stimulation or by direct application of GABA) by several drugs which bind to benzodiazepine (BZ) receptors: diazepam, zolpidem and abecarnil. Patch-clamp recording in whole cell configuration was used. Experiments were performed on hippocampal slices (stimulation electrodes were positioned in stratum radiatum and stratum oriens) and on cells isolated from hippocampus (CA1 pyramidal cells), cerebellum (Purkinje cells, PC) and striatum (giant cholinergic interneurons, GCN). It was found that all the drugs under investigation potentiated responses to GABA. However, the degree of potentiation and its time course were different for different substances and various types of neurons. The input specificity in the degree of potentiation was observed in slices: IPSCs induced by stimulation of stratum radiatum were more sensitive to diazepam and zolpidem (207±11%, 238±36%, respectively) than those induced by stratum oriens (107±4%, 125±6%, respectively). Comparison of the effect of zolpidem on isolated cells also revealed difference in sensitivity to this drug in Purkinje and striatal cells. Thus, zolpidem EC_{50} value was 58±6 nM and maximal enhancement of current induced by 2 μM GABA – 456±13% in PC, whereas in GCN these characteristic were 185±67 nM and 257±16%, respectively. The enhancement of the amplitude of GABA responses by diazepam and zolpidem was fast and reversible. In contrast, the potentation induced by abecarnil (a full agonist at $GABA_A$/BZ receptor on PC somatic membrane) although found to be of similar efficacy to diazepam (241% and 217%, respectively) developed gradually even after cessation of drug application, and depended on both abecarnil concentration and exposure time. These data taken together suggest a difference in functional properties of BZ binding sites of $GABA_A$ receptors in different types of central neurons.

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P.3.026 Pharmacokinetics of peptide anxiolytic Selank in rat blood and brain after intranasal administration

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The study deals with the distribution of peptide anxiolytic Selank (TKPRPGP), and its fragments, in rat brain and blood after intranasal administration, as well as with the degradation pathways of this peptide in blood plasma (in vitro). Solid state catalytic isotope exchange [1] was 3 used for producing [G-^{3}H]Selank (110 Ci/mmol) with the isotope label in all its amino acid residues. [G-^{3}H]Selank was administered to the rat or incubated with rat blood plasma in vitro. After that HPLC of brain tissues and blood extracts, enriched with nonlabeled Selank and its fragments in UV-detectable concentration, was performed. The concentration of [G-^{3}H]Selank and its fragments was evaluated by measuring radioactivity of appropriate chromatographic fractions. At the first minute after intranasal administration Selank was detected in bulbus olfactorius, in brain cortex and in brainstem. At the third minute the peptide concentration in above-mentioned brain regions was several times higher than in blood. Selank biodegradation in blood plasma was mainly due to c-end dipeptides restriction (90%) leading, in series, to the formation of TKPRP and finally to the long-living TKP and RP fragments (see Table).

Concentration of Selank and its hydrolysis products in rat blood plasma

Peptide	Ci/mmol	Concentration of radioactive peptides, nMol						
		0.3 min	1 min	1.5 min	2 min	2.5 min	4 min	23 min
TKP	71	0	39	82	125	137	166	19
RP	18	0	38	69	123	185	207	31
PRPGP	60	12	37	46	53	35	37	5
TKPR	80	0	0	0	0	0	0	0
TKPRPG	101	0	0	0	0	0	0	0
TKPRP	89	109	151	171	164	145	118	6
TKPRPGP	110	895	731	555	410	321	158	8
RPGP	68	0	0	0	0	0	0	0
KPRPGP	99	0	0	0	0	0	0	0

Contribution of dipeptidylaminopeptidases to Selank biodegradation in blood plasma was about 10%, while amino- and carboxypeptidases did not take part in Selank hydrolysis at all. It was also shown that the sets of Selank biodegradation products formed in the blood plasma of rat and man are similar, although the rate of hydrolysis in rat blood plasma is three times as rapid as that in human blood plasma. Selank biodegradation in blood *in vivo* was similar to that in blood plasma *in vitro*; however, some products of amino- (4%) and carboxypeptidase (0.4%) activities were detected in the blood. In conclusion, we demonstrate that after intranasal administration, Selank quickly enters the brain and the blood. The main biodegradation pathway of Selank in rat blood is hydrolysis by dipeptidylcarboxypeptidases.

References

[1] Yu.A. Zolotarev et al. New development in the tritium labelling of peptides and proteins using solid-state catalytic isotopic exchange with spillover-tritium. Amino Acids 2003; 24(4): 325–333.