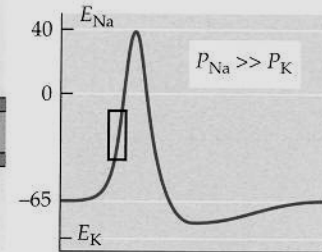
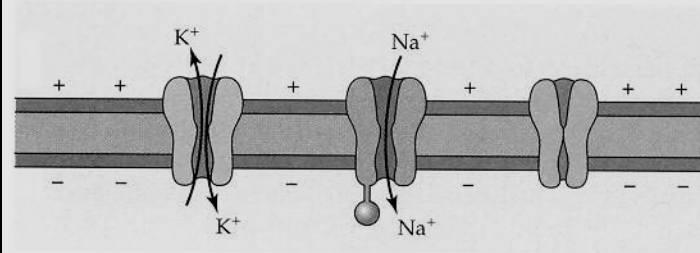


# Schopný inaktivace

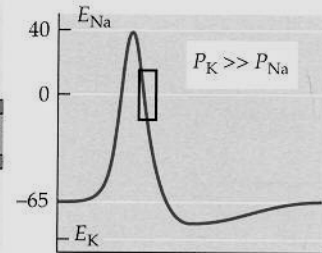
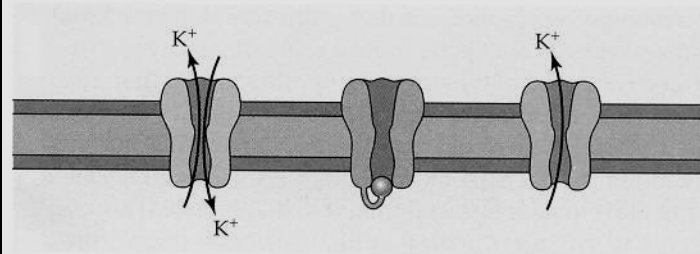
(a) Resting membrane potential



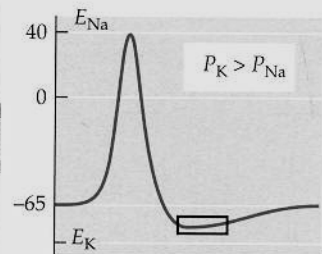
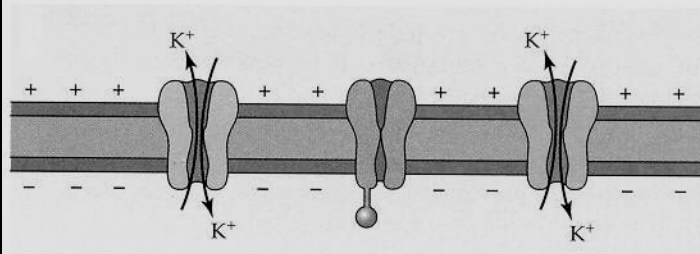
(b) Rising phase



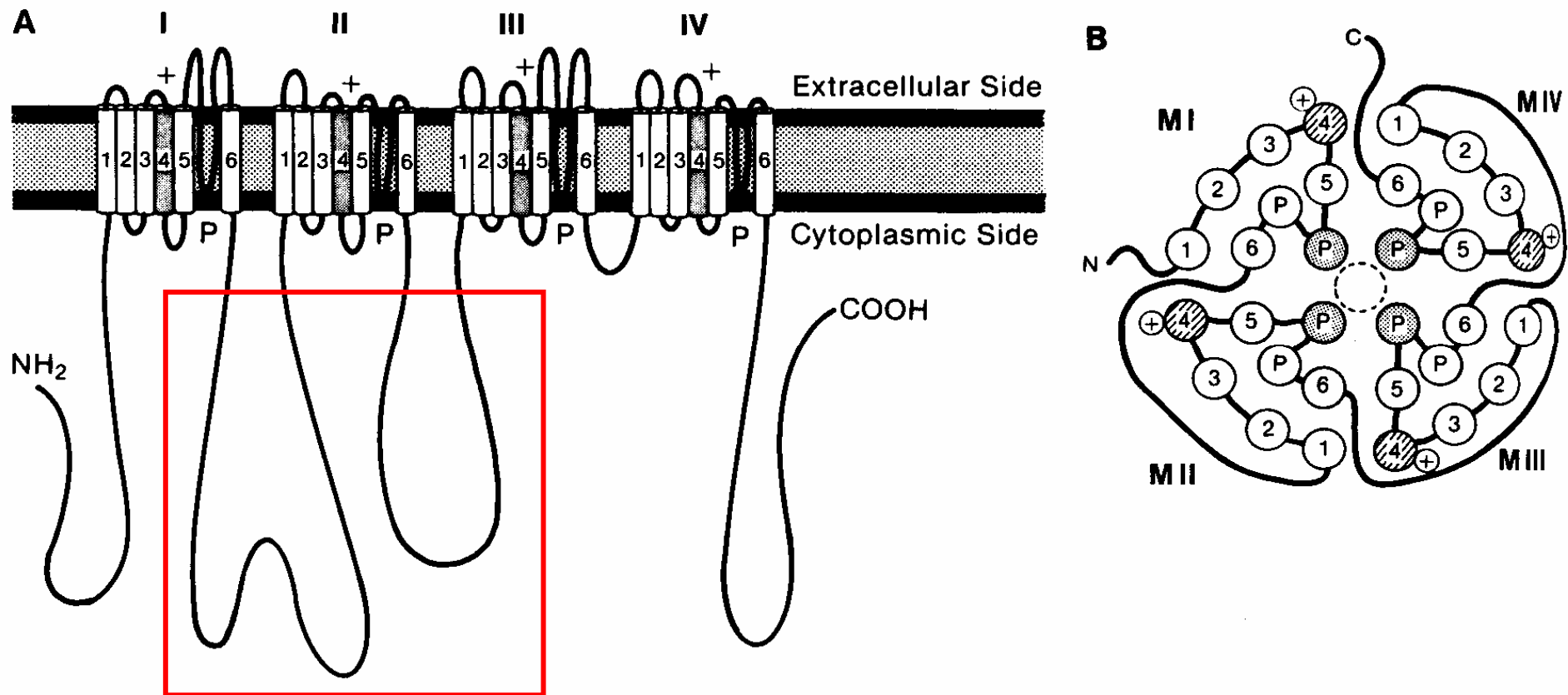
(c) Falling phase



(d) Recovery



## Schopný inaktivace



**Fig. 5.3** Presumed tertiary structure of the Na<sup>+</sup> channel protein based on hydropathicity plots of the primary amino acid sequence. **A.** The channel protein consists of four repeating subunits, each containing six presumed transmembrane segments. Segment 4 contains an excess of positively charged residues and is assumed to be the voltage sensor. A long loop between segments 5 and 6 is believed to dip into the membrane and form the face of the pore. A cytoplasmic loop contains the inactivation gate. **B.** View looking down on the membrane to see the arrangement of the four subunits around the central pore. The Ca<sup>2+</sup> channel protein is similar in its construction. (Modified from Catterall, 1988, and Stevens, 1991, in Kandel et al., 1991)





The Nobel Prize in Physiology or  
Medicine 1991

# Patch Clamp – Technika, která „vidí“ kanály při práci

"for their discoveries concerning the function of single ion  
channels in cells"



