Distribution of GABA in *Aplysia* ganglia

Ganglion	Animal number A15	A16	A17	A18	A24	Mean ± SD
Abdominal	0.30	0.72	0.75	0.48	0.34	0.51 ± 0.21
L-pedal	2.66	1.47	1.29	2.41	0.94	1.75 ± 0.74
R-pedal	2.40	1.70	2.48	5.09	1.57	2.65 ± 1.42
L-pleural	1.46	1.52	1.49	1.73	1.11	1.46 ± 0.22
R-pleural	1.08	1.44	2.58	2.09	1.53	1.74 ± 0.59
L-buccal	2.03	5.33	4.01	3.25	3.04	3.53 ± 1.23
R-buccal	3.97	5.90	6.16	6.14	3.36	5.11 ± 1.37
Cervical	2.39	1.72	2.22	3.31	2.45	2.42 ± 0.58
Connective nerve	0.53	1.48	1.03	1.50	0.70	1.05 ± 0.44
Buccal muscle	–	–	–	–	–	0.04 ± 0.03*

GAGA contents are shown as mmoles/kg protein. *Buccal muscle was dissected out from 3 other animals.

15 °C and fed with dried sea weed (*Porphyra* sp.) for 1–4 weeks before the experiments. Ganglia were dissected from the animals without anaesthesia using fine ophthalmological scissors and transferred to a dish containing filtered sea water at 2–8 °C. The connective tissue and sheaths were removed with a special care not to damage their neurons. Each ganglion was homogenized with a glass homogenizer containing 50–200 µl of 0.01 N HCl at 4 °C. The homogenates were centrifuged at 1000 × g for 15 min at low temperature. Resulting supernatants were stored at −20 °C until the assay of GABA. To the precipitates 200–400 µl of 0.5 N NaOH was added and they were dissolved and kept −20 °C for the protein determination.

An aliquot (3.8 µl) of the supernatants was transferred to the oil well[12] making small droplets and heated at 60 °C for 10 min to destroy NADPH in the extracts. After heating 4 µl of GABA assay reaction mixture containing 0.2 M tris-HCl buffer (pH 8.9), 10 mM α-ketoglutarate, 0.5 mM NADP, 0.01% β-mercaptoethanol and 0.2 mg protein/ml of bacterial enzymes (GABA-transaminase and succinic semialdehyde dehydrogenase) was added to the droplets and they were incubated at 30 °C for 30 min. After the addition of 0.9 µl of 1.0 N NaOH, the droplets were heated at 60 °C for 20 min and the mixtures were transferred to 45 µl of cycling reagent in 1 ml tubes. The detail of NADPH cycling procedure is described elsewhere[14]. The protein content of ganglia was determined by the method of Lowry et al.[15].

8 distinctive ganglia (abdominal, left (L)- and right (R)-pedal, L- and R-pleural, L- and R-buccal and cervical), connective nerve and buccal muscle were dissected out. The GABA content in each preparation is shown as mmoles/kg protein in the table. GABA was detected in all ganglia and connective nerve. Abdominal ganglion contained the lowest level of GABA among the ganglia examined. The highest level of GABA was found in buccal ganglia, and a fairly high concentration of GABA was also observed in cervical and pedal ganglia.

Concerning the putative transmitters in *Aplysia*, many physiological and pharmacological investigation used the abdominal ganglion and acetylcholine has been found to have both an excitatory and inhibitory action on the postsynaptic membranes[3,16]. In the present investigation, lower concentration of GABA was found in the abdominal ganglion, whereas it was relatively concentrated in the buccal ganglia. In the *Aplysia* ganglia, the level of GABA was 0.3 to 6.2 mmoles/kg protein. If the protein content of the ganglia is assumed to be about $^1/_{10}$ of their wet weight as in other tissues, the values reported here correspond to 0.03–0.62 mmoles/kg wet weight. This value seems to be comparable to the GABA content in mammalian CNS[17]. However, in mammalian spinal cord which contains low concentration of GABA, Miyata et al. found relatively high concentration in the dorsal part of dorsal horn[18]. Although the concentration of GABA in the ganglia is not as high as in the mammalian CNS, GABA can be distributed in the specifically localized areas or neurons of the *Aplysia* ganglia as observed in the lobster ganglion[19].

