ZOO332H1S
Lecture 9
2nd Messenger Add-in Notes and Topics
(AJE 2003)

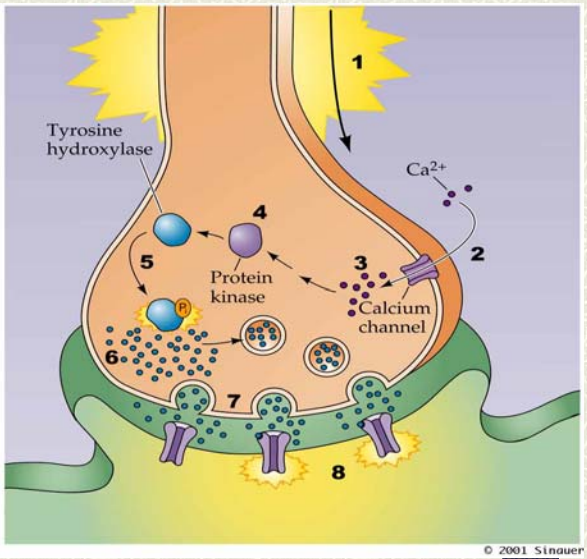
(Ref: Ch. 10, 16 (in part), other)

1

Presynaptic terminal – Effects of Ca^{2+} on local catecholamine production

1. AP
2. v-gated Ca channels
3. Increase 2nd messenger
4. Activ'n PK
5. TH phosph'ated
6. Increase catecholamine synth.
7. Increase NT release
8. Increase postsynaptic response

(9.) ?



2

Neuronal 2nd messengers

(A)

Second messenger	Sources	Intracellular targets	Removal mechanisms
Ca^{2+}	Plasma membrane: Voltage-gated Ca^{2+} channels; Various ligand-gated channels; Endoplasmic reticulum: IP_3 receptors; Ryanodine receptors	Calmodulin; Protein kinases; Protein phosphatases; Ion channels; Synaptotagmin; Many other Ca^{2+}-binding proteins	Plasma membrane: Na^+/Ca^{2+} exchanger; Ca^{2+} pump; Endoplasmic reticulum: Ca^{2+} pump; Mitochondria
Cyclic AMP	Adenylyl cyclase acts on ATP	Protein kinase A; Cyclic nucleotide-gated channels	cAMP phosphodiesterase
Cyclic GMP	Guanylyl cyclase acts on GTP	Protein kinase G; Cyclic nucleotide-gated channels	cGMP phosphodiesterase
IP_3	Phospholipase C acts on PIP_2	IP_3 receptors on endoplasmic reticulum	Phosphatases
Diacylglycerol	Phospholipase C acts on PIP_2	Protein kinase C	Various enzymes
Nitric oxide	Nitric oxide synthase acts on arginine	Guanylyl cyclase	Spontaneous oxidation

Missing: PLA > PIP2 > arachadonic acid > 12-HPETE

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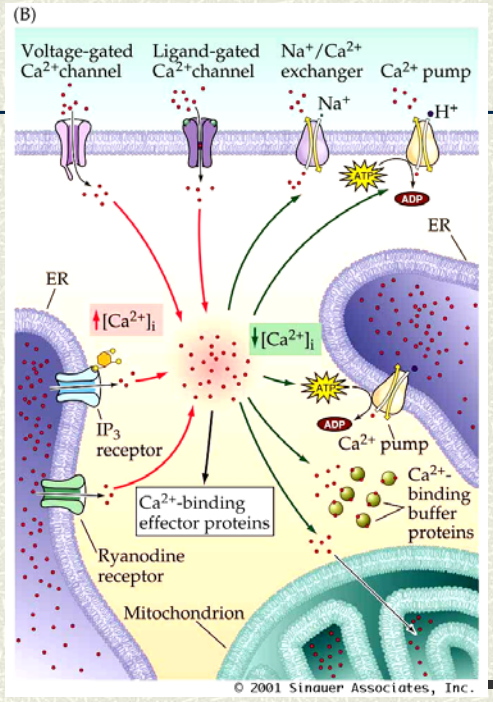
Proteins involved in moving calcium to and from the cytoplasm



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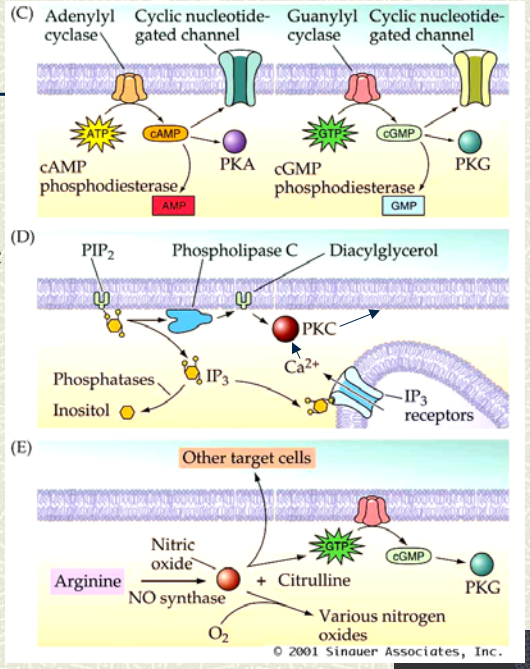
2nd messenger production and degradation - cyclic nucleotides, DAG/IP3, and nitric oxide –

