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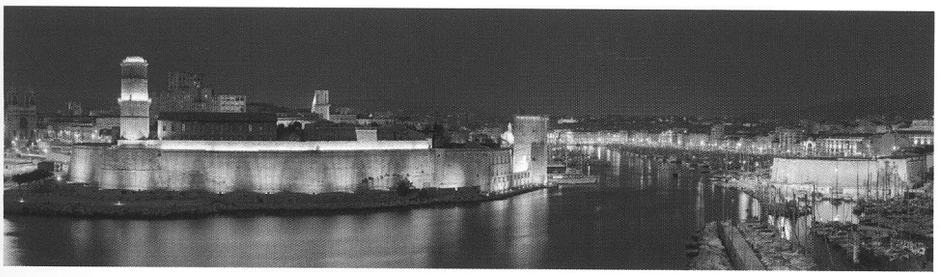

Abstract book

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L38, P46

Complex regulation of ColQ, a major anchor of AChE

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Acetylcholinesterase (AChE) terminates the action of acetylcholine at the neuromuscular junction, when AChE is clustered by the collagen Q. To localize AChE in the basal lamina, the muscle cells organize complex hetero-oligomeric forms, A_{12}, in which each chain of ColQ trimer assembles a tetramer of $AChE_T$. We have analyzed a novel allele of ColQ ($ColQ^{\delta E14}$) in which the deletion of a highly conserved sequence in the C-terminus encoded by exon 14, prevents the assembly of A_{12}, but not the formation of the trimer of ColQ when expressed alone. In $ColQ^{\delta E14}$ mice, A_{12} levels are very low but detectable, and AChE tetramers are linked to a full-length, single-stranded collagen. Consequently, $AChE_4/ColQ_1$ cannot interact with heparan sulfate proteoglycan, because the heparin binding domain requires a collagen trimer. $AChE_4/ColQ_1$ presumably interacts through the C-terminus of ColQ with MuSK and other proteins of the extracellular matrix, because we observe a dominant effect in $ColQ^{\delta E14}$ heterozygotes. Despite the partial deficit in AChE in $ColQ^{\delta E14}$ mice, synaptic transmission and muscle contraction are affected as severely as in ColQ KO mice, where ColQ cannot interact with AChE, or in $AChE^{1irr}$ mice, in which AChE is not produced by the skeletal muscle. Despite the similar severity of synaptic transmission defects, ColQ KO mice are grossly more severe than $AChE^{1irr}$ and $ColQ^{\delta E14}$, but less affected than AChE KO mice. To further explore the discrepancy between the alteration of the synaptic transmission at the NMJ and the overall health of the mice, we have analyzed AChE molecular forms in different tissues. We found high level of A_{12} in ciliary ganglion, in urinary bladder, in trachea and in salivary glands. We observed that A_{12} forms are transformed into G4 in some tissues including the muscle of $ColQ^{\delta E14}$ mice, suggesting that the interaction with the C-terminal domain of ColQ significantly retains AChE in some, but not all, tissues. Moreover, we found that the level of A_{12} is differently affected when perlecan is reduced. Reduction of perlecan, or of heparan sulfate in perlecan, dramatically decreases the quantity of A_{12} extracted from the skeletal muscle, but not from salivary glands. Together, these analyses reveal an unsuspected function of AChE in several tissues, presumably related to the hydrolysis of Ach, but apparently not in the context of the classical synaptic transmission.

L59, P97

Slow-binding inhibition of acetylcholinesterase by an alkylammonium derivative of 6-methyluracil: molecular modeling, X-ray crystallography and kinetic study of inhibition mechanism

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Slow-binding inhibitors present considerable advantages over classical reversible inhibitors in pharmacology [1]. Inhibition of human AChE and BChE by an alkylammonium derivative of 6-methyluracil, C-547, a potential drug for the treatment of *myasthenia gravis* was studied. Progressive AChE inhibition was observed. Molecular docking, steered molecular dynamics (SMD) and free energy profile calculations of binding/dissociation processes of C-547 showed that the inhibitor rapidly binds to the AChE PAS. This is followed by a slow step for crossing the AChE bottleneck. The slow step is reflected in high force peaks for SMD simulations and a 4 kcal/mol energy barrier in the free energy profile. Then tight complex below the AChE gorge bottleneck is established. This complex was observed by X-ray crystallography (3.13 Å resolution) [2]. For the dissociation process, passing through the bottleneck was more hindered than for binding. This mechanism corresponds to slow-binding inhibition of type B, i.e. after formation of the initial enzyme-inhibitor complex (K_i = 140 pM), an induced-fit step allowed establishment of the final complex (K_i^* = 22 pM). Slow k_{off} = 0.05 min^{-1} determines a long residence time on target, τ = 20 min, much longer than for other reversible inhibitors used in the treatment of *myasthenia gravis*. This makes C-547 a promising drug for the treatment of this disease.

On the other hand, due to the absence of bottleneck in BChE gorge, SMD simulations of C-547 binding and dissociation processes did not display a slow step. Kinetic studies showed that inhibition of human BChE is a reversible fast-binding process of mixed-type (K_i = 1.77 μM; K_i = 3.17 μM). The non-charged analog of C-547, compound C-35 was not a slow-binding inhibitor of AChE. It did not cross the bottleneck because it is not sensitive to the electrostatic driving force that pulls charged ligands to the bottom of the gorge. These results demonstrated that slow-binding inhibition of AChE by C-547 is determined both by the existence of a bottleneck in the enzyme active gorge and by the cationic nature of this ligand.

