



Baština Akademije nauka i umjetnosti Bosne i Hercegovine

Međunarodni simpozijum o eksperimentalnom tremoru: 1-3. april 1971. godine

Stern, Pavao

1972

Akademija nauka i umjetnosti Bosne i Hercegovine

<https://bastina.anubih.ba/items/05bee08e-28ec-4b12-b10e-5c9e89b50461>

Preuzeto s Baštine Akademije nauka i umjetnosti Bosne i Hercegovine

<https://bastina.anubih.ba/>

AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE

POSEBNA IZDANJA

KNJIGA XVII

ODJELJENJE MEDICINSKIH NAUKA

KNJIGA 4.

MEĐUNARODNI SIMPOZIJUM

O EKSPERIMENTALNOM TREMORU

1 — 3. APRIL 1971. GODINE

Urednik
PAVEL STERN,
redovni član Akademije nauka i umjetnosti
Bosne i Hercegovine



SARAJEVO
1972

DRAGAN STANKOVIĆ, AMIR PLEHO, JELA GRUJIĆ-VASIĆ,
BRANKO NIKOLIN AND JELENA MIRIĆ

CHE ACTIVITY IN WHOLE BRAIN AND BASAL GANGLIA OF RATS TREATED WITH MANGANESE

In our earlier experiments we investigated the influence of manganous chloride and manganous sulfate on ChE activity in whole brain of rats treated with manganous salts (4). The reason for this investigations was the fact that in chronical poisoning of manganous salts which may cause extrapyramidal syndrome, the role of holinergeric system is not enough investigated up to date. In our work we found in rats treated by manganous chloride and manganous sulfate increase of ChE activity in whole brain, but differences were not significant with regard to the control. Now the question is whether manganous ions present in brain of animals treated with Mn^{++} — ions may influence the ChE activity.

The subject of our present study is the investigation of influence of Mn^{++} — ions on ChE activity in homogenate of whole brain in vitro.

In addition to this our study in whole brain we carried out also the control. Now the question is whether manganous ions present in basal ganglia.

MATERIALS AND METHODS

For preparation of samples we used a supernatant of whole brain's homogenate, prepared in following sheme. Concentration of manganous chloride in examined samples was 0,20 — 0,60‰ in whole brain homogenate and 0,45 ml distilled water. The experimental group presents the following mixtures:

Samples	Brain homogenate in ml	ml of 1‰ $MnCl_2$	ml of water
A	0,30	0,15	0,30
B	0,30	0,30	0,15
C	0,30	0,45	—

Acknowledgments. We are indebted to the Federal Research Fund Belgrade for the financial support granted for this investigation.

As blank for brain homogenate was used solutiones made by following sheme:

Sample	ml 1% $MnCl_2$	ml H_2O
A	0,15	0,60
B	0,30	0,45
C	0,45	0,30

Cholinesterase activity was determined by Hestrin's method (1).

For histochemical investigations we used Wistar albino rats, female, body weight 100 — 115 g treated with manganese chloride in doses 15 mg/kg during 21 days. As control same sort of Wistar albino rats were used. In experiment brain first was washed with Ringer via left ventricule and than basal ganglia was removed. Frozen brain were cut in slides and Gomori's procedure were used for determination of ChE activity (3). Incubation medium were prepared fresh and for preincubation we used three specific inhibitors — iso-OMPA in concentration 1×10^{-4} , BW 284 C 51 in concentration of 1×10^{-5} and eserine in concentration 1×10^{-5} (2). Preincubation was carried out in medium without supstrate when inhibitor was not added during 15 minutes. As supstrates AThCh and BuThCh were used.

RESULTS

The results have presented in table 1—3.

Table 1.

EFFECTS OF Mn^{++} — IONS ON ChE ACTIVITY IN BRAIN HOMOGENATE

Samples	Experimental group N-4	Control N-4
	1	2
A	72,54	7,05 = 100%
B	67,03	
C	62,57	

1. ChE activity in percentage of the mean value of the control.

2. The mean value of ChE activity = 100%

The brain homogenate exposed to Mn^{++} — ions one hour.

