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MEASUREMENT OF γ-AMINOBUTYRIC ACID IN ISOLATED NERVE CELLS OF CAT CENTRAL NERVOUS SYSTEM

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Abstract—A sensitive method for measuring γ-aminobutyric acid (GABA) has been developed. This method consists of a combination of the enzymic GABA assay of JAKOBY and SCOTT (1959) with the enzymic cycling technique of LOWRY, PASSONNEAU, SCHULZ and ROCK (1961) and permits the measurement of as little as 2×10^{-14} mol of GABA. Using this method, GABA analyses were made on single isolated nerve cell bodies of different types from the CNS of the cat. Average GABA concentrations in these cell bodies were: spinal ganglion cells, 0·2 mM; spinal mononeurons, 0·9 mM; large cells of the ventral part of Deiters' nucleus, 2·7 mM; large cells of the dorsal part of Deiters' nucleus, 6·3 mM; cerebellar nuclei cells, 6·0 mM; cerebellar Purkinje cells, 6·6 mM; cerebral Betz cells, 2·5 mM.

The GABA concentrations in the isolated dorsal Deiters' cells were greatly reduced (1·7 mM) after the removal of the cerebellar vermis while those of the ventral Deiters' cells were unaffected by the denervation. These results suggest that GABA is concentrated within axon terminals, probably of Purkinje neurons, synapsing with the dorsal Deiters' cells. The results of GABA analyses on isolated nerve cells are discussed in relation to the relevant neuronal functions and the possible role of GABA as an inhibitory transmitter.

γ-AMINOBUTYRIC acid (GABA) has been shown to be an inhibitory transmitter in the crustacean nervous system (KRAVITZ, KUFFLER and POTTER, 1963; TAKEUCHI and TAKEUCHI, 1965; OTSUKA, IVERSEN, HALL and KRAVITZ, 1966). Inhibitory neurons of the lobster contain GABA in much higher concentrations than the excitatory neurons (KRAVITZ *et al.*, 1963; OTSUKA, KRAVITZ and POTTER, 1967). Conversely, if a neuron with an unknown function is shown to contain GABA in a high concentration, this suggests that the neuron is an inhibitory one with GABA as the transmitter. This suggestion has so far been confirmed in the chemical studies of single neurons of the lobster (KRAVITZ and POTTER, 1965; OTSUKA *et al.*, 1967).

In the mammalian CNS it is also probable that GABA serves as a neuronal index with regard to the transmitter and function. There is an accumulating evidence which suggests that GABA functions as an inhibitory transmitter in the mammalian CNS (OBATA, ITO, OCHI and SATO, 1967; OBATA and TAKEDA, 1969; KRNJEVIĆ and SCHWARTZ, 1967). Here, however, the analysis of the GABA in single neurons is limited by the sensitivity of chemical assay. Since the sizes of mammalian neurons are in general smaller than those of Crustacea, it is to be expected that nerve cells of the mammalian CNS will contain much smaller amounts of GABA than the large crustacean neurons analysed in previous work. In the present study, we have, therefore, developed a method for microdetermination of GABA which, by combining the enzymic GABA analysis (JAKOBY and SCOTT, 1959) and the enzymic cycling method (LOWRY *et al.*, 1961), permits the measurement of as little as 2×10^{-14} mol of material, and using this method GABA analyses were made on several types of isolated nerve cells of cat CNS. Preliminary reports of this work have been published (OBATA, OTSUKA and TANAKA, 1970; MIYATA, OBATA, TANAKA and OTSUKA, 1970).

METHODS

Macro-analysis of GABA in tissue slices

Spinal cords and brain slices were removed from cats anaesthetized with pentobarbitone sodium. From these slices selected areas were dissected out with the aid of a dissecting microscope. For the dissection of vestibular and cerebellar nuclei, slices were stained with 0·1% methylene blue–Locke solution, but other tissues were dissected out without staining. After weighing the tissues, 1 ml of 0·1 N-HCl was added per 4–25 mg of fresh tissue. The above procedures were performed at room temperature (about 23°C) and the time taken from removing the tissues from the animal until they were placed in 0·1 N-HCl was less than 30 min, usually 4–10 min. Preliminary experiments showed that the post mortem increase in GABA concentration of the nervous tissues was about 20 per cent in 30 min (cf. ELLIOTT, TARIQ KAHN, BILODEAU and LOVELL, 1965). Tissues in 0·1 N-HCl were homogenized and the GABA contents of the extracts were measured by enzymic assay (HIRSCH and ROBINS, 1962; KRAVITZ *et al.*, 1963). When the tissues were stained with methylene blue, the dye slightly inhibited the NADPH production by GABA conversion. Therefore, the values of GABA contents obtained were corrected using standard curves obtained in the presence of methylene blue.

Micro-analysis of GABA by the enzymic cycling method

Biochemicals. GABA–glutamate transaminase and succinate semialdehyde dehydrogenase were extracted from *Pseudomonas fluorescens* ATCC-13430 following the method of SCOTT and JAKOBY (1959) through the first part of step 4. The specificity of the enzyme preparation was checked for glycine, β-alanine, DL-β-hydroxy-γ-aminobutyric acid, L-ornithine, L-lysine, L-glutamic acid and L-glutamine. In the presence of the enzyme preparation, these compounds did not produce any detectable amount of NADPH under the condition used in the present study (see below). Glucose-6-P dehydrogenase, glutamate dehydrogenase and 6-P-gluconate dehydrogenase were obtained from Boehringer and Sons, Mannheim, Germany. Sulphate was removed by centrifugation from the suspensions of glucose-6-P dehydrogenase and glutamate dehydrogenase before they were added to the cycling reagent.

Constriction pipettes. Constriction pipettes of 0·1 to 0·2 μl (Fig. 1A) were constructed from commercially available 1 μl Lang–Levy pipettes. The pipettes were calibrated as described by LOWRY, ROBERTS, LEINER, WU and FARR (1954).

Principle of method. GABA–glutamate transaminase and succinate semialdehyde dehydