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THE ACTIVITY OF CHOLINESTERASES AND MONOAMINE OXIDASE IN SINGLE NERVE CELLS AND GROUPS OF GLIAL CELLS ISOLATED FROM BASAL GANGLIA

The study of cholinesterase (ChE) and monoamine oxidase (MAO) activity in nerve and glial cells of basal ganglia might contribute to the present knowledge on basal ganglia functions and diseases. The activity of the two enzymes was already measured in patients suffering from Parkinson's disease and some authors found no difference from normal values (1,8). On the other hand, Weber (10) reported that in postencephalitic parkinsonism the values of ChE activity in basal ganglia were extremely low. In all these experiments, the enzyme activity was measured in homogenates of single nuclei or even of larger regions of the brain so that their results must represent the mean enzyme activity of all constituents of tissue homogenates.

The microdiver technique offers a possible approach to the study of enzyme activity in a single nerve cell or group of glial cells. The aim of our work is to find out whether there is a difference in ChE or MAO activity of single nerve cells and groups of glial cells isolated from different basal ganglia of 1. normal brain and 2. pathological brain (experimental parkinsonism or Parkinson's disease). This report deals with the first part of the program.

The experiments were done with healthy adult rats. The nerve cells and groups of glial cells were isolated according to Hydén and Pigon (5) and their ChE and MAO activity measured with the microdiver technique (2, 7, 11).

Figure 1

THE MEAN HYDROLYSIS RATE OF 3.0 mM ACh PER SINGLE NERVE CELL OR GLIAL CLUMP OF THE RAT; μMOLES x 10^{-5} ± S. E. M. SAMPLE/HOUR; n = > 10

Region	Cell	Glia
Caudate nucleus	1.4 ± 0.11	1.6 ± 0.04
Putamen	1.6 ± 0.11	1.3 ± 0.10
Globus pallidus	2.5 ± 0.25	1.3 ± 0.94
Substantia nigra	2.1 ± 0.11	1.4 ± 0.12
Reticular formation	2.0 ± 0.06	1.6 ± 0.08

Figure 1 shows the hydrolysis of acetylcholine (ACh) in single nerve cells and glial clumps isolated from a single basal ganglion. The enzyme activity seems to be rather uniform in all nuclei tested in both nerve cells and glia, whereas in the nerve cells of globus pallidus and substantia nigra a higher hydrolysis rate was observed. A comparison of the ChE activity of basal ganglion nerve cells with that of the reticular formation of the brain stem reveals practically no difference. The high rate of hydrolysis of ACh in glial tissue is striking.

Figure 2

THE MEAN HYDROLYSIS RATE — μMOLES OF SUBSTRATE x 10^{-5} ± S. E. M. SAMPLE/HOUR

	Substrate (mM)		Caudate nucleus	Putamen	Globus pallidus	Substantia nigra	Reticular formation
Nerve cells	ACh	3.0	1.43	1.56	2.50	2.05	1.96
	BuCh	10.0	0.71	0.80	1.92	0.76	0.67
	MCh	3.0	0.16	0.89	0.89	1.38	1.38
Glial clumps	ACh	3.0	1.61	1.34	1.29	1.38	1.61
	BuCh	10.0	0.31	0.40	1.34	0.98	0.31
	MCh	3.0	0.80	0.67	1.29	1.20	0.13

Figure 2 shows hydrolysis rates of some choline esters. The glia of globus pallidus and substantia nigra as well as nerve cells from globus pallidus hydrolyze butyrylcholine at a higher rate than do the nerve cells and glia from other nuclei.

When compared with other nuclei globus pallidus is an exception also in respect to tyramine (Ty) oxidation (figure 3). Its nerve cells

Figure 3

THE OXIDATION RATE OF 10.0 mM Ty PER SINGLE NERVE CELL OR GLIAL CLUMP OF THE RAT; μMOLES x 10^{-5} ± S. E. M. SAMPLE/HOUR; n = 10

Region	Cell	Glia
Caudate nucleus	4.5 ± 0.53	1.5 ± 0.26
Globus pallidus	2.8 ± 0.20	1.4 ± 0.15
Substantia nigra	4.1 ± 0.52	1.5 ± 0.13
Reticular formation	4.8 ± 0.23	1.6 ± 0.16

oxidize Ty at lower rate than do the cells isolated from other basal or reticular nuclei where a relatively uniform rate was found. The oxidation rate of Ty in cells is 2—3 times higher than the hydrolysis rate of ACh. The oxidation of Ty by glial tissue is very slow but uniform in all tested nuclei.

Measuring the Ty oxidation in cells and glia isolated from one nucleus, one obtains a picture similar to that obtained with ACh hydro-

lysis (7): a wide variation in enzyme activity (figure 4). In glial samples the MAO activity is sometimes absent or below the sensitivity of the method.