**Erwin Neher**

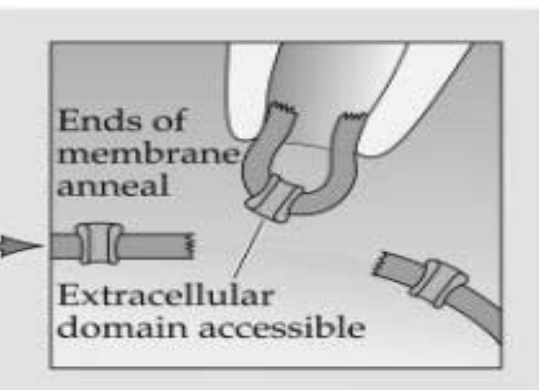
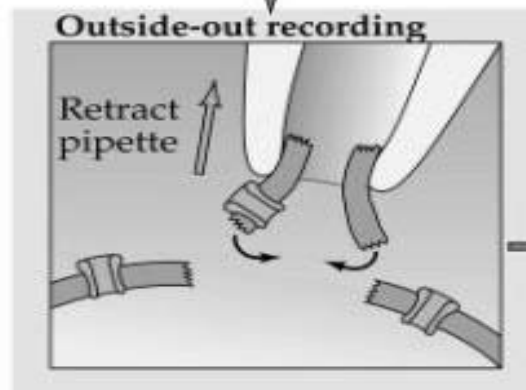
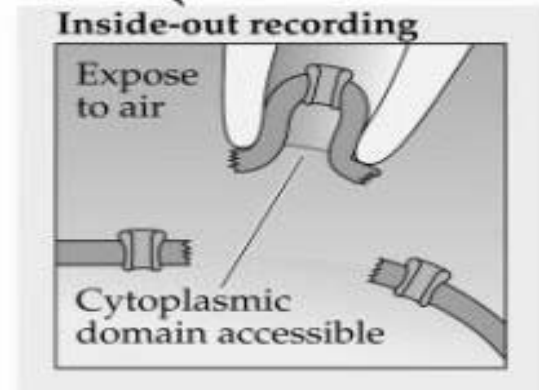
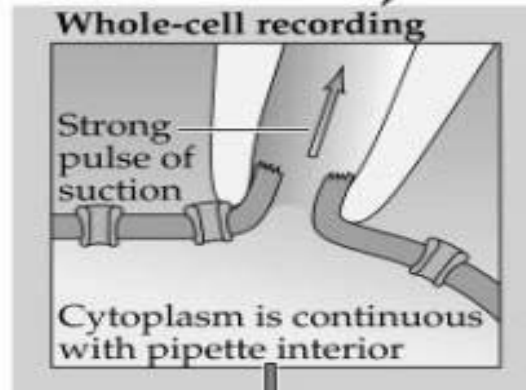
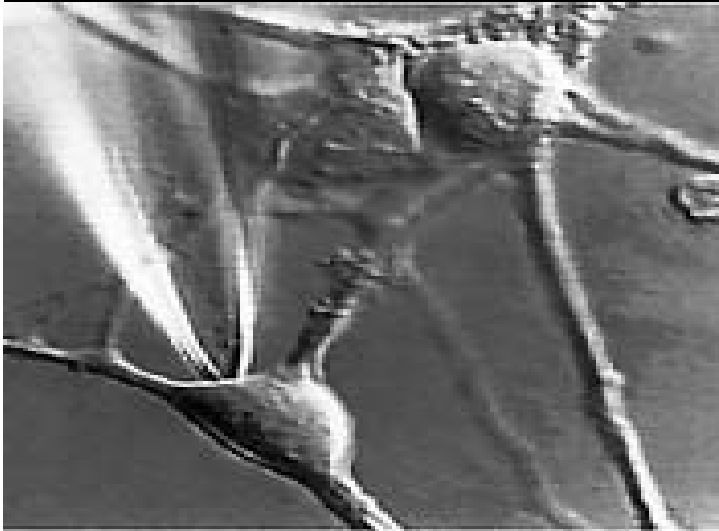
1/2 of the prize  
Federal Republic of  
Germany  
Max-Planck-Institut für  
Biophysikalische  
Chemie  
Goettingen, Federal  
Republic of Germany  
b. 1944

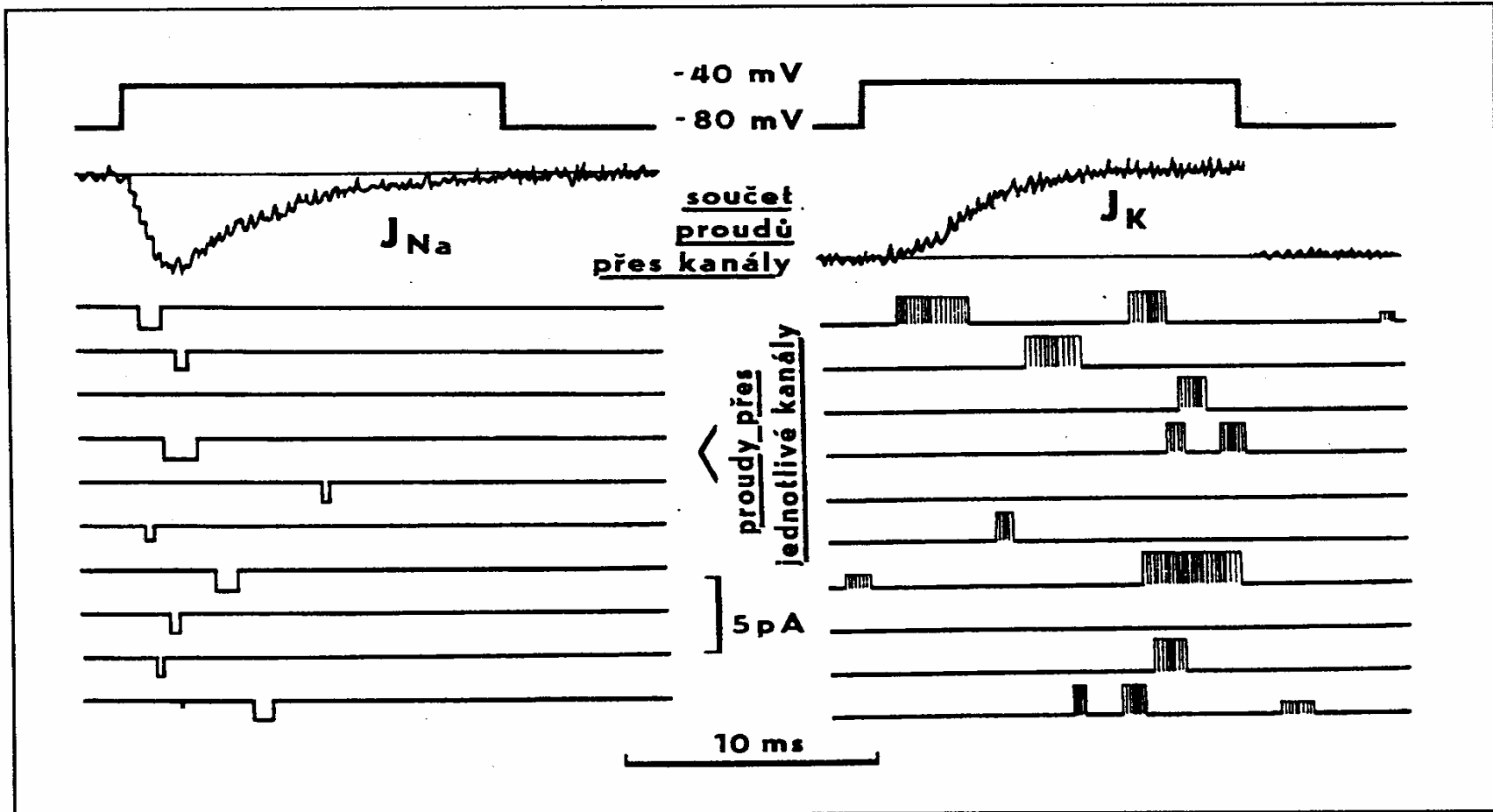


**Bert Sakmann**

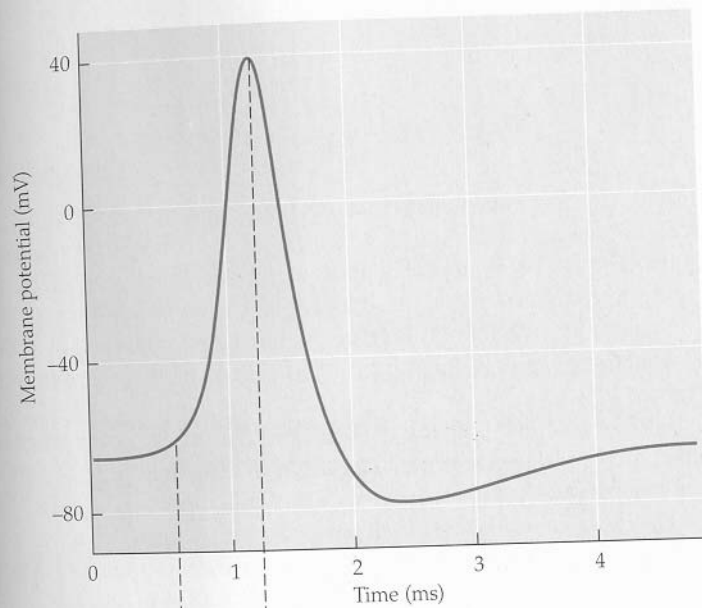
1/2 of the prize  
Federal Republic of  
Germany  
Max-Planck-Institut für  
medizinische Forschung  
Heidelberg, Federal  
Republic of Germany  
b. 1942