It should be noted that a relatively high amount of GABA was found in the buccal ganglia. Considering the motor behavior of *Aplysia*, buccal movement seems to be the most striking. It might be worthwhile to study systematically the function of GABA in the *Aplysia* nervous system using the buccal ganglia. Further electrophysiological and pharmacological studies are needed to give light on the role of GABA in the ganglia.

14 Y. Okada, Brain Res. *75*, 362 (1974).
15 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
16 E. R. Kandel and H. Wachtel, in: Physiological and Biochemical Aspects of Nervous Integration, p. 7. Ed. F. D. Carson. Prentice-Hall, New Jersey 1967.
17 Y. Okada, C. Nitsch-Hassler, J. S. Kim, I. J. Bak and R. Hassler, Expl Brain Res. *13*, 514 (1971).
18 Y. Miyata and M. Otsuka, J. Neurochem. *19*, 1833 (1972).
19 M. Otsuka, E. A. Kravitz and D. P. Potter, J. Neurophysiol. *30*, 725 (1967).

The levels of metallothionein-like proteins in animal tissues[1]

A. J. Zelazowski and J. K. Piotrowski

Department of Toxicol. Chemistry, Institute of Environmental Research and Bioanalysis, Medical Academy, Narutowicza 120a, 90-145 Lódz (Poland), 4 April 1977

Summary. The level of metallothionein-like proteins was determined in different tissues of 6 animal species. The highest concentrations were found in pig and rat tissues. The organs richest in metallothionein-like proteins included: kidneys (101–305 µg/g), intestine (127–257 µg/g) and liver (54–496 µg/g).

Metallothionein and related proteins play a regulatory role in uptake and metabolism of zinc and copper and are of considerable importance in binding heavy metals such as cadmium, mercury and bismuth in animal organs[2]. Estimations of the physiological levels of metallothionein yielded values of 100–260 µg/g for the rat liver and 180–700 µg/g for the rat kidneys[3–8] when using the radiochemical method of estimation of metallothionein[9] or its more specific, modified version[8]. Chen and Ganther[10] obtained values of 42 µg/g and 87 µg/g for the liver and kidneys, respectively, using a method based on molecular filtration with the ^{109}Cd label. Metallothionein levels in

Physiological levels of MTP in tissues of several animal species

Organ	Species Pig	Rat	Cow	Mice	Guinea-pig	Rabbit
Kidneys	305 ± 104	207 ± 35	101 ± 26	104 ± 25	188 ± 15	193 ± 34
Intestines	127 ± 40	205 ± 47	257 ± 117	217 ± 26	178 ± 88	133 ± 39
Liver	496 ± 150	106 ± 14	169 ± 60	104 ± 28	54 ± 2	54 ± 22
Spleen	89 ± 21	204 ± 80	97 ± 29	125 ± 38	106 ± 22	188 ± 47
Lung	105 ± 11	171 ± 19	105 ± 27	68 ± 39	82 ± 17	171 ± 55
Brain	108 ± 11	92 ± 33	92 ± 7	89 ± 12	76 ± 12	70 ± 12
Heart	32 ± 7	97 ± 40	36 ± 11	68 ± 4	59 ± 8	59 ± 14
Muscles	12 ± 6	16 ± 6	20 ± 9	14 ± 4	16 ± 1	7 ± 2

μg MTP/g tissue, wet weight; means ± SD, 5–6 samples in each group.

other rat tissues and in tissues of other animal species have not hitherto been determined. Multiple exposure to some metals (Cd, Zn, Cu, Hg, Bi) resulted in an increase in the level of metallothionein in the liver and kidneys by a factor of several up to several orders of magnitudes[4–6, 11, 12]. In this paper, the levels of metallothionen-like proteins in different organs of several species are reported.