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[1] P. Masson and S. Luschekina. Slow-binding inhibition of cholinesterases, pharmacological and toxicological relevance. *Arch. Biochem. Biophys.*, **2016**, 593, pp. 60-68.

[2] A.D. Kharlamova *et al.* Slow-binding inhibition of acetylcholinesterase by an alkylammonium derivative of 6-methyluracil: mechanism and possible advantages for *myasthenia gravis* treatment. *Biochem. J.*, **2016**, 473(9), pp. 1225-1236.

P54

α7 nicotinic receptor a novel sensor for spillover of acetylcholine adjusted by butyrylcholinesterase at the terminal Schwann cell

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The neuromuscular synaptic transmission is indispensable for life because it transfers the complex cerebral commands to the muscular twitches. At the vertebrates neuromuscular junction (NMJ), acetylcholine (ACh) is released from motor nerve, diffuses through synaptic cleft, activates postsynaptic nicotinic acetylcholine receptors (nAChRs) of muscle type ((α1)2β1δε) and subsequently transiently depolarizes the post-synaptic membrane, forming the excitatory postsynaptic potential (EPSP). The exposure time of ACh on nAChRs is controlled by cholinesterases.

The mammalian cholinesterases family includes two closely related enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) that hydrolyze ACh. In mature NMJs, the obvious functional distinctions between these enzymes are not related to their structure but to their respective localizations. AChE at the NMJ is clustered by ColQ mainly in synaptic cleft, whereas BChE is essentially anchored by PRiMA on Terminal Schwann cells (TSCs) outside of synaptic cleft. It appears obvious that AChE controls the ACh lifetime and its time exposure on receptors in the synaptic cleft. BChE on the TSC controls the lifetime of ACh outside the synaptic cleft. We have revealed that BChE regulates the activation of α7 nAChRs. When α7 nAChRs are activated by spillover of ACh, the release of ACh by the nerve terminal is depressed. This regulation loop works as an ACh sensor on the surface of TSCs. Thus, it adapts continually the synaptic release of ACh to the ACh spillover outside the synaptic cleft. Indeed, this loop may explain some paradoxical effects of non-specific cholinesterase inhibitors used for rescuing muscle strength in *myasthenia gravis*. On one hand, AChE inhibition increases the lifetime of ACh in the synaptic cleft and increases EPSP amplitude until the threshold of action potential generation, and thus, improves muscle twitch. One the other hand, BChE inhibition increases the lifetime of ACh in the vicinity of TSC where 7 nAChR triggers a negative feedback loop in which less quanta of ACh are released and the amplitude of EPSP decreases. This novel knowledge should help to improve the use of specific AChE inhibitors in neuromuscular pathological conditions.

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P70

Novel non-charged acetylcholinesterase inhibitors based on 1,3-bis(w-ethylaminoalkyl or aminoalkyl)-6-methyluracil derivatives for the treatment of Alzheimer's disease

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A series of 1,3-bis[ω-(NO_2, CF_3, F-substituted benzylethylamino or amino)alkyl]-6-methyluracils were designed, synthesized, and evaluated as selective acetylcholinesterase (AChE) inhibitors. The aimed compounds were synthesized starting from NO_2, CF_3, F-benzyl bromides or benzaldehydes and 1,3-bis(ω-ethylaminoalkyl)-6-methyluracils or 1,3-bis(ω-aminoalkyl)-6-methyluracils, in its turn the latter being obtained from 1,3-bis(ω-bromoalkyl)-6-methyluracils and ethylamine or by introduction of these bisbromides in Staudinger reaction. In these compounds the number of methylene groups in alkyl chains and the electron withdrawing substituents on benzyl rings were varied. The compounds are reversible inhibitors of cholinesterases of mixed type, and some of them show a remarkable selectivity for human AChE, with inhibitory power in nanomolar range, more than 10000 times higher than for human butyrylcholinesterase.

Molecular modeling study of the compounds indicated that they are bifunctional AChE inhibitors spaning the enzyme active site gorge and strongly binding to its peripheral anionic site (PAS) regardless their protonation state. Moreover, the cleft at the PAS edge facilitates this binding. Thus, binding of these 6-methyl derivatives blocks the entrance of the gorge, causing inhibition of the esterase activity of AChE.

In vivo experiments show that the 6-methyluracil derivatives are able to penetrate the blood brain barrier inhibiting the brain AChE up to 70% of the enzyme. The most potent AChE inhibitors exhibit LD50 values less than 100 mg/kg. In addition, by masking the PAS area, they impair the role of PAS in chaperoning Aß aggregation. *In vivo* experiments showed that the most potent AChE inhibitor, namely 1,3-bis[5-(*ortho*-nitrobenzylethylamino)pentyl]-6-methyluracil improved working memory in scopolamine and transgenic APP/PS1 models of Alzheimer's disease, and significantly reduced the number and area of Aß plaques in the brain. In particular, treatment with this compound (5 mg/kg) during 14 days reduced percentage of summary area and number of ß-amyloid peptide deposits visualized in sections of cerebral cortex, dentate gyrus, and hippocampal CA3 area in APP/PS1 mice. Meanwhile, treatment with reference drug Donepezil (1 mg/kg) during 14 days significantly reduced Aß load in cerebral cortex but not in dentate gyrus and CA3 area. Thus, this compound is a promising candidate as a bifunctional inhibitor of AChE for treatment of AD [1].

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1. Semenov V.E., Zueva I.V., Mukhamedyarov M.A., et al. ChemMedChem. 2015. Vol. 10. P. 1863-1874.