Neher & Sackman  
Terčíkový zámek, 1991



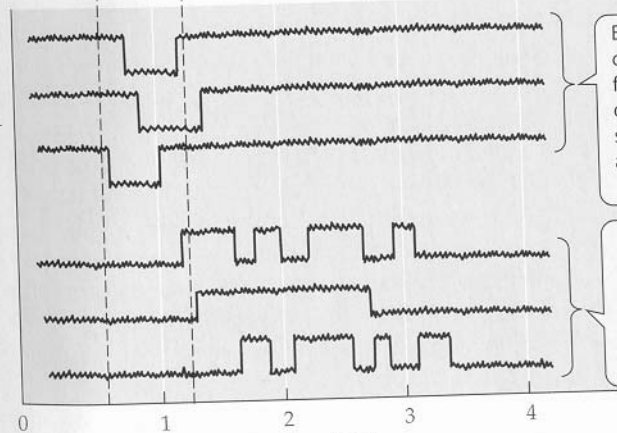


Obr. 14.18 Proudý tekoucí  $Na^+$ -kanály (vlevo) a  $K^+$ -kanály (vpravo). Membránové napětí bylo po dobu 14 ms experimentálně skokem přestaveno z -80 mV na -40 mV (horní křivka), a to bylo desetkrát opakováno. Přitom byly měřeny membránové proudy (10 křivek v dolejší části obr.). Proudý proteklé jednotlivými kanály se objevují porůznu během depolarisace a trvají různě dlouho. Sumací takovýchto záznamů vzniká záznam sumačního proudu,  $J_{Na}$  popř.  $J_K$  (zubatá křivka). Je vidět, že u  $Na^+$ -kanálů je otevření nejpravděpodobnější krátce po změně napětí na membráně a že pak dochází k pozvolné inaktivaci.  $K^+$ -kanály se naproti tomu otvírají v průměru s jistým zpožděním, pak se však ustavuje určitá střední častota otevření, která zůstává konstantní po celou dobu depolarisace. (Z DUDELA 1990b)



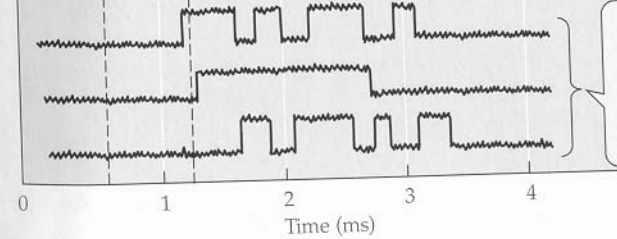
ing current, the e  
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 potential is cla  
 current-measu  
 required to se  
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 not ionic curre  
 a shift in accur

(a)  
 Inward  
 currents  
 through Na<sup>+</sup>  
 channels



Each Na<sup>+</sup> channel  
 opens with little delay  
 following initial  
 depolarization, and  
 stays open for less than  
 a millisecond before  
 becoming inactivated.

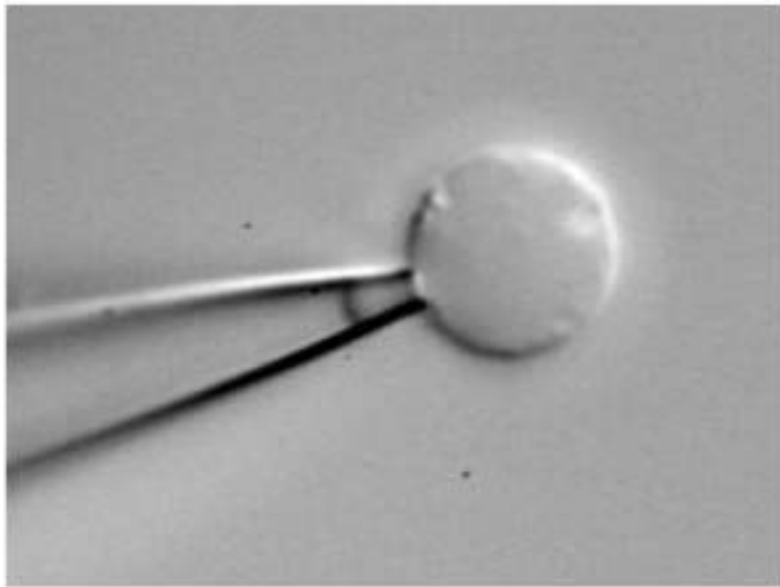
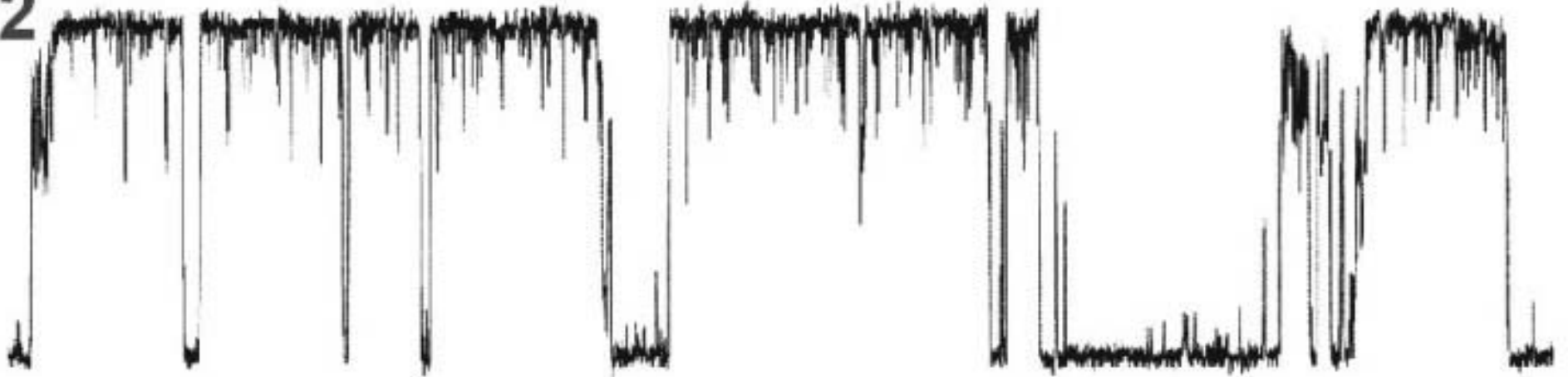
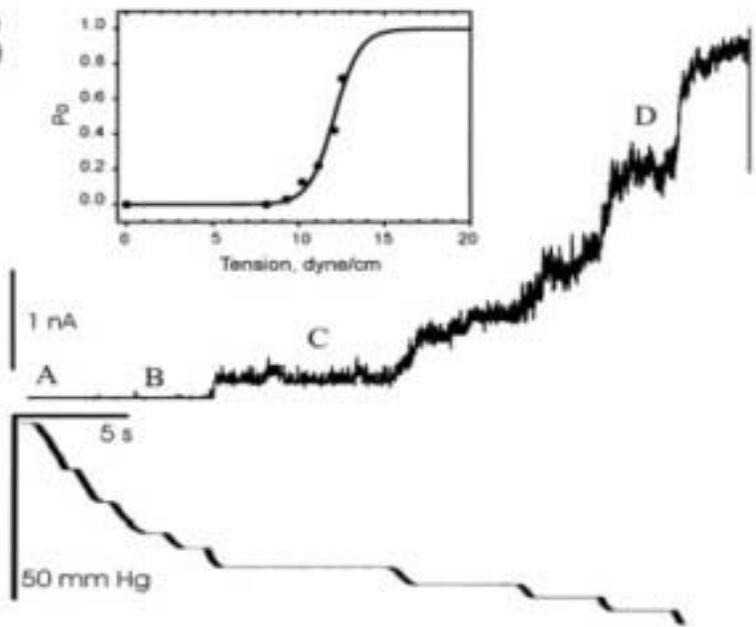
(b)  
 Outward  
 currents  
 through K<sup>+</sup>  
 channels



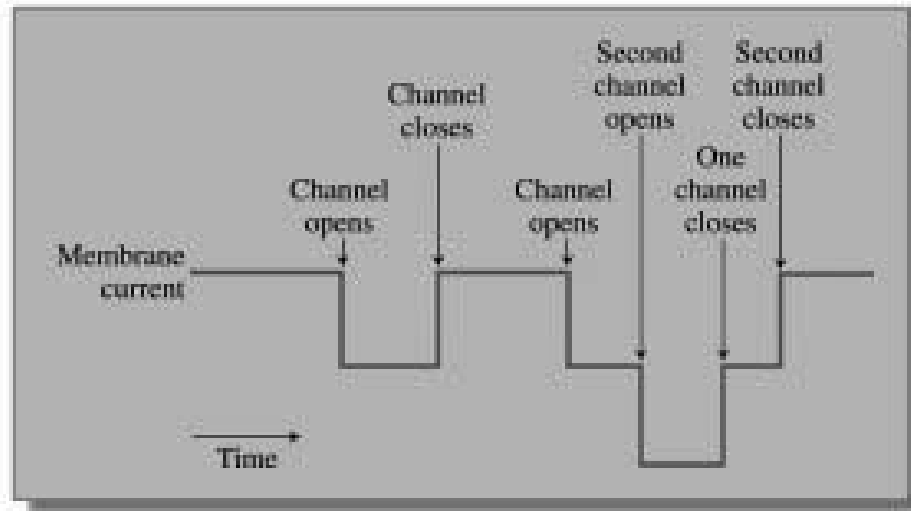
The voltage-gated K<sup>+</sup>  
 channels open slightly  
 later and can stay  
 open until shortly  
 after membrane  
 repolarization.



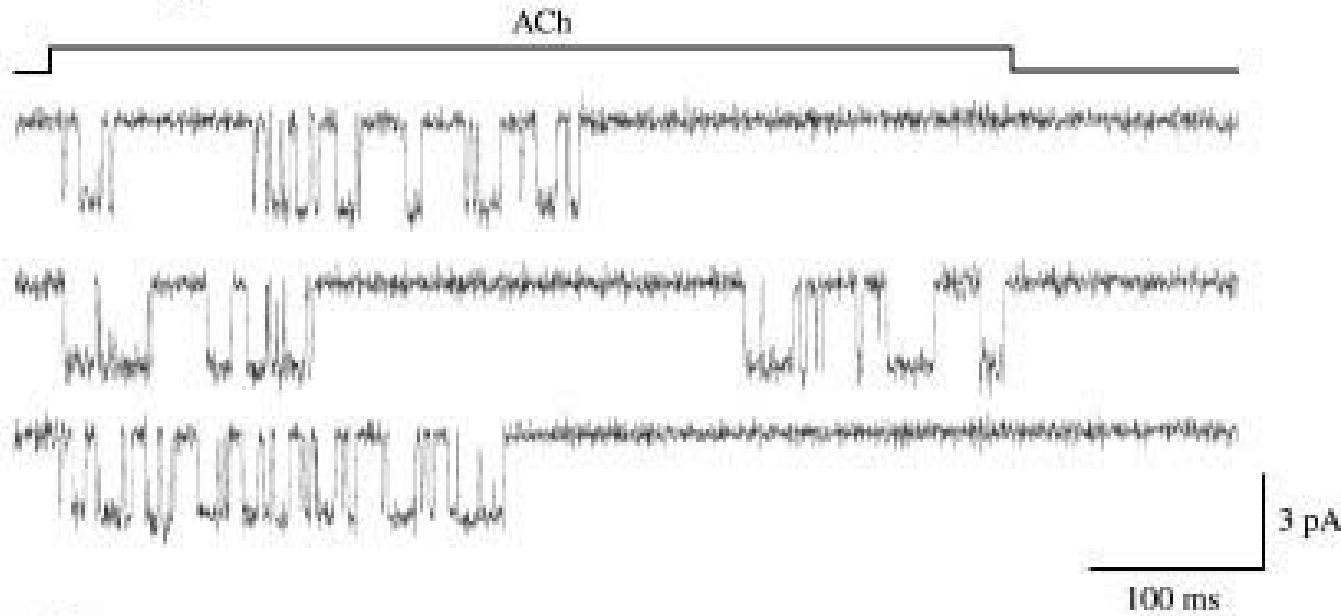
Animace patch clamp

**1****2****3**

A



B



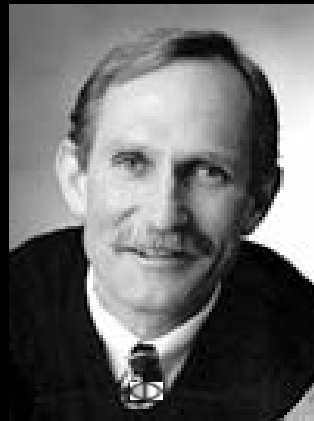
The Nobel Prize in Chemistry 2003



"for discoveries concerning channels in cell membranes"

"for the discovery of water channels"

"for structural and mechanistic studies of ion channels"



**Peter Agre**

1/2 of the prize

USA

Johns Hopkins University  
School of Medicine  
Baltimore, MD, USA

b. 1949



**Roderick MacKinnon**

1/2 of the prize

USA

Rockefeller University  
New York, NY, USA; Howard  
Hughes Medical Institute

b. 1956



# Roderick MacKinnon and Ion Channels

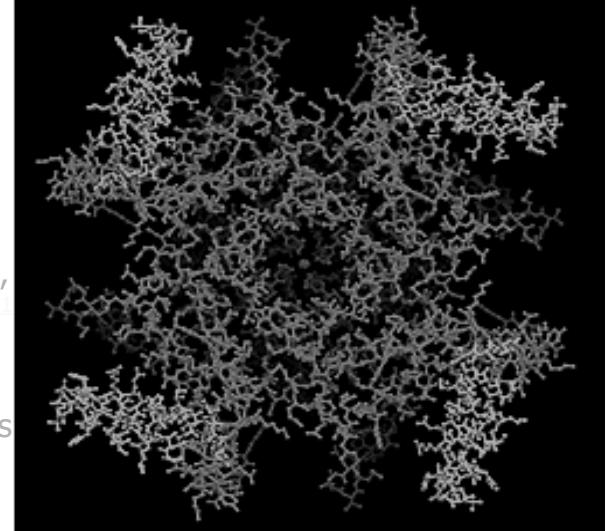


Roderick MacKinnon, M.D., a visiting researcher at the U.S. Department of Energy's Brookhaven National Laboratory, is a recipient of the 2003 Nobel Prize in Chemistry 'for structural and mechanistic studies of ion channels.,

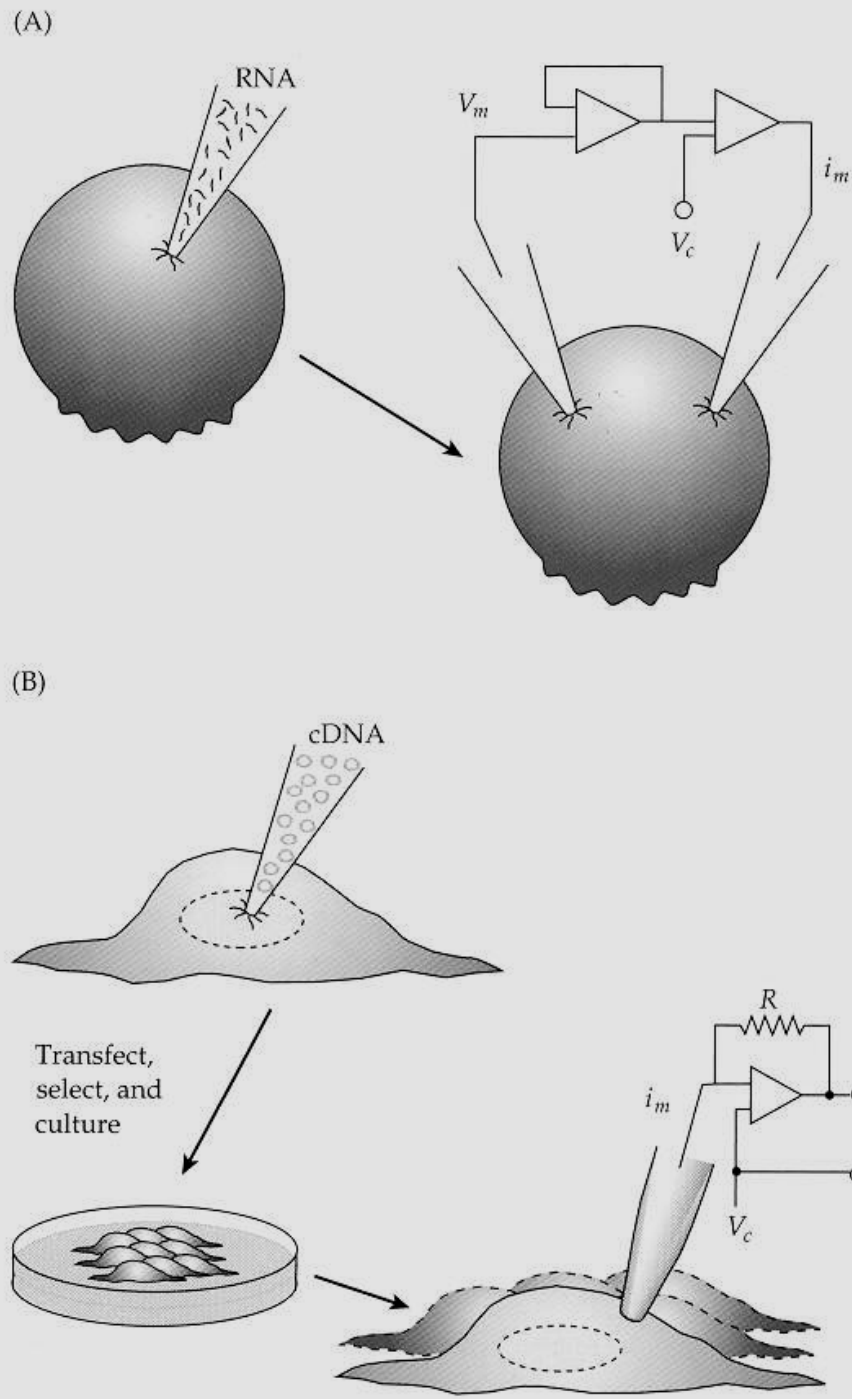
His research explains "how a class of proteins helps to generate nerve impulses – the electrical activity that underlies all movement, sensation, and perhaps even thought. The work leading to the prize was done primarily at the Cornell High Energy Synchrotron Source [CHESS] and the National Synchrotron Light Source [NSLS] at Brookhaven.

The proteins, called ion channels, are tiny pores that stud the surface of all of our cells. These channels allow the passage of potassium, calcium, sodium, and chloride molecules called ions. Rapid-fire opening and closing of these channels releases ions, moving electrical impulses from the brain in a wave to their destination in the body."

"Potassium channels act as both gateways and gatekeepers on cell membranes, controlling the flow of ions and enabling brains to think, muscles to move, and hearts to beat. Malfunctioning ion channels contribute to epilepsy, arrhythmia, and other diseases."<sup>2</sup>

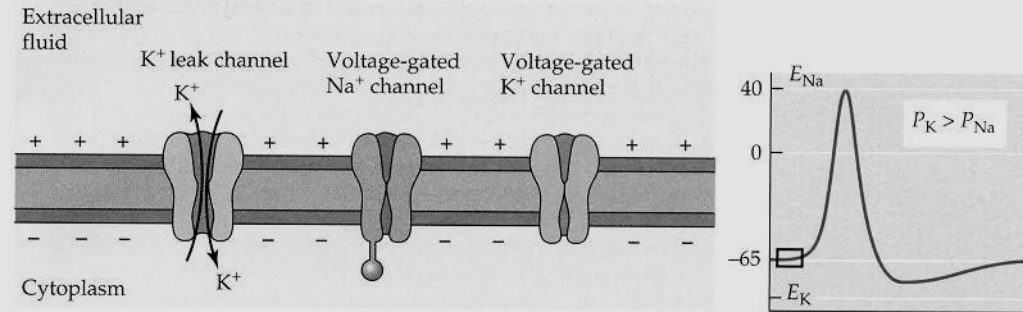


An overhead view of a voltage-dependent potassium ion channel shows four red-tipped "paddles" that open and close in response to positive and negative charges. This structure, discovered by Rockefeller scientists, shows for the first time the molecular mechanism by which potassium ions are allowed in and out of living cells during a nerve or muscle impulse.

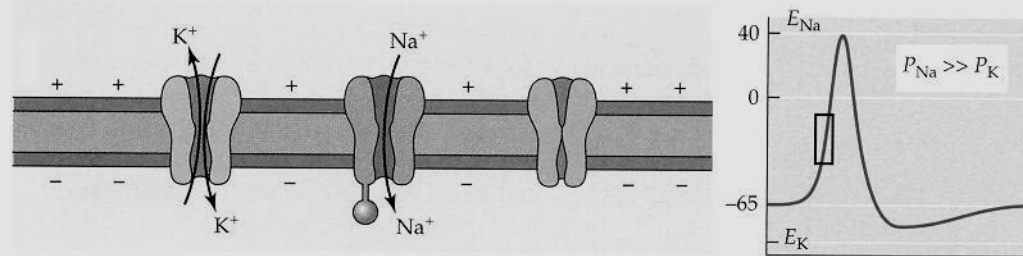


**Figure 1.9**  
**Common methods of gene expression for recording the electrical activity of ion channels and other membrane proteins** (A) Injection into a *Xenopus* oocyte, which can then be studied by voltage clamping. (B) Transfection. DNA incorporated into plasmid or viral vector is introduced into the cell by electroporation,  $\text{Ca}^{2+}$  shock, or direct injection into the nucleus (as shown). Cell line may then be used for patch-clamp recording.  $V_m$ , membrane potential;  $V_c$ , command potential;  $R$ , feedback resistance of patch amplifier;  $i_m$ , membrane current. After Fain (1999).

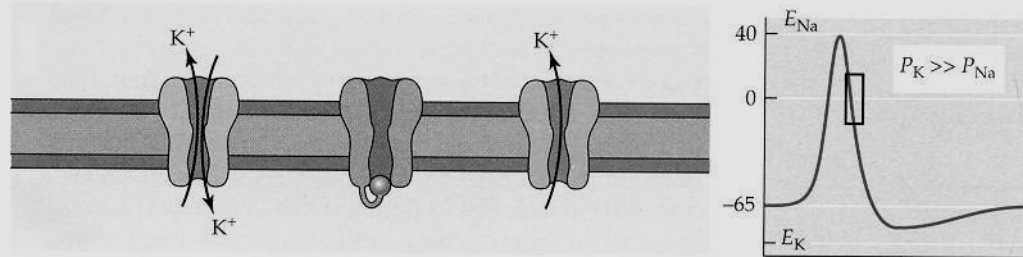
(a) Resting membrane potential



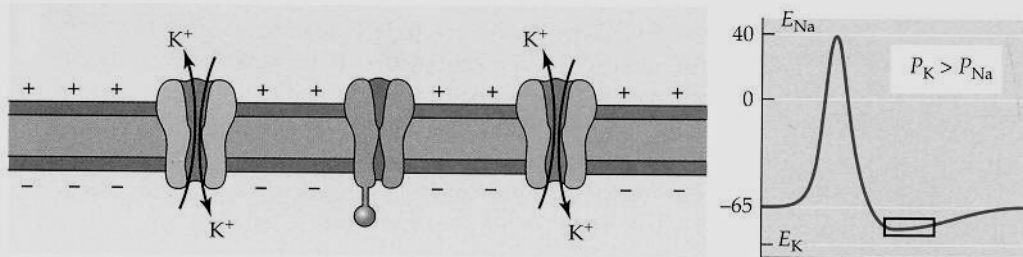
(b) Rising phase

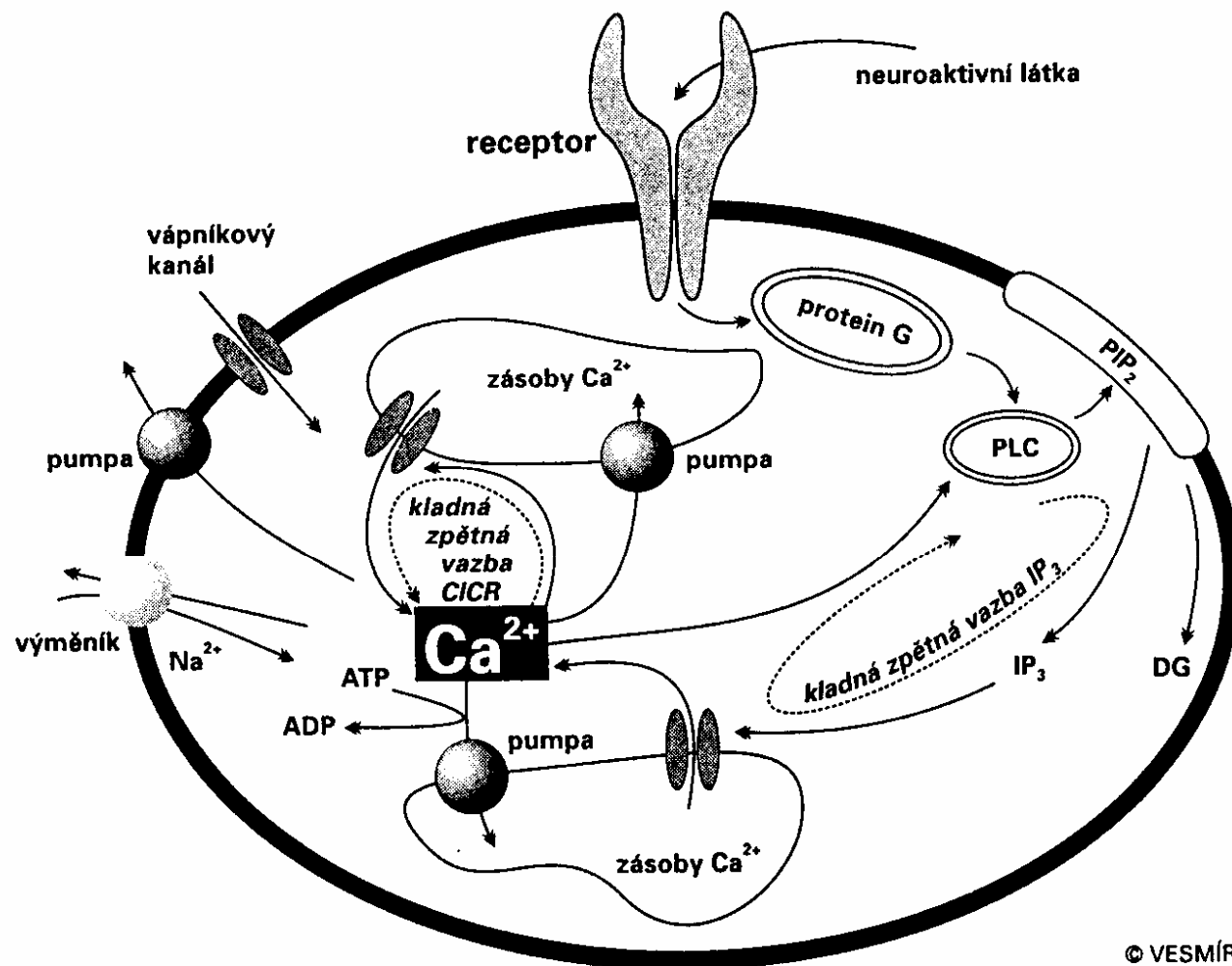


(c) Falling phase



(d) Recovery



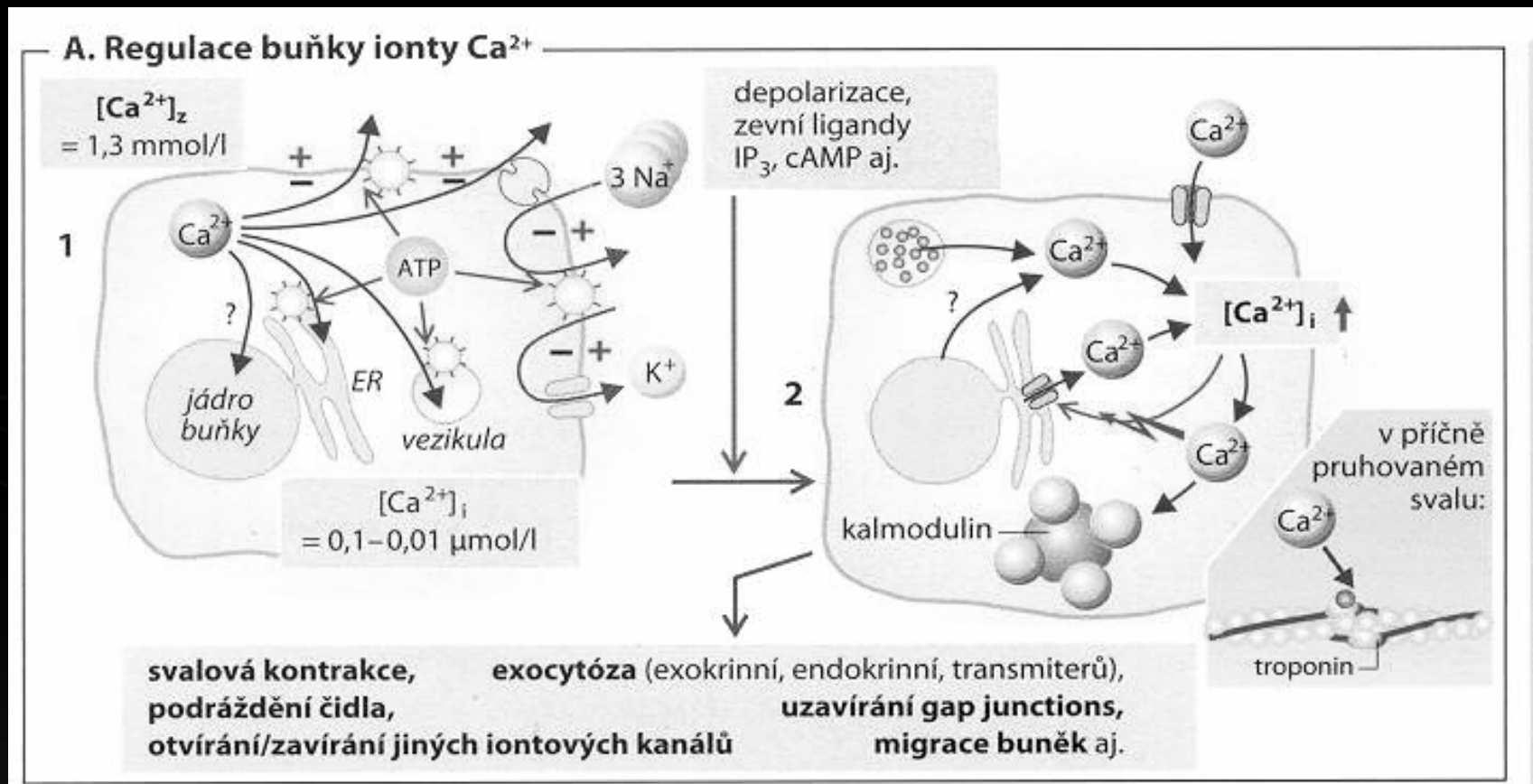


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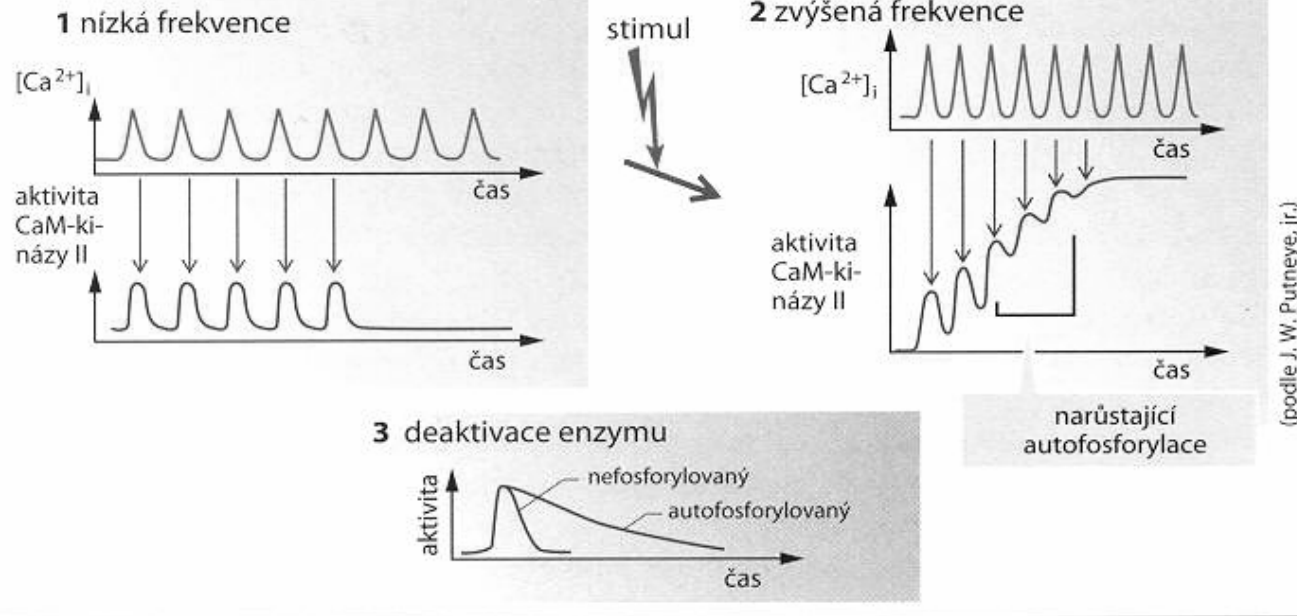
Mechanismy regulující v buňce koncentraci vápníku: PLC – fosfolipáza C, DG – diacylglycerol, CICR – indukované uvolňování vápníku

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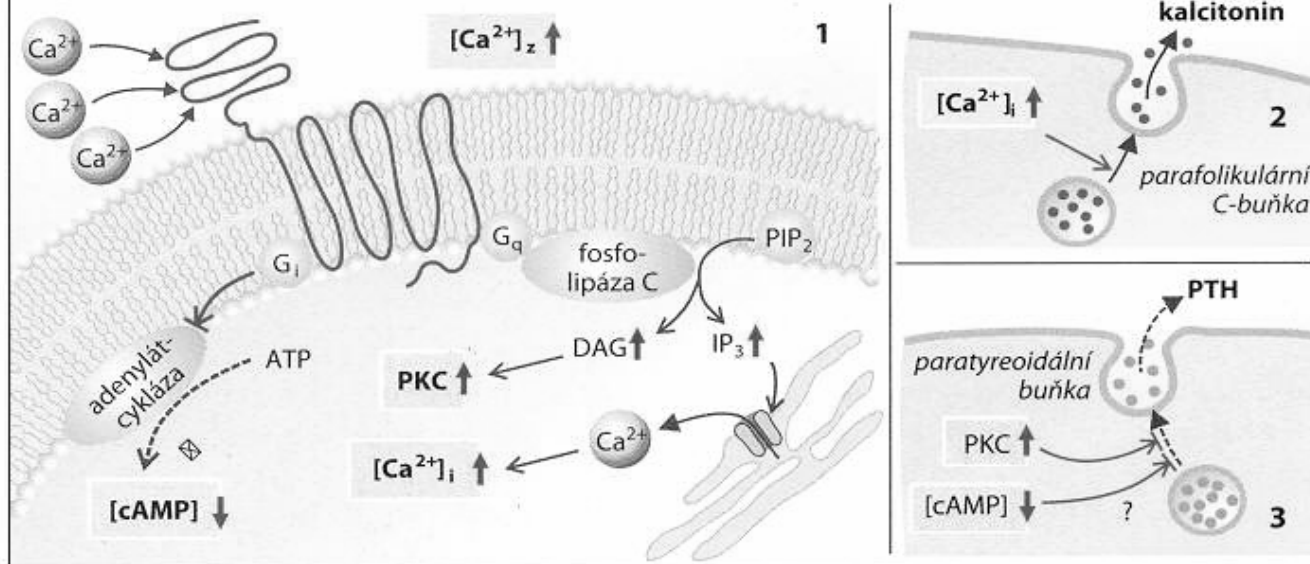
# Vápníková komunikace a Ca kanály



## B. Oscilace $Ca^{2+}$

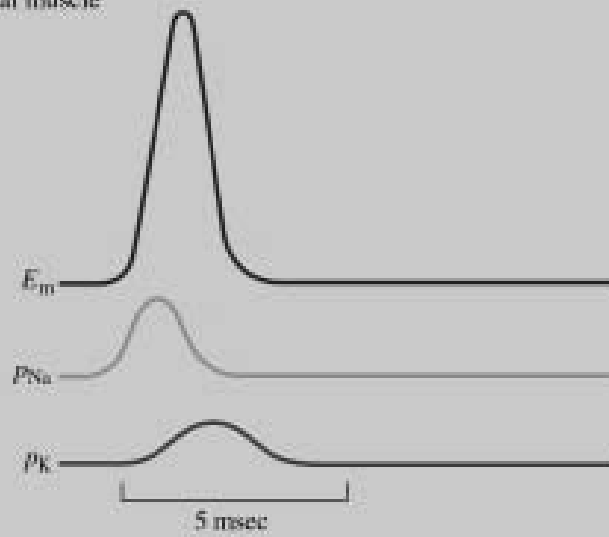


## C. $Ca^{2+}$ -senzor

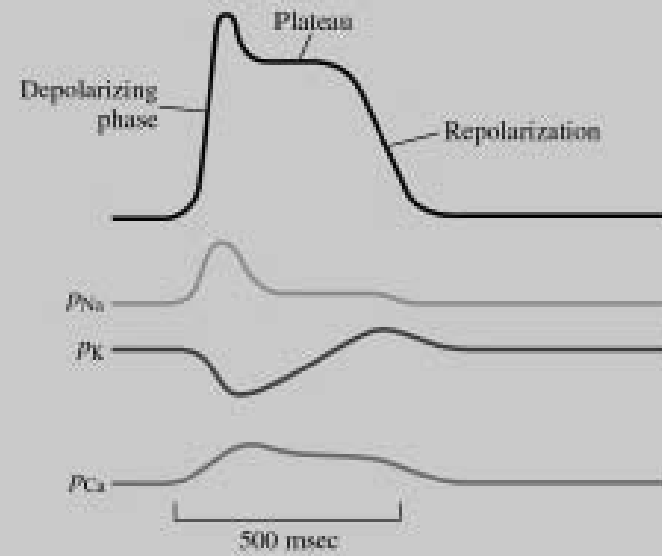


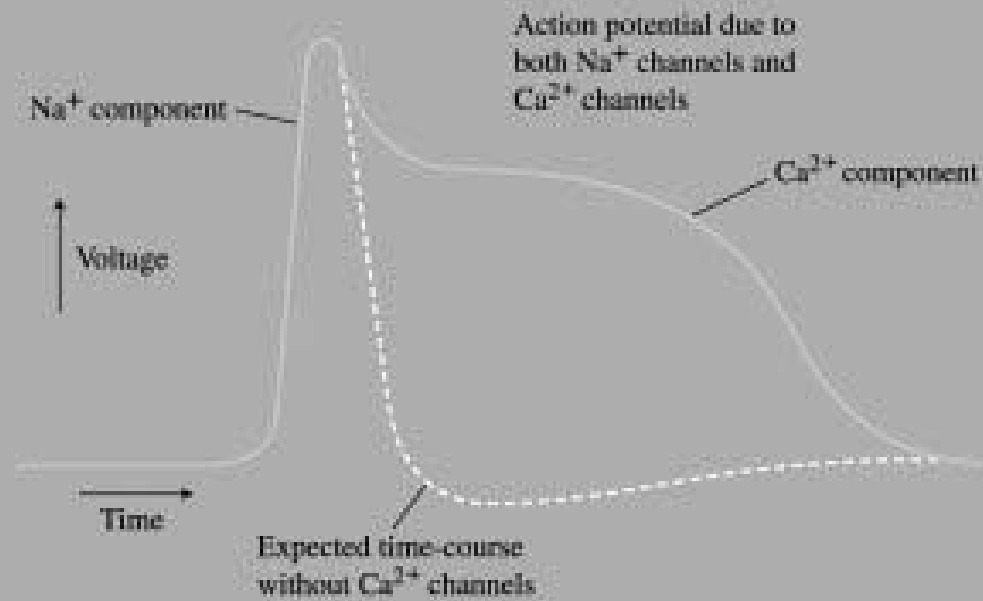
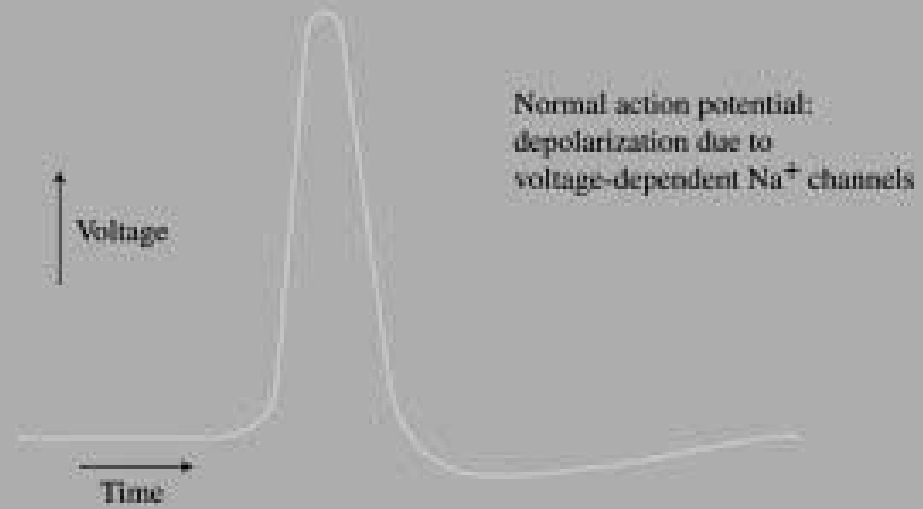
# Ca v myokardu

