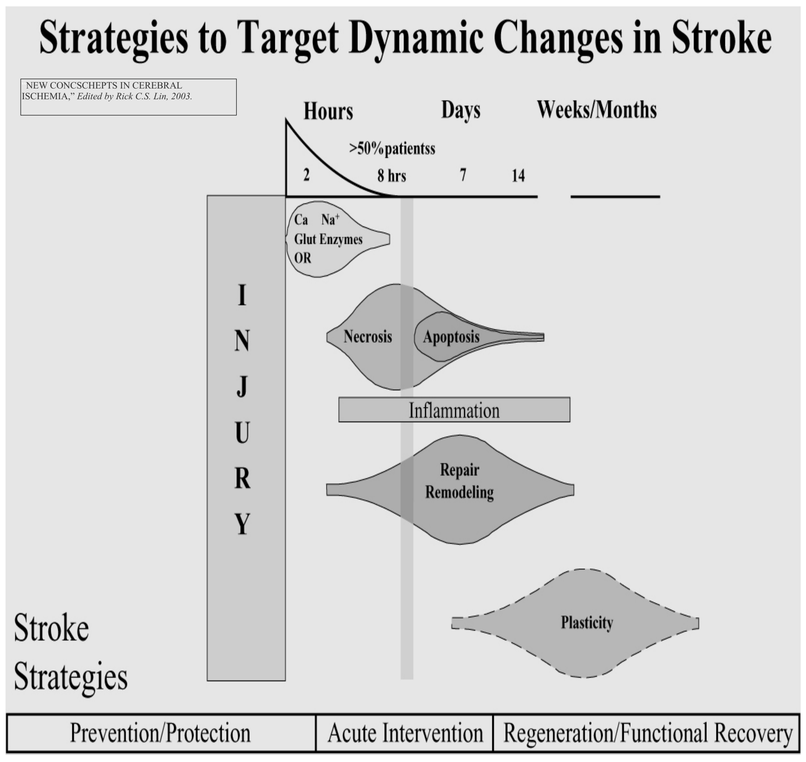
Influence of afobazol on neuromediator amino acids level in rat brain caused by global ischemia

V.S. Baykova, I.A. Kadnikov

Zakusov's Instiute of Pharmacology of Russian Academy of Medical Sciences, Department of Pharmacogenetics, Moscow, Russia

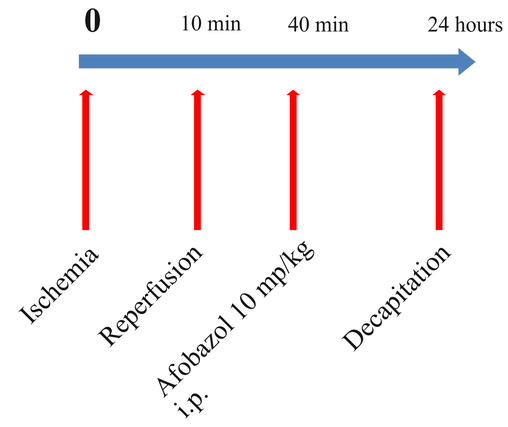
OBJECTIVE

Afobazol (5-etoxy-2[2-(morpholino)ethilthio]benzimidazole dihidrochloride) is selective anxiolytic drug designed in Institute of Pharmacology RAMS. In recent studies we found out that afobazol among with its anxiolytic effect possess neuroprotective properties. It is common knowledge that the depth of ischemic brain damage depends on drift of balance between excitatory and inhibitory amino acids down to excitation. The concern of our study was to determine the tardive effects of Afobazol on neuromediator amino acids in model of global ischemia.



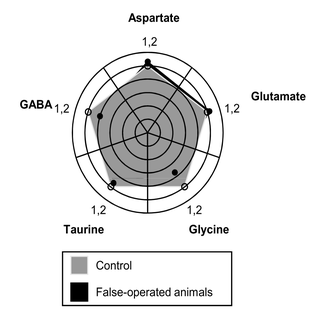
METHODS

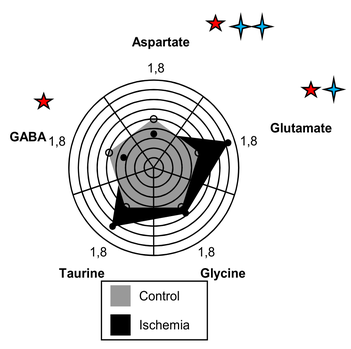
Global ischemia was formed in white male randomly-bred rats weighing 260-300 g. using model by M.L. Smith (1984). Afobazol was intraperitoneally administrated (10 mg/kg) 30 minutes after reperfusion.
Rats were anesthetized with chloral hydrate (325 mg/kg, i.p.) with natural breathing. Reperfusion performed 10 minutes after ischemia.
24 hours after reperfusion rats were decapitated, brains were quickiy removed. We extracted hypothalamus, frontal cortex, striatum, hippocampus and nucleus accumbens.
Structures homogenisation was performed in 0,1 M $HClO_4$ with the addiction of homoserine as internal standard in a concentration of 1 nM. Derivatization time was 21 minutes in the presence of 20 µl 0.1 M borate buffer and 10µl ortho-phtalaldehyde. Amino asids were separated on a Hypersil BDS C18 column using 2 mM phosphate buffer containing 3% acetonitrile as mobile phase (pH 5.6).
As an internal standard for monoamines used 3,4-dihydroxibenzilamin (DHBA) in a concentration of 0.5 nM. Monoamines (NA, DA, 5-HT) and their metabolites (DOPAC, HVA, 5-HIAA) were separated on a Zorbax SB C18 column using as mobile phase 0.1 M citrate-phosphate buffer containing 0.3 mM sodium octansulphonate, 0.1 mM EDTA and 8% acetonitrile (pH 3.0).
Level of neuromediator amino asids and monoamines was measured using HPLC-ED technique.
Assessing the reliability of the results was performed using unifactor analysis of variance (ANOVA; post-hoc: Fisher LSD test).



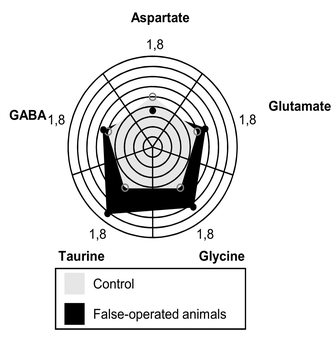
RESULTS

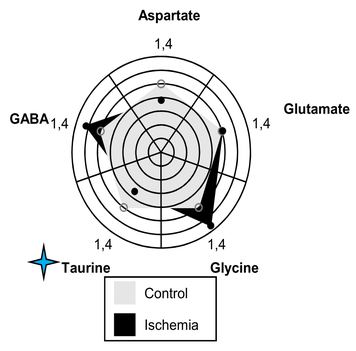
The concentration of amino acids in the striatum of random-bread rats. Relative values

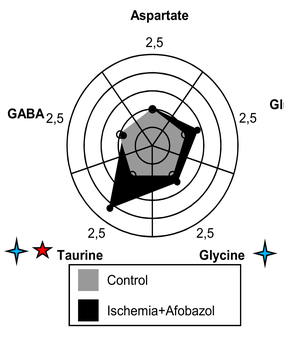




The content of amino acids in the hippocampus of random-bread rats. Relative values

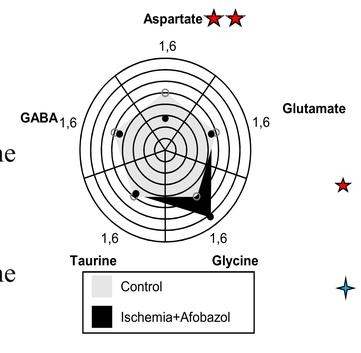






★ No significant differences compared to the control group (intact animals)

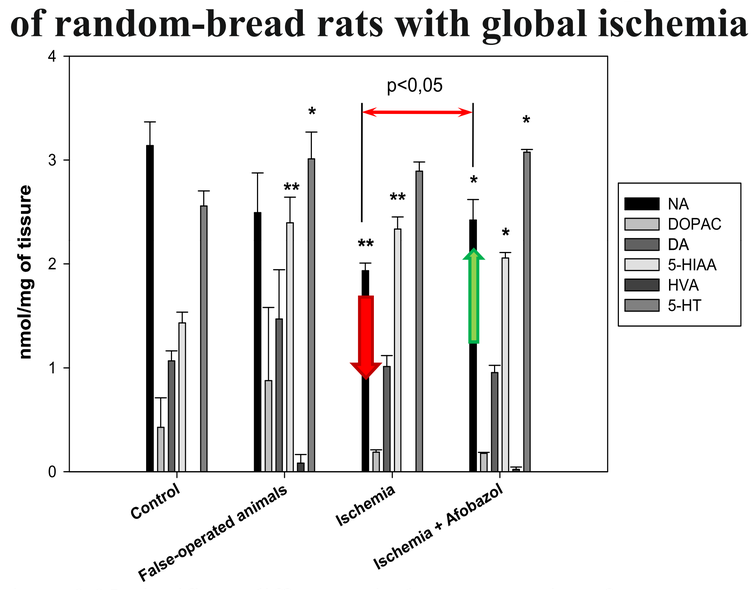
✦ No significant differences compared to the false-operated group of animals



★ No significant differences compared to the control group (intact animals)