Figure 4

NERVE CELLS AND GLIAL CLUMPS ISOLATED FROM THE CAUDATE NUCLEUS OF THE RAT. 10.0 mM Ty; μMOLES x 10^{-5} SAMPLE/HOUR

	Cell	Glia
	4.7	0.7
	4.2	0.6
	5.5	2.2
	3.4	1.7
	5.4	2.3
	2.1	0
	2.7	1.6
	4.4	2.2
	3.0	3.5
	2.7	0.7
	4.8	0.4
	4.1	0.5
	4.3	0
	2.6	1.6
	1.8	0
Mean ± S. E. M.:	4.5 ± 0.53	1.5 ± 0.26

Figure 5

ONE AND THE SAME RETICULAR PONTINE NERVE CELL, TWO SUBSTRATES IN SUCCESSION: 3.0 mM ACh AND 10.0mM Ty. THE ENZYME ACTIVITY IN μMOLES x 10^{-5} CELL/HOUR

Cell. No.	First determination: hydrolysis of ACh	Second determination: oxidation of Ty
1	3.4	2.1
2	4.2	5.7
3	2.2	8.9
4	3.4	3.9
5	1.7	1.7
6	2.4	3.0
7	2.9	2.4
Mean ± S. E. M.:	2.9 ± 0.58	4.0 ± 0.70

Cell No.	First determination: oxidation of Ty	Second determination: hydrolysis of ACh
1	7.2	3.6
2	6.8	4.9
3	5.9	1.6
4	4.2	5.8
5	3.6	2.6
6	3.8	3.6
7	4.2	2.5
Mean ± S. E. M.:	5.1 ± 0.44	3.5 ± 0.56

Those experiments in which the hydrolysis of ACh and the oxidation of Ty were measured in succession in the same nerve cell revealed that every nerve cell studied had the ability of inactivating either substrate (figure 5).

It has long been known that the basal ganglia constitute the brain region with the highest ChE activity (6, 3). It is possible that the considerable capacity of glial tissue of basal ganglia to hydrolyze choline esters (as seen in figure 2) substantially contributes to the high ChE activity obtained with the homogenates of this region.

The somewhat peculiar behaviour of ChE and MAO in globus pallidus as compared with that of the other basal ganglia can not be interpreted in the light of the existing information on function of this nucleus.

Consolo, Giacobini and Karjalainen (4) measured the oxidative deamination of Ty in single ganglion cells of the cat using a radioactive method and found 5.5×10^{-12} moles of deaminated Ty per cell per hour. This result is about 10 times lower than that yielded by our experiments; the fact however must not be lost sight of that they used a different technique, another species and another brain region. The above authors observed a wide variation in the results, a variation ranging from 1 to 40×10^{-12} moles of substrate (cell) hr. The former observation was made also in our experiments (figure 3).

The finding in the same nerve cell of two active enzymes which are involved in neurotransmission might be related to the observation of Salmoiraghi and Stefanis (9) who in microelectrophoretic experiments evoked a response in the same nerve cell with the injection of ACh or noradrenaline.

To summarize, the hydrolysis rate of ACh and the oxidation rate of Ty in single nerve cells and groups of glial cells isolated from basal ganglia of the rat were measured. The rate of activity of these enzymes in cells of different basal nuclei was found to be rather uniform. An exception was the globus pallidus where a higher rate of ACh hydrolysis and a lower one of Ty oxidation was obtained. Every investigated cell had the ability to hydrolyze ACh and oxidize Ty.

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RUDI PAVLIN

AKTIVNOST HOLINESTERAZA I MONOAMINOOKSIDAZE U POJEDINIM NERVNIM ĆELIJAMA I GLIOZNIM GRUDVICAMA IZOLOVANIM IZ BAZALNIH GANGLIJA

KRATAK SADRŽAJ

Poznavanje aktivnosti holinesteraza i monoaminooksidaze u pojedinim nervnim ćelijama izolovanim iz bazalnih ganglija doprinelo bi našem poznavanju mehanizama bolesti bazalnih ganglija. U pojedinim

nervnim ćelijama i glioznim grudvicama iz bazalnih ganglija mjerili smo hidrolizu nekih holinskih estera i oksidaciju tiramina. Veći deo merenja obavili smo na zdravim mozgovima štakora, a neke i na zdravom čovečjem mozgu. Upotrebljavali smo mikromanometričnu kartezijsku tehniku. Našli smo da je brzina hidrolize acetilholina u gliji bila skoro jednaka onoj u nervnim ćelijama, međutim oksidacija tiramina u gliji dostigla je otprilike jednu trećinu brzine u nervnim ćelijama. Nervne ćelije i glija iz globusa pallidusa i supstance nigre hidrolizirale su butirilholin brže od nervnih ćelija i glije izolovanih iz drugih jezgara. Eksperimenti u kojima smo merili hidrolizu acetilholina i oksidaciju tiramina naporedo u istoj nervnoj ćeliji pokazali su da je svaka nervna ćelija imala enzimske aktivnosti za hidrolizu acetilholina i za oksidaciju tiramina.

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Bruggencate: Dr Pavlin, could you give me the dimension of the orifice of the pipette whith which aspirrated you the cells? Furthermore, it is difficult to discern neurones from glial cells?

Pavlin: The dimension of the glass pipette is about 50 microns. To the second question: staining the tissue with methylene blue allows taking an easy difference betwen these two types of cells.