"PKA" a.k.a. cAMP-dependent protein kinase



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Transcriptional regulation by CREB

- multiple signalling pathways converge (common end point via CREB) by activating kinases that phosphorylate CREB (not only cAMP)
- CREB is a ubiquitous transcriptional activator, when phosphorylated can greatly potentiate transcription
- *eg*., PKA, Ca^{2+}/calmodulin kinase IV, and MAP kinase (when increased intracellular Ca^{2+} induces phosphorylation of CREB, CRE site referred to as CaRE)
- phosphorylation of CREB allows it to bind co-activators, which then stimulate RNA polymerase to begin synthesis of mRNA
- RNA processed and exported to cytoplasm
- mRNA > translation into protein

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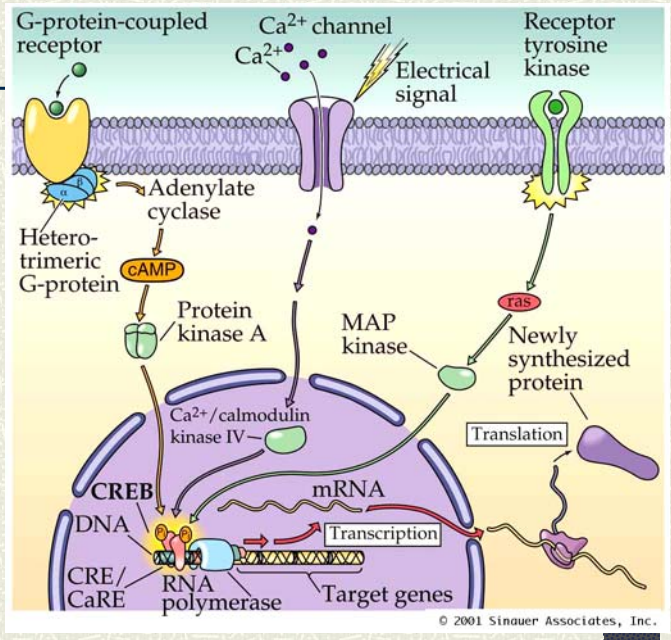
Transcriptional regulation by CREB



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FMRFamide Related Peptides - Squid

Background: various FaRPs already identified in molluscs

various effects: changes in membrane conductance to different ions, 2nd messenger activation and G proteins, effects without change in membrane permeability, ligand-gated ion channel

Prep: Squid stellate ganglion – giant synapse

Recordings: voltage clamp (postsynaptic currents - EPSC); intracellular recording of APs pre- and post-synaptically

Application of peptides: microinjection in ASW within stellate ganglion; arterial perfusion (aorta cannulated)

After Cottrell *et al*., 1992

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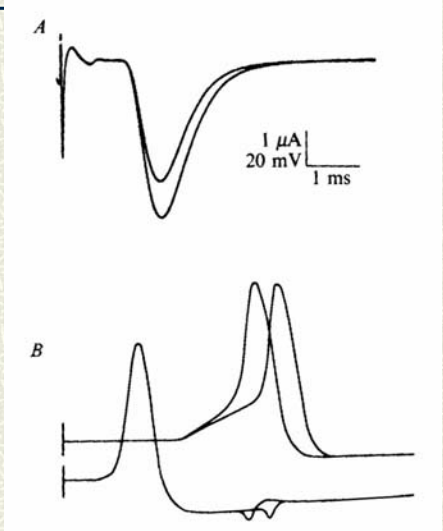
EPSCs and APs

A – EPSCs before and after FLRFamide (V/C)

B – APs pre- and post-synaptic



After Cottrell *et al*., 1992

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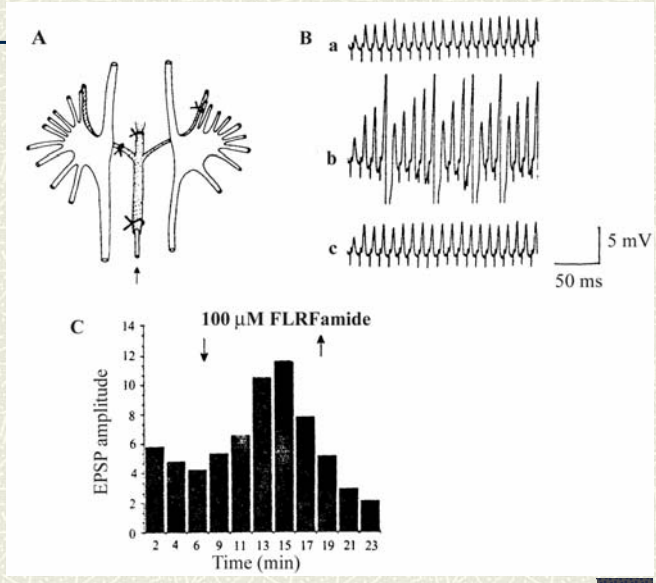
Arterial perfusion

A - Two stellate ganglia

Ligatures

B – EPSPs recorded

C – time course of potentiation



After Cottrell *et al*., 1992

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Summary

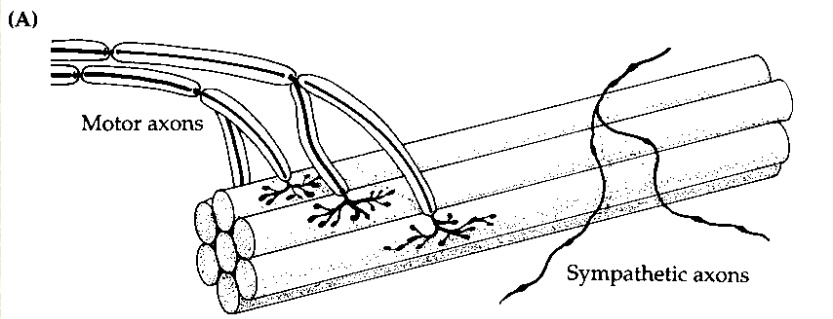
FLRFamide potentiates transmission at giant synapse:

- increase in rate of rise of EPSP
- increase in amplitude of EPSP
- increase in EPSC (v/c)

Fatigability of this synapse

Mechanism?

Modulation of skeletal muscle contraction –
2nd messenger Story Continues -



- Earliest prep to show neuromodulation (1923)
- NE facilitates neuromuscular transmission
- Presynaptic and postsynaptic

Some Specific Effects of Adrenergic Receptors

Skeletal NMJ, α-adrenergic receptors presynaptically
(increase number of quanta released (curare))

(recall an opposite effect - activation of α2 adrenergic receptor in presynaptic terminal (noradrenergic neuron) 'closed' Ca^{2+} channel)

β- adrenergic receptors postsynaptically (activates Na-K pump - what happens ?)

Hyperpolarization

Decreased resting membrane conductance

Specificity of action: general release on muscle, specific receptors on target cell

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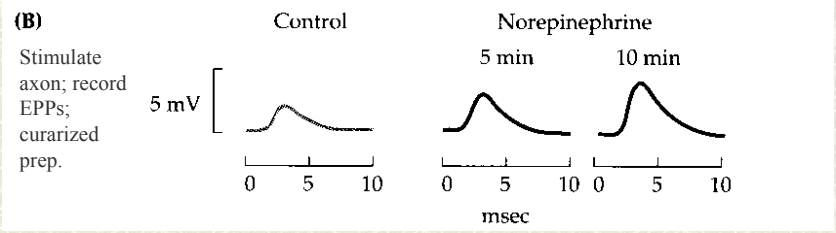
Noradrenalin at NMJ
Isolate Pre- and Post-Synaptic effects



- NA (noradrenalin) increases EPP amplitude
- Increase in quanta released show presynaptic effect
- How causes increase in NT release?
- Slow time course (*i.e.*, time before see effect)
- Effect blocked by α-adrenergic receptor antagonists

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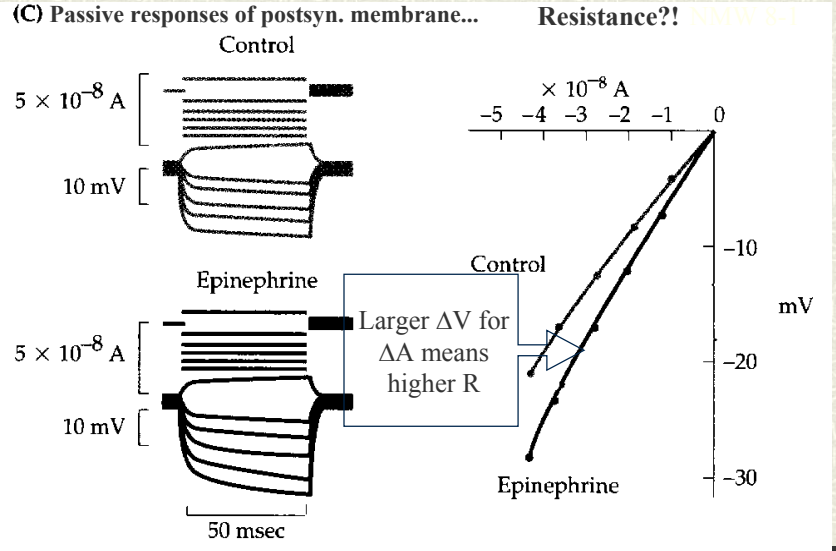
Also decrease in muscle membrane conductance, producing larger EPP's -- so *both* *pre- and post-synaptic*!



How do we test for *indirect* action of NT?

- Action is ***slow***: seconds to minutes, not milliseconds
- Action can be enhanced or inhibited by application of appropriate compounds
- Action can be mimicked using components of pathway
- Known components of 2nd messenger systems can be assayed
- Site of action of NT is usually distant from ion channels (but recall P/C experiments in heart atrial muscle and mAChRs)

In vivo example of combined effects of two types of AChR and a peptide in a frog sympathetic ganglia

- preparation location: outside spinal cord
- input to B and C cells of sympathetic ganglion
- electrical recordings

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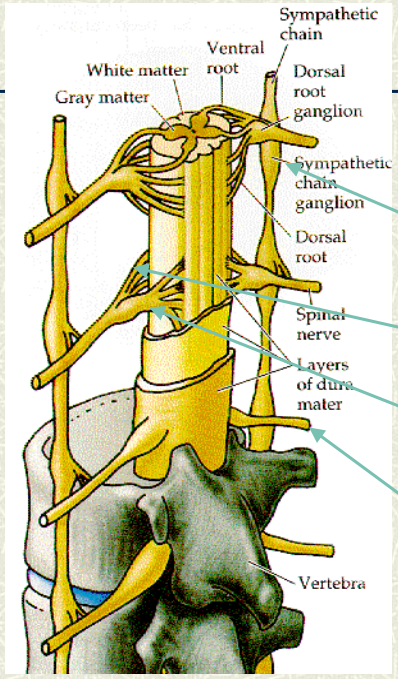
Spinal cord structure



Sympathetic ganglion

Ventral root

Dorsal root

Spinal nerve

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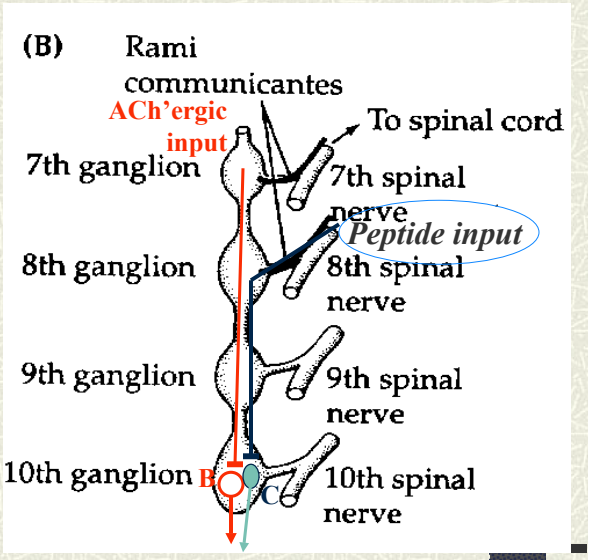
Frog sympathetic ganglia

Sympathetic ganglia with adrenergic neurons, innervated by presynaptic spinal neurons (cholinergic)



Cholinergic input

- **Initially (A):**

ACh - fast EPSP; single in, single out

- **Prolonged activation (B):**

Complex PSP (10Hz, 5sec)

Long duration, increased excitability

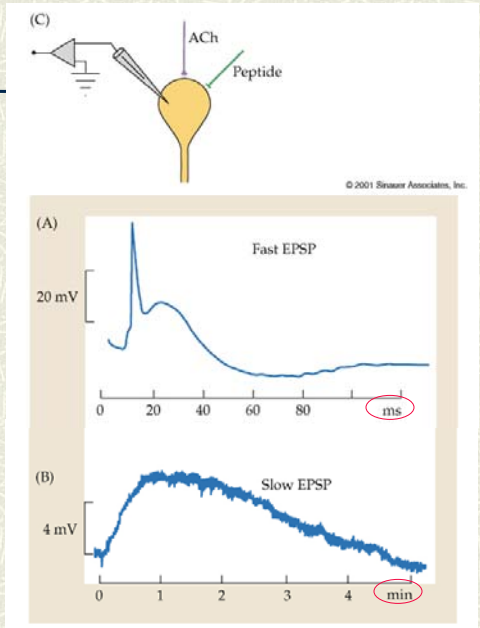


Fig. 16.2

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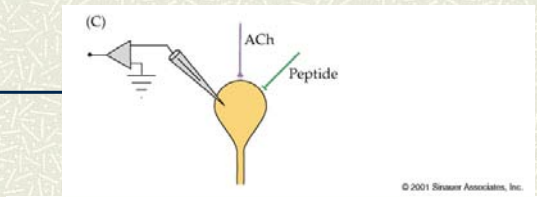
Peptidergic input

System becomes a little more complex

"Late" slow EPSP – evoked by stimulating presynaptically at 20Hz for 5sec

Question of where is peptide coming from?



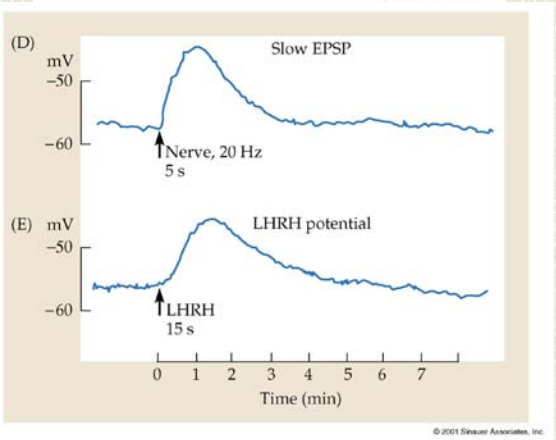


Fig. 16.2

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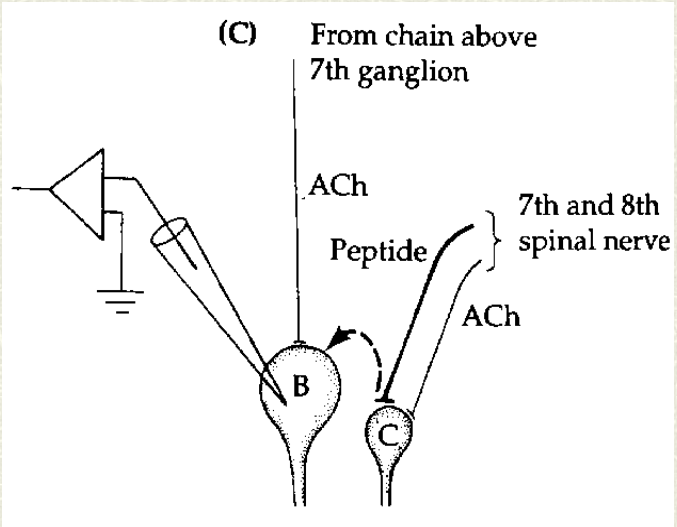
Record from ganglion cells

• Peptide NT can diffuse to affect neighbouring cells

• Can selectively activate direct (ACh) or indirect (peptide) input to "B"



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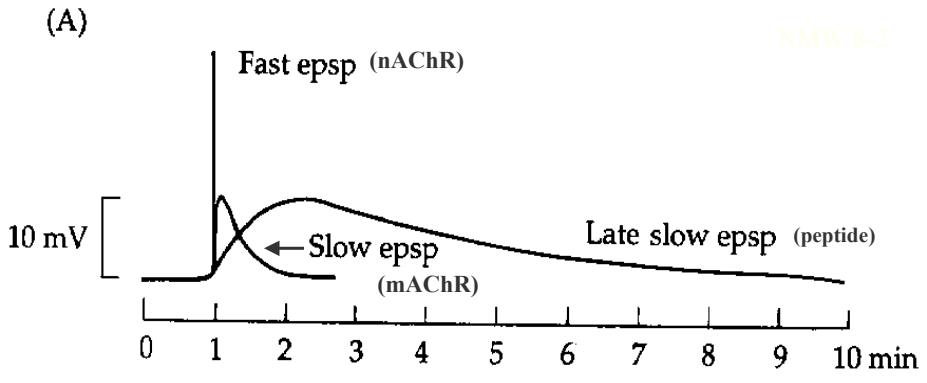
Slow action: frog sympathetic ganglia recording from cell "B"



- Recordings show 3 time courses of PSPs

What is the mechanism responsible for the various PSPs?

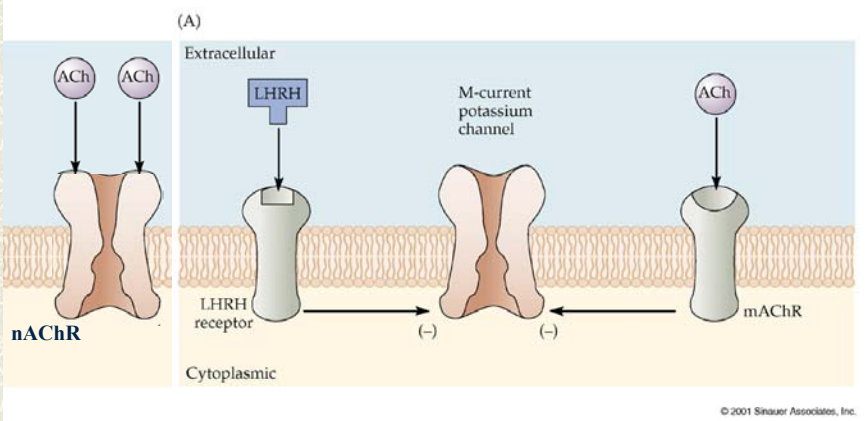


Fig. 16.4

cont. 3 time courses of PSPs – modulation fo a K^+ channel

- mAChR activated, effect on M-current K^+ channel; closes

- M-current K^+ channels voltage activated - threshold for activation near resting potential (***i.e.*, some open at rest**)

what happens when close these channels ?

- resting conductances no longer matched (Na^+ *vs*. K^+)
- cell depolarizes (Na^+), causing the slow EPSP; this is small, insufficient to evoke AP….**BUT...**

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Modulation of postsynaptic responsiveness

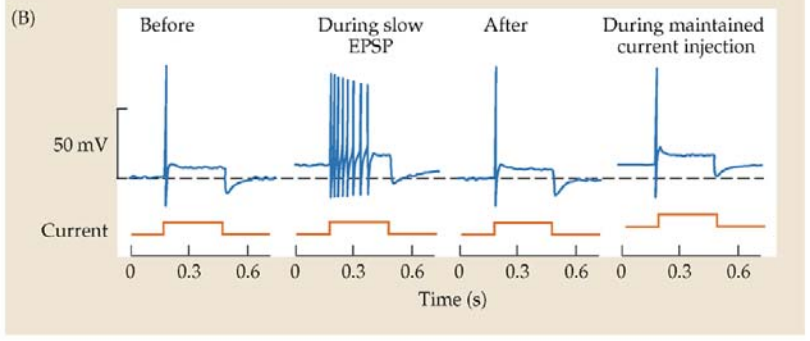

Fig. 16.4

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Properties of M-Channels

- Neuroblastoma cell line
- Contribute to resting g_{K+} - why is this important?
- Channel voltage sensitivity
- muscarine – shows influence by mAChR
- Balance – K^+ <-> Na^+