The decrease of ChE activity in the brain homogenate treated with Mn^{++} — ions is evident.

The distribution of enzyme activity in basal ganglia is presented in table 2.

Table 2.
ACTIVITY OF AChE IN RATS BASALE GANGLIA

Experiments	Localisation and AChE-activity		
	Nuc. caudatus	Putamen	Gl. pallidus
1. Incub. medium without the addition of substrate	—	—	—
2. Incub. medium+substrate AThCh	++	++	+
3. Incub. med. + isoOMPA 10^{-4} + substrate AThCh	++	++	+
4. Incub. med. + BW 10^{-5} + AThCh	—	—	—
5. Incub. med. + eserine 10^{-5} + AThCh	—	—	—
6. Incub. med. + BuThCh	+	+	+
7. Incub. med. + iso-OMPA 10^{-4} + BuThCh	—	—	—
8. Incub. med. + BW 10^{-5} + BuThCh	+	+	+
9. Incub. med. + eserine 10^{-5} + BuThCh	—	—	—

From these histochemical results we can make conclusion that the incubation medium with substrate gives characteristic color reaction, which was more intensive with AThCh than by BuThCh.

With specifics inhibitors of AChE, BW284C51 and eserine color reaction was not expressed. By using inhibitors of BuChE, iso-OMPA and eserine the tissue was without the color reaction, and by BW284C51 inhibitors color reaction was observed.

Color intensity was more pronounced in Putamen and nucleus caudatus than in Gl. pallidus, as had been noted in literature. The tissue color in the basal nuclei was more times intensive than in the cortex (Fig. 1).

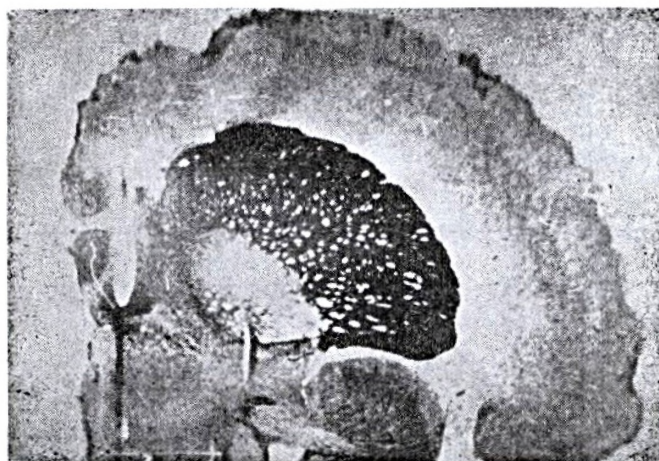
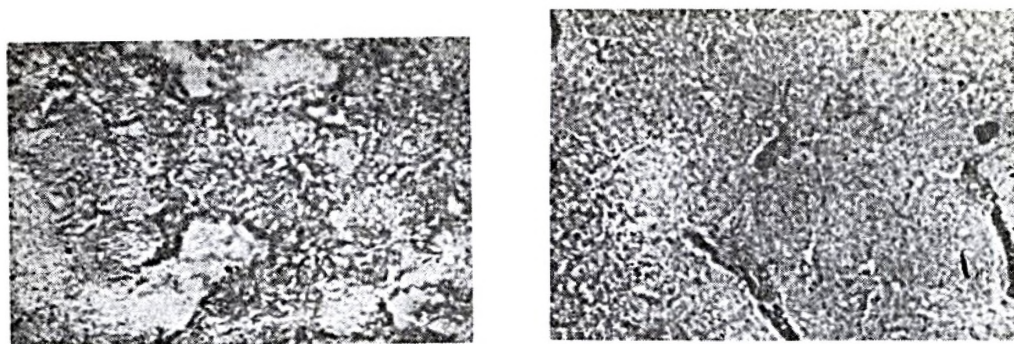


Fig. 1

The distribution of ChE in basal ganglia and cortex.

Localisation of AChE and BuChE in basal ganglia was quite different. AChE were in neuropil and BuChE were localised in the neuron cells and dendrites (Fig. 2, a and b). The intensity of tissue color of examined group comparison to control was not significantly different.



a)

b)

Fig. 2

a) AChE localisation in neuropil,

b) BuChE localisation in dendrites and neuron.

The enzyme activity was determined with Warburg respirometer. This results are in agreement with histochemical findings. The yielded activity ChE is higher, than BuChE (Table 3).

Table 3.

ACTIVITY AChE and BuChE IN BASAL GANGLIA

№ Sample	Substrates			
	AThCh		BuThCh	
	Experimental group in % of control	Control ml CO ₂	Experimental group in % of control	Control ml CO ₂
1	124,86	Average=354,66	106,33	Average=24,45
2	121,04		139,14	
3	117,47		98,15	
4	120,77		84,82	
5	116,17		97,90	
Average = 120,06		100%	Average = 105,44	100%

CONCLUSIONS

1. Mn^{++} — ions inhibit ChE activity in brain's homogenate of rats in vitro.
2. Corelation between decrease of ChE activity and concentration of Mn^{++} — ions could be established.
3. The discordance in results noted in paper as regarding ChE activity in the brain tissue points at possibility that there are probably in the brain some regions with high and other with low or without ChE activity. Therefore it would be necessary in animals treated with manganese to make detail mapping of ChE activity in brain.

AKTIVNOST HOLINESTERAZE U MOZGU I BAZALNIM GANGLIJAMA PACOVA TRETIRANIH MANGANHLORIDOM

KRATAK SADRŽAJ

Ispitivana je aktivnost ChE u homogenatu mozga i posebno u regijama bazalnih ganglija albino pacova tretiranih manganhloridom. Uporedo s ovim istraživanjem određivana je aktivnost ChE u homogenatu mozga normalnih životinja in vitro nakon dodavanja homogenatu $MnCl_2$ u više poznatih koncentracija. Utvrđena je smanjena aktivnost ChE, iako se u potpunosti nije mogla dokazati korelacija između stepena smanjenja aktivnosti ChE i dodanih koncentracija $MnCl_2$.

U histohemijskom istraživanju aktivnosti ChE u izolovanoj regiji bazalnih ganglija nisu se sa sigurnošću mogle dokazati značajne promjene. U toku su dalja istraživanja ChE u pojedinim bazalnim ganglijama.

REFERENCES

1. Hestrin, S. I.: The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and analytical application. *J. Biol. Chem.* 180: 249—261, 1949.
2. Karnovsky, M. J.: A »direct-coloring« thiocholine method for cholinesterases. *J. Histochem. and Cytochem.* 12: 219—221, 1964.
3. Koelle, G. B.: The elimination of enzymatic diffusion artefacts in the histochemical localisation of cholinesterases and a survey of their cellular distributions. *J. Pharmacol. Exp. Ther.* 103: 153—171, 1951.
4. Stanković, D., Grujić-Vasić, J., Pleho, A., Mirić, J.: Cholinesterase activity in brain plasma and erythrocytes *Folia Medica, Fac. Med. Univ. Sarajevo*, 4: 99—111, 1969.

DISCUSSION

Funcke: Have you seen any signs of tremor or other neurologic deficit in your manganese poisoned rats? For in monkeys manganese poisoning causes tremor.

Stanković: In our experiments in albino rats treated with $MiCl_2$ (21—30 days 15 mg/kg body weight by intraperitoneal injection) we could get rigidity without tremor. As we know, tremor with manganese was in experimental animal caused only in monkey *Macacus rhesus* (Mell, 1942, van Bogaert and Dallemagne, 1943, 1945).

Vojvodić: Do you have some evidence since you use Koelle's technic, about the relationship between functional and non-functional cholinesterase in your experimental conditions?

Pleho: 1) In experimental group expressed color which showed activity ChE by histochemical Koelle's procedure in comparison to control group was not evidently more intensive and because of it we used Warburg's quantitative method for determination of ChE activity. 2) We did not control restoration ChE activity after adding eserine. Eserine, as noted in literature, inactivated ChE activity in brain homogenate in high per cent and histochemical procedure inactivation is about 95%.