Gamma-Aminobutyric Acid in Purkinje Cells and Motoneurones

Recent electrophysiological studies demonstrated that the cerebellar Purkinje cells make inhibitory synaptic contact with Deiters neurones[1] and the intracerebellar nuclei neurones[2]. It has also been shown that the effect of gamma-aminobutyric acid (GABA) applied iontophoretically to Deiters neurones mimics the inhibitory action by Purkinje cell axons[3], and that during activation of Purkinje cells GABA is released into the cat's fourth ventricle at a rate about 3 times as high as that on the background[4]. In Crustacea, GABA concentration is much higher both in the inhibitory nerve fibres[5] and in their somas[6] than that in the excitatory neurones. It is, therefore, interesting to see whether there is such a difference between inhibitory and excitatory neurones in mammalian central nervous system. In fact, GABA content of the layer containing Purkinje cells is higher than that of molecular and granular layers of the cerebellum[7]. In the present study, enzymic assay of GABA was performed on isolated Purkinje cells and spinal motoneurones. The concentration of GABA in the former was much higher than that in the latter.

The cerebellum and lumbar segment of the spinal cord were removed from cats under anaesthesia with pentobarbitone sodium (30 mg/kg, i.p.). Rats were decapitated and then specimens were obtained. Thin slices were prepared with a razor blade from the tissues and their cut surface was stained faintly with 0.05% methylene blue. Under a dissecting microscope ($\times$ 60) individual cells were isolated by free-hand dissection with the tip of a glass capillary which was made by the same method as used for fabricating intracellular microelectrode. The neurones thus picked up were pooled on a tiny piece of plastic and then transferred to 5 µl of 0.1 *N* HCl for extraction. Tissues from 1 cat and 2 or 3 rats were used, fresh slices being dissected 2 to 4 times to collect sufficient number (500–1000) of the cells as one sample. In order to minimize the postmortem change of GABA content, the cell isolation was performed at 5–10 °C within 90 min after the tissue was deprived of its blood supply. GABA was measured enzymatically[8], the assay procedures being similar to those used for the crustacean nerves[5]. Total incubation volume was 15 µl and GABA standards (0.2 to 10×10^{-10} moles) were run with each assay. For correction of tissue blank levels of fluorescence, a parallel assay was run in the presence of aminooxyacetic acid. The fluorescence was measured in a Turner fluorometer, Type 110. GABA contents per single cells thus obtained are shown in the second column of the Table.

GABA content of isolated neurones

	Volume of single cell ($\times 10^{-12}$ l)	GABA/cell ($\times 10^{-14}$ moles)	GABA/volume[a] (m*M*)
Purkinje cells, Cat	13.4 ± 0.8	7.7 ± 2.1 (3)	5.8 ± 1.5[b] (3)
Rat	5.8 ± 0.3	4.8 ± 1.0 (4)	8.3 ± 1.8 (4)
Motoneurones, Cat	100 ± 11	15.2 ± 5.3 (5)	1.5 ± 0.5 (5)

Values are means ± standard errors of means; number of the experiments indicated in parentheses. [a] Calculated from GABA content divided by the average cell volume. No allowance for variation of the cell volume was made on the calculation. [b] $P < 0.02$ when compared with cat motoneurones.

In view of the large difference in cell sizes between Purkinje cells and motoneurones, it seems appropriate to compare the GABA concentration per unit cell volume. The volume of a cell soma is best approximated by the equation $1.04 \times {}^1/_6 \pi ab\sqrt{ab}$, where a is the major and b the minor axis of the soma passing through the nucleolus[9]. In the present study, the major and minor axes were measured on frozen section of unfixed tissues. From their average values obtained in 100 Purkinje cells and spinal motoneurones, the cell volume was estimated with the above equation (the first column in the Table). GABA concentration in the third column was thus calculated from the content per single cell and cell volume. It is seen that the GABA concentration in Purkinje cells of cats is 4 times higher than that in spinal motoneurones, the difference being statistically significant.

The present result is consistent with the hypothesis that the inhibitory transmitter liberated by Purkinje cells is GABA[3]. However, it must be considered that the isolated cell preparation in this study includes the presynaptic nerve terminals on the soma surface. Actually, Purkinje cells are enclosed by a dense terminal net of basket cell axons which are also inhibitory in nature[10]. A possibility thus remains that GABA in Purkinje cells arises from axon terminals of basket cells but not from the cytoplasm of Purkinje cells. Further investigation is needed to discriminate the presynaptic and postsynaptic GABA. On GABA content of several types of mammalian isolated neurones, further studies are now in progress with improved assay procedures[11,12].

Zusammenfassung. Enzymatische Messungen von Gamma-Aminobuttersäure (GABA) ergaben in den isolierten Purkinje-Zellen der Katze eine viermal höhere GABA-Konzentration als in den Motoneuronen.

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[1] M. Ito and M. Yoshida, Expl. Brain Res. *2*, 330 (1966).
[2] M. Ito, M. Yoshida, K. Obata, N. Kawai and M. Udo, Expl. Brain Res., in press (1969).
[3] K. Obata, M. Ito, R. Ochi and N. Sato, Expl. Brain Res. *4*, 43 (1967).
[4] K. Obata and K. Takeda, J. Neurochem. *16*, 1043 (1969).
[5] E. A. Kravitz, S. W. Kuffler and D. D. Potter, J. Neurophysiol. *26*, 739 (1963).
[6] M. Otsuka, E. A. Kravitz and D. D. Potter, J. Neurophysiol. *30*, 725 (1967).
[7] K. Kuriyama, B. Haber, B. Sisken and E. Roberts, Proc. natn. Acad. Sci. USA *55*, 846 (1966).
[8] W. B. Jakoby and E. M. Scott, J. biol. Chem. *234*, 937 (1959).
[9] J. P. Schadé, in *Organization of the Spinal Neurones* (Ed. J. C. Eccles and J. P. Schadé; Elsevier, Amsterdam 1964), p. 261.
[10] P. Andersen, J. C. Eccles and P. E. Voorhoeve, J. Neurophysiol. *27*, 1138 (1964).
[11] K. Obata, M. Otsuka and Y. Tanaka, J. Neurochem., in press (1970).
[12] This work was supported in part by Grant No. NB-07440 from U.S. Public Health Service. The author thanks Dr. S. Ishikawa and Professor S. Ebashi for advice on assay procedure and Dr. M. Ito and Professor M. Otsuka for encouragement and discussion.