A Skeletal muscle



B Cardiac muscle

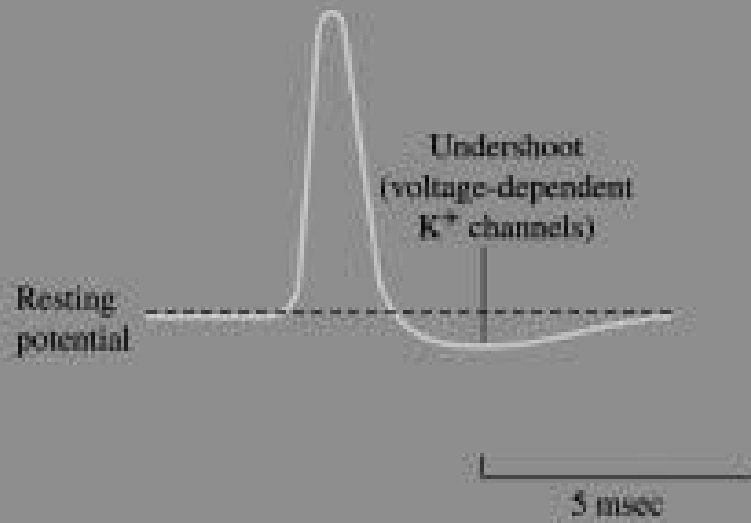




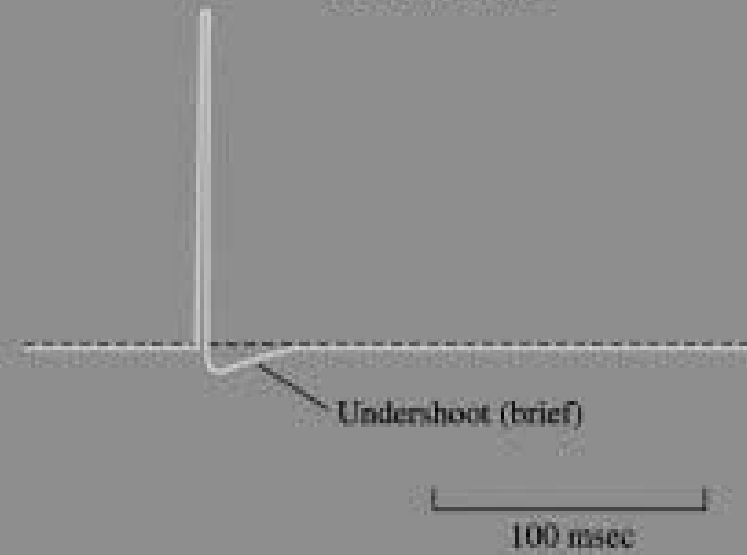


A

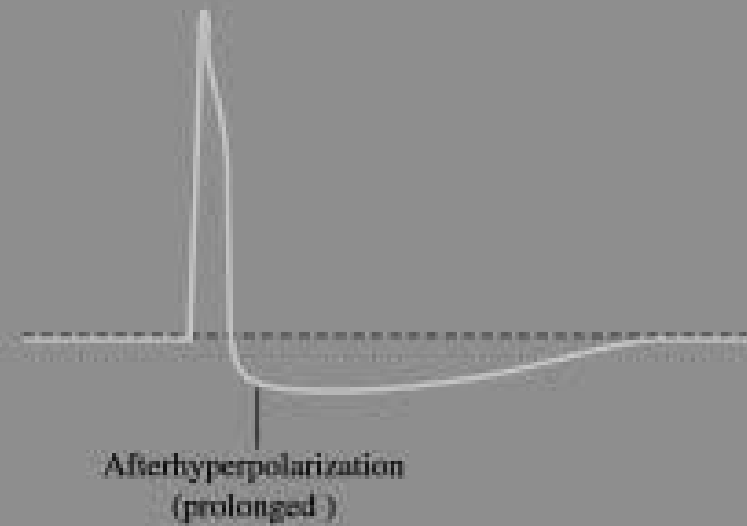
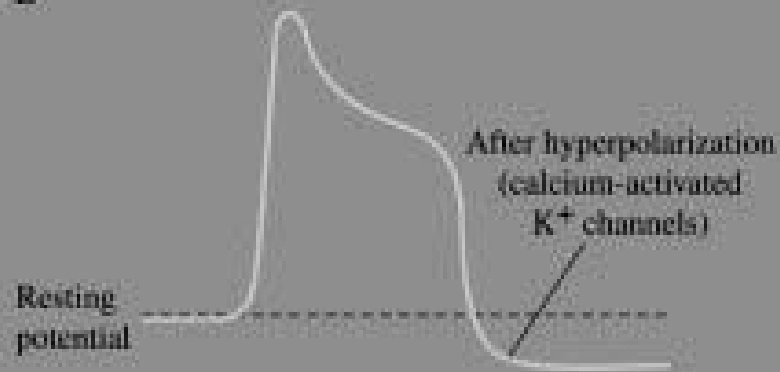
Fast time scale

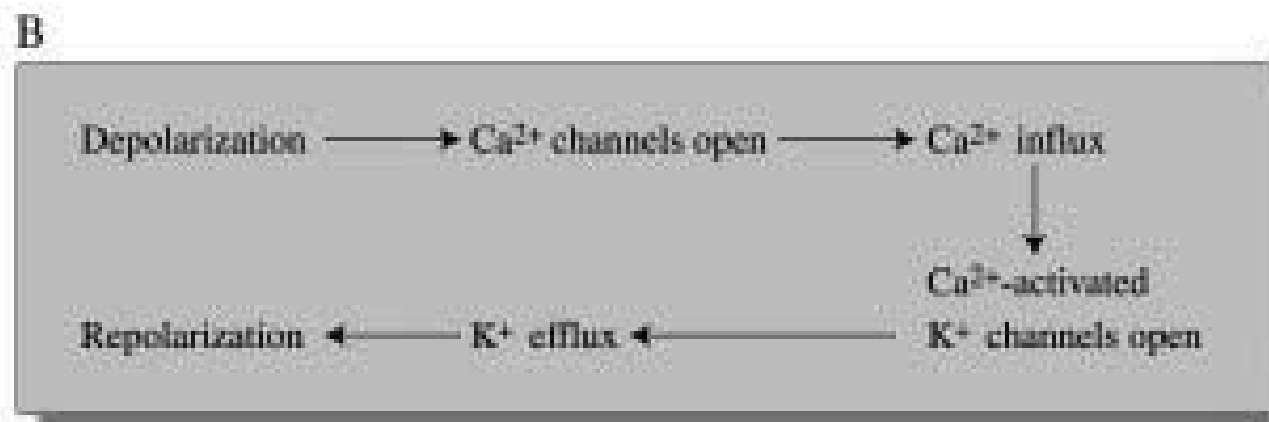
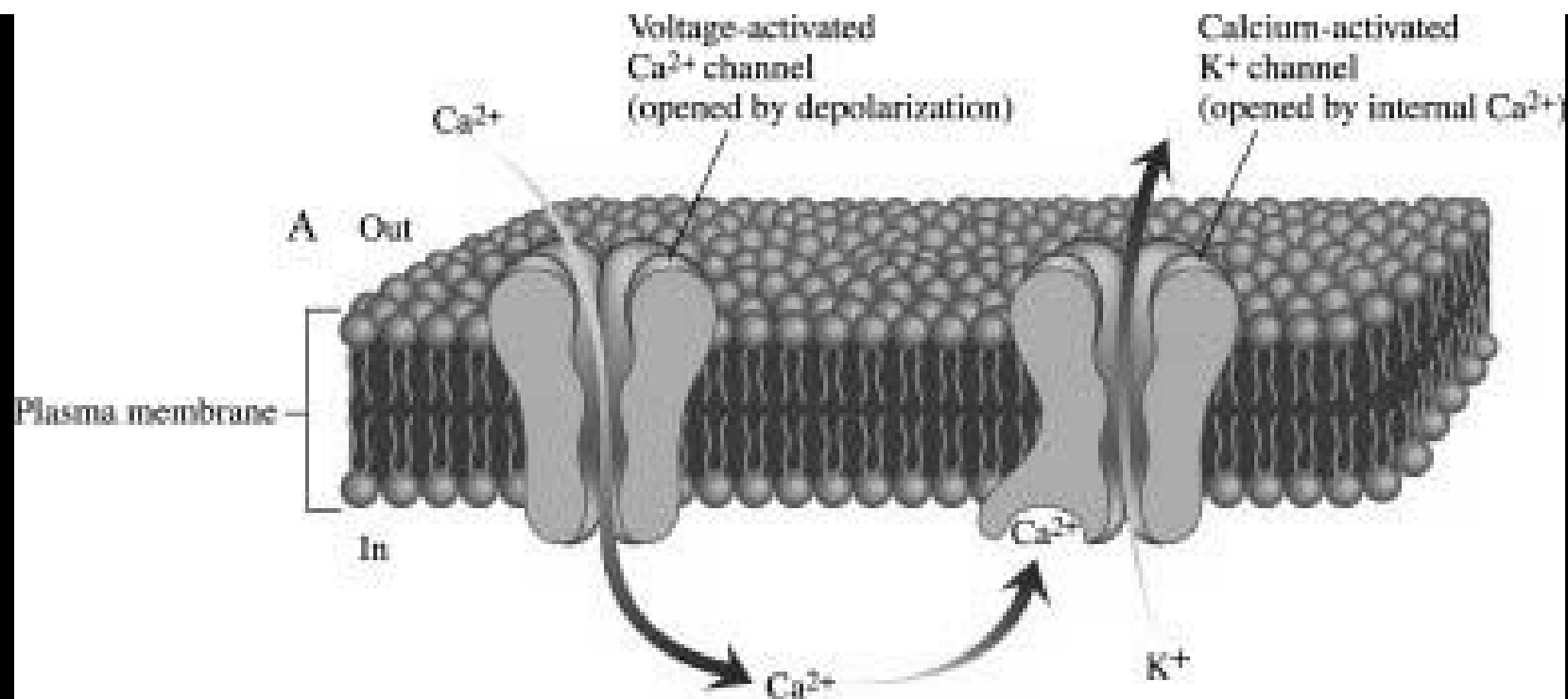


Slow time scale



B





Akční potenciál kanály

Akční potenciál cartoon

Propagace, Voltage clamp



- Leukocyty, stejně jako rakovinové buňky mají napětově vrátkované kanály