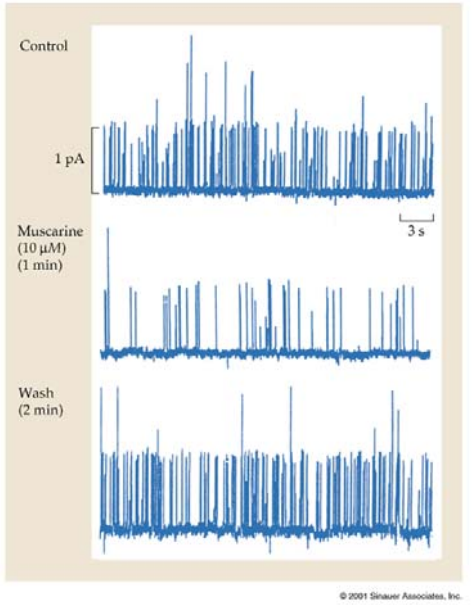

Fig. 16.3

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cont…M-current K^+ channels

- broad distribution in nervous system (SC, hippo, cerebral cortex)
- acute control – strong m-current, one-to-one (dilatation of pupil)
- broader, more continuous downstream effects; suppress m-current, tonic activity, up or down (more or less ***vasoconstriction***)

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Table 16.1

Table 16.1 (Part 1)
Characteristic actions of adrenergic sympathetic and cholinergic parasympathetic nervous systems

	Effect of		
	Adrenergic sympathetic		Cholinergic parasympathetic
Organ	**Action[a]**	**Receptor[b]**	**Action**
Eye			
Iris			
Radial muscle	Contracts	α_1	—
Circular muscle	—	—	Contracts
Ciliary muscle	(Relaxes)	β	Contracts
Heart			
Sinoatrial node	Accelerates	β_1	Decelerates
Contractility	Increases	β_1	Decreases (atria)
Vascular smooth muscle			
Skin, splanchnic vessels	Contracts	α	—
Skeletal muscle vessels	Relaxes	β_2	—
Nerve endings	Inhibits release	α_2	—
Bronchiolar smooth muscle	Relaxes	β_2	Contracts

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Table 16.1

Table 16.1 (Part 2)
Characteristic actions of adrenergic sympathetic and cholinergic parasympathetic nervous systems

	Effect of		
	Adrenergic sympathetic		Cholinergic parasympathetic
Gastrointestinal tract			
Smooth muscle			
Walls	Relaxes	α_1, β_2	Contracts
Sphincters	Contracts	α_1	Relaxes
Secretion	—	—	Increases
Myenteric plexus	Inhibits	α	Activates
Genitourinary smooth muscle			
Bladder wall	Relaxes	β_2	Contracts
Sphincter	Contracts	α_1	Relaxes
Metabolic functions			
Liver	Gluconeogenesis	α/β_2	—
	Glycogenolysis	α/β_2	—

Adrenalin acts on all adrenergic receptors, noradrenalin does not act on β2.

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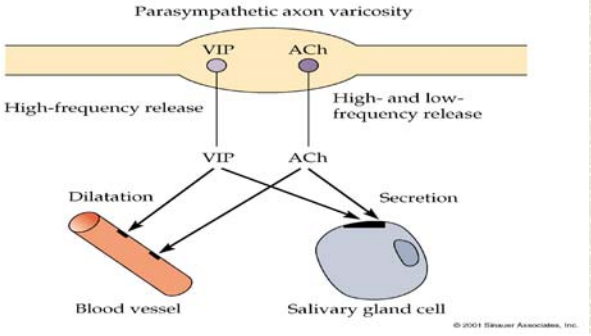
Co-transmitter release and modulation

Parasympathetic

- ACh and peptides
- *Eg*., salivary gland – co-release of VIP under high-frequency stimulation
- Atropine



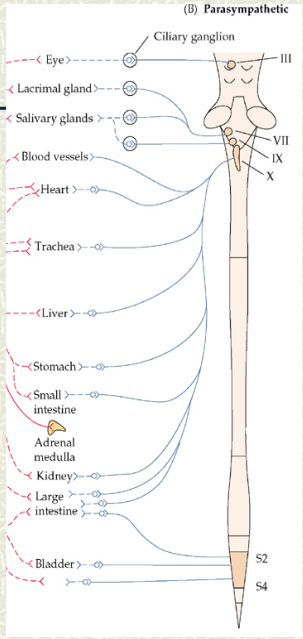


Fig. 16.1, 16.5

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Purinergic Transmission - ATP and Adenosine

- **Sympathetic transmitters** (co-transmitters) (with noradrenalin or ACh (special cases where ACh released from sympathetic)
- unusual – ATP can activate ionotropic receptor (MEPPs in sm muscle uterus)
- two main families of receptors for purines: P1, P2
- P1 – adenosine
- P2 - ATP

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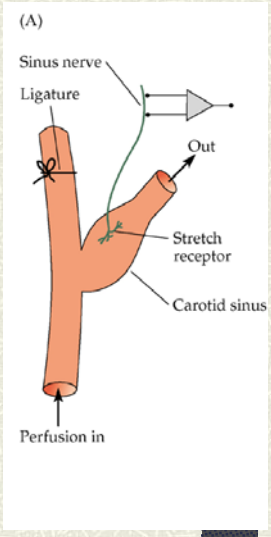
Eg., Reflex arc controlling blood pressure