Material and methods. Rats, weighing 200–250 g, and mice, weighing 30–40 g, were derived from own stock, rabbits, weighing 3–4 kg, and guinea-pigs, weighing 400–600 g, were purchased from a breeder. Prior to the experiment the animals were kept under standard conditions; the intake of cadmium from the food and environment was not controlled. Hog and ox tissues were obtained from a slaughter house. Estimations of metallothionen were performed by the radiochemical method with the ^{203}Hg label. Specificity of the method was achieved by precipitation of metallothionen-like proteins from the TCA-supernatant using tannic acid[8]. ^{203}Hg of specific activity of about 5,000,000 cpm/mg Hg was obtained from the Institute of Nuclear Research, Świerk (Poland). Activity was measured in an USB counter. Horse kidney metallothionein was used as a standard[13]. The preparation (mol. wt of 10,300) contained 3.2 μmole SH groups per mg protein, as determined by amperometric titration[14]. Under the applied analytical conditions, 1 mg protein bound 210 μg Hg. All the reagents used were of analytical grade.

Results and discussion. Concentration of metallothionein-like proteins in tissues of different animal species are reported in the table. Among the animal species studied, the highest levels of metallothionein-like proteins were found in the tissues of pig, and, among laboratory animals, in the tissues of rat. The highest concentrations, of these proteins were found in the kidneys, intestine and liver, and in some species the spleen and lungs were also rich in these proteins.

Absolute levels of metallothionein-like proteins may exhibit considerable variation resulting from the dependence on the uptake of heavy metals, especially cadmium[7]. Physiological role of metallothionein contained in the liver and intestine may consist in its regulatory function with respect to the uptake of zinc and copper[15, 16]. Metallothionein was discovered firstly in the horse kidney[17]. According to our present understanding, metallothionein-like proteins contained in that organ are responsible for the cumulation of cadmium and are also of primary importance in binding of mercury and bismuth[18, 19]. The role of metallothionein-like proteins in other tissues has not been studied in detail hitherto. Binding of inorganic mercury by these proteins in the brain has been observed by Sapota et al.[3]. The presence of metallothionein-like proteins in tissues not studied previously in this respect, reported in the present study, require additional identification studies with would verify the identity or similarity of the proteins estimated by the radiochemical method with metallothionein, as was done for analogous proteins in the kidneys and liver.

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2 J. K. Piotrowski and E. M. Mogilnicka, Post. Biochem. *22*, 401 (1976).
3 A. Sapota, J. K. Piotrowski and B. Barański, Med. Pracy *25*, 129 (1974).
4 B. Trojanowska and J. K. Piotrowski, Bromat. Chem. Toksyk. *7*, 137 (1974).
5 J. K. Piotrowski and J. A. Szymańska, J. Toxic. Envir. Hlth *1*, 991 (1976).
6 E. M. Mogilnicka, J. K. Piotrowski and B. Trojanowska, Med. Pracy *26*, 147 (1975).
7 W. Kaszper, J. K. Piotrowski, W. Marciniak and M. Sielczyńska, Bromat. Chem. Toksyk. *9*, 515 (1976).
8 A. J. Żelazowski and J. K. Piotrowski, Acta biochim. pol. *24*, 97 (1977).
9 J. K. Piotrowski, W. Bolanowska and A. Sapota, Acta biochim. pol. *20*, 207 (1973).
10 R. W. Chen and H. E. Ganther, Envir. Physiol. Biochem. *5*, 378 (1975).
11 J. K. Piotrowski, B. Trojanowska and A. Sapota, Archs Tox. *32*, 351 (1974).
12 E. M. Mogilnicka, J. K. Piotrowski and A. Tomaszewski, Bromat. Chem. Toksyk. *9*, 357 (1976).
13 A. J. Żelazowski, J. K. Piotrowski, E. M. Mogilnicka, J. A. Szymańska and W. Kaszper, Bormat. Chem. Toksyk., in press.
14 R. E. Benesch, H. A. Lardy and R. Benesch, J. biol. Chem. *216*, 663 (1955).
15 G. W. Evans, Physiol. Rev. *53*, 535 (1973).
16 M. Richards and R. J. Cousins, Biochem. biophys. Res. Commun. *64*, 1215 (1975).
17 J. H. R. Kägi and B. Vallee, J. biol. Chem. *235*, 3460 (1960).
18 J. K. Piotrowski, B. Trojanoska, J. M. Wieśniewska-Knypl and W. Bolanowska, Toxicol. appl. Pharmac. *27*, 11 (1974).
19 J. A. Szymańska, E. M. Mogilnicka and B. W. Kaszper, Biochem. Pharmac. *26*, 257 (1977).