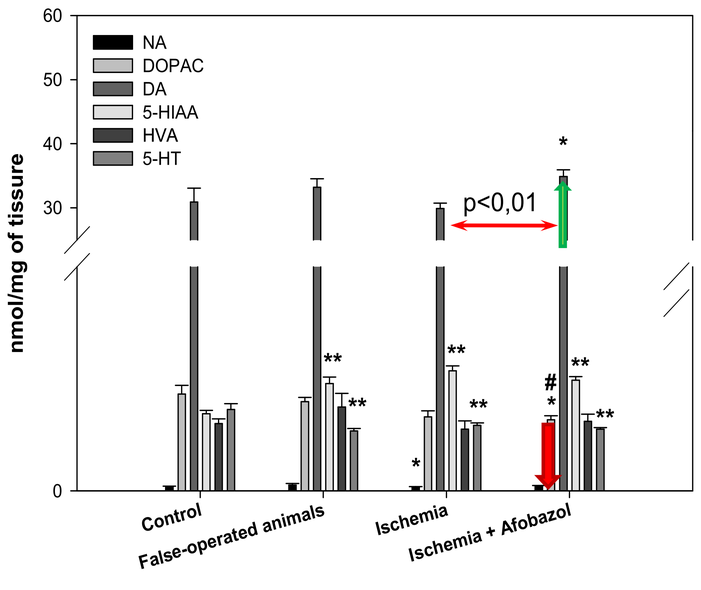
✦ No significant differences compared to the false-operated group of animals

Influence of afobazol on the concentration of neurotransmitter monoamines in the hypothalamus of random-bread rats with global ischemia



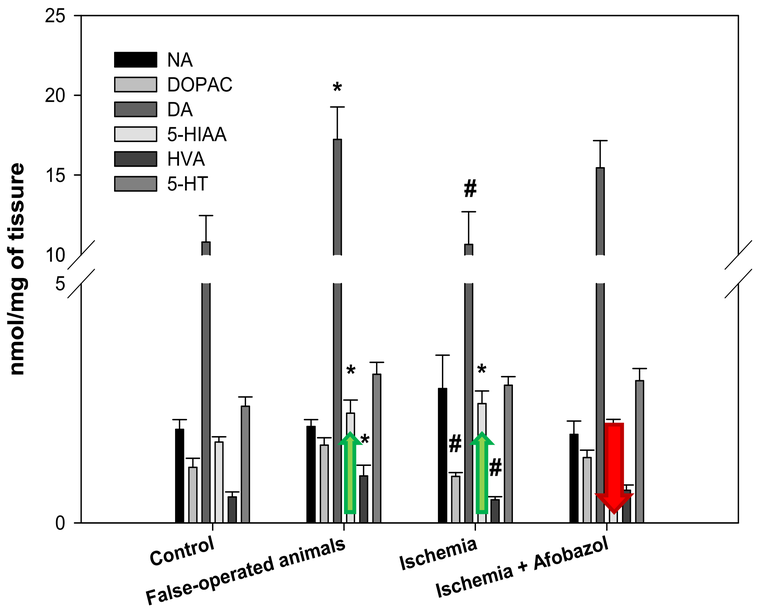
* - $p < 0.05$; significant differences in the concentration of neurotransmitters in the group (ANOVA; Fisher LSD) compared with control
** - $p < 0.01$; significant differences in the concentration of neurotransmitters in the group (ANOVA; Fisher LSD) compared with control

Influence of afobazol on the concentration of neurotransmitter monoamines in the striatum of random-bread rats with global ischemia



* - $p < 0.05$; significant differences in the concentration of neurotransmitters in the group (ANOVA; Fisher LSD) in comparsion with control
** - $p < 0.01$; significant differences in the concentration of neurotransmitters in the group (ANOVA; Fisher LSD) in comparsion with control
\# - $p < 0.05$; significant differences in the concentration of neurotransmitters in the group (ANOVA; Fisher LSD) in comparsion with false-operated animals

Influence of afobazol on the concentration of neurotransmitter monoamines in the NAC of random-bread rats with global ischemia



* - $p < 0.05$; significant differences in the concentration of neurotransmitters in the group (ANOVA; Fisher LSD) as compared with control
\# - $p < 0.05$; significant differences in the concentration of neurotransmitters in the group (ANOVA; Fisher LSD) as compared with false-operated animals

CONCLUSION

The most notable changes in neurotransmitter pattern were observed in striatum. We detected significant increase of glutamate level up to 65% and decrease of GABA level down to 67% in ischemic animals striatum in comparison to intact rats. In afobazol treated aninals level of glutamate was 27% lower and level of GABA 32% higher than observed in ischemic animals. Concentrations of glutamate and GABA observed in afobazol treated aninals were of no significant difference between control rats. Taurin level was 50% and 114% higher in ischemic and afobazol administrated animals respectively than observed in intact rats.
It was proved that in the postischemic period in mammals characterized by reduced functional activity of a number of monoaminergic systems, because of lack of oxigen. The result of ischemia is an uncontrolled release of neurotransmitters monoaminergic systems, with a subsequent decrease in their functional activity in the postischemic period.
We can assume that afobazol prossessing membrane stabilizing effect, prevents the uncontrolled release of neurotransmitters and depletion of neurotransmitter systems. Afobazol restore distrupted balance in striatum between excitatory and inhibitory amino acids caused by global ischemia and activate taurin dependant neuroprotection.

There is no potential conflict of interest