- Maintaining blood pressure in head
- Stretch receptors in carotid artery (sinus)
- Lying down stretches sinus stretch receptor, increased firing R8, inhibition of sympathetic output – cardiac output decreased, bp down, heart R8 decreased
- Standing – drop in sinus pressure, decreased firing R8, release of inhibition of sympathetic arm of reflex
- basics of reflex, much more complex ("black box")

CS > brainstem nucleus (solitary tract) > project to brainstem reticular formation > autonomic preganglionic neurons (high rate of firing) > inhibition of cardiovascular sympathetic outputs



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Myasthenia gravis - History MG

- muscle fibres generally unaffected - record CAP
- curare
- motor unit - jitter
- raise antibodies against AChR in rabbits
- experimental autoimmune myasthenia gravis
- safety factor in generating APs in muscle fibres

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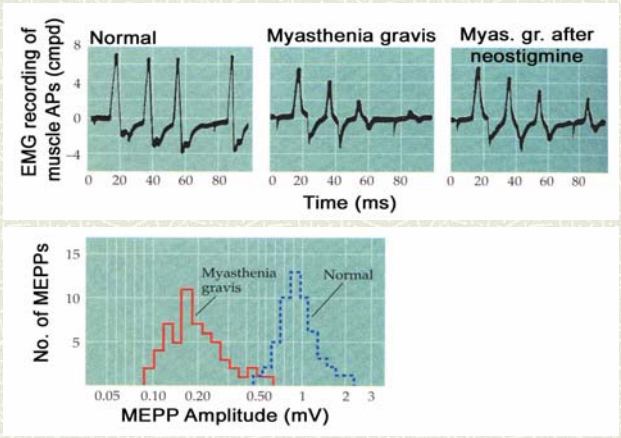
EMGs after stimulating motor nerves

- Train stimuli
- rapid fatigue
- partially restored
- smaller size of MEPPs in Mya. gravis >>fewer AChR postsynap.



From KSJ (17-2) & Purves et al. (1997)

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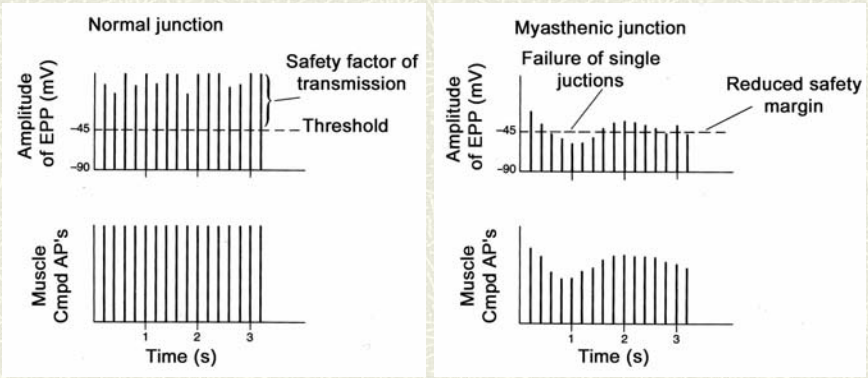
Failure of transmission at NMJ



KSJ 17-5

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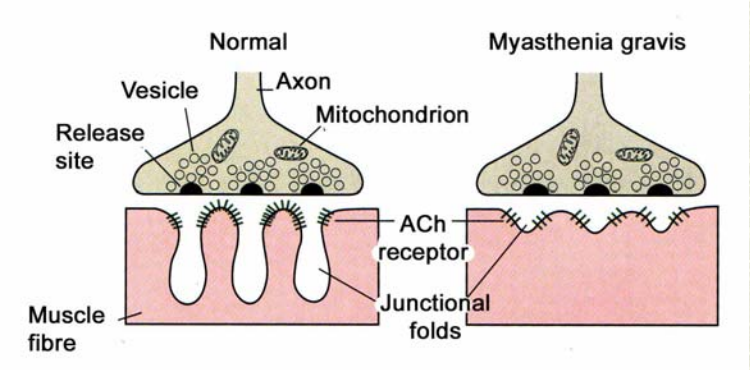
Morphological changes at the NMJ



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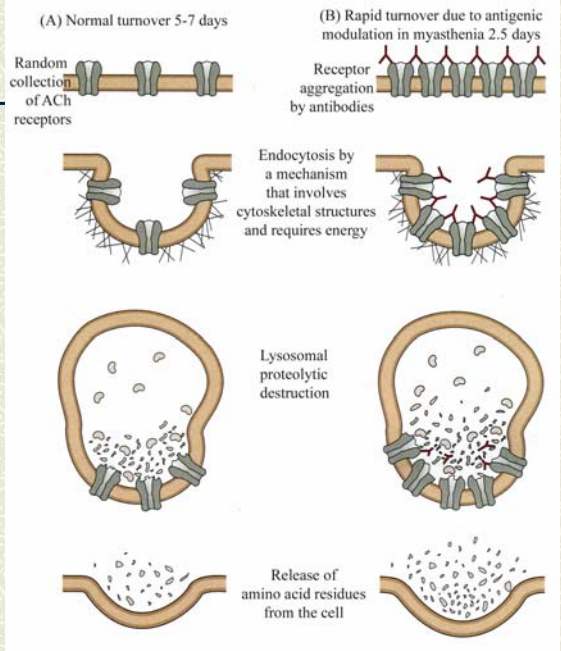
AChR turnover rate increased in myasthenia



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Etiology

- antibodies usually directed against one of two sites
  - α-bungarotoxin binding site (also ACh binding site)
  - α-subunit area (*main immunogenic region*)

Myasthenic antibodies not usually bind to receptor site (α-BTX)
- may hinder interaction of ACh with AChR
- cross linking of AChR's >> degradation
  turnover too rapid

- persistent viral infection (alters membrane properties)
- bacterial or viral infection - antigenic epitope to which antibodies made similar to peptide sequence in ACh receptor α-chain
- Thymus gland abnormalities are usually present in MG patients

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Other notes of interest

- onset of symptoms may be gradual or abrupt
- any skeletal muscle
- patients with more severe disease weak even at rest
- MG can be remitting, static, or progressive
- elevated level of AChR-Ab in up to 90% of patients
- correlates well with decrement of compound motor AP of muscle following repetitive nerve stimulation (90%)
- muscarinic side effects of anticholinesterase medications (low doses of atropine)

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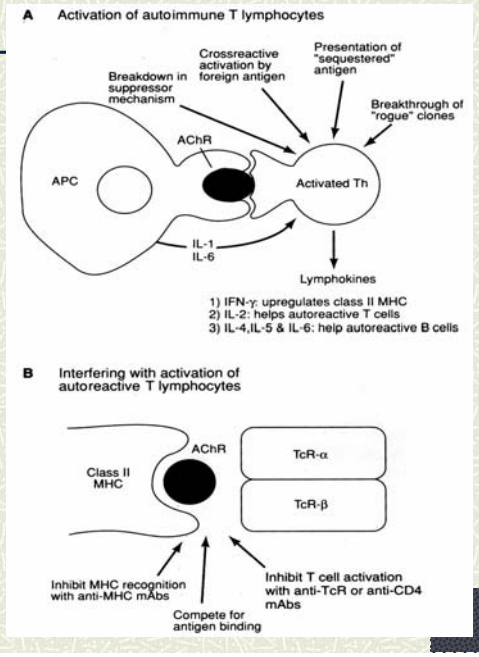
Mechanisms of the autoimmune reaction

see lecture and notes by Dr. Bonnard



Abbreviations: APC, Antigen-presenting cell; AChR, acetylcholine receptor; Th, thymocyte; MHC, major histocompatibility complex; TcR, T-cell receptor; IFN, interferon; IL, interleukin; mAb, monoclonal antibody.

Extra slides after here

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Cont…. Etiology

•MG - more complex disease than merely autoimmune
ACh receptors

•Two distinct types MG: (1) acquired autoimmune form,
(2) hereditary form (no Ab)

(2): affects other aspects transmitter release, metabolism of ACh, AChR (numbers, structure and function), *etc.*

eg. (a) lack of AChEase: phenotype….
Repetitive firing
MEPPs
EPPs

eg. (b) "slow channel syndrome" - reverse symptoms of autoimmune-type (limbs rather than eyes, speech, swallowing).

• Kinetics of opening/closing of AChR channel; developmental transition not achieved

• low ampl. MEPPs (T-tubule system, loss of receptors)

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Catecholamines

- act exclusively by activating G-protein-coupled receptors
- includes: molecules with catechol ring (benzene ring with two hydroxyl groups position 3 and 4) and amine (NH_2) off C1(ring C1-C-C-NH_2)
- many contribute to complex behaviours - hyperactivity and repetitive behaviour pattern; vomiting (antagonists to DA receptors induce vomiting; can also induce catalepsy (DA receptor subtypes activate or inhibit adenylyl cyclase (see later))
- adrenalin and noradrenalin - each act on α– and β- adrenergic receptors
- activation of α1-receptors usually elicits slow depolarization linked to inhibition of K+ channels; α2-receptors produces slow hyperpolarization due to activation of different type of K+ channel
- 3 subtypes of β-adrenergic receptors; most blockers ("β-blockers") have action in heart and respiratory system

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Indirect Mechanisms of Synaptic Transmission - Story Summary

- some neurotransmitters act on metabotropic receptors which influence ion channels and pumps indirectly through membrane-associated or cytoplasmic 2nd messengers
- action can be to modulate direct synaptic transmission or indirect neurotransmission can act alone at a synapse
- often mediated (after NT acts on receptor) G-proteins (because they bind guanine nucleotides), composed of 3 subunits (α–, β–, γ–) which dissociate when activated and act on intracellular targets (s.a.: directly on an ion channel; or indirectly on an ion channel by activation of enzymes that upregulate a 2nd messenger pathway which usually leads to phosphorylation of a target channel)
- prime targets are K^+ and Ca^{2+} channels
- action on presynaptic terminal to modify NT release
- action on postsynaptic terminal to alter spontaneous activity and responses to synaptic input
- note that there is an element of controversy over whether α–subunit dissociates and acts on the target, or all three dissociate and the βγ subunits act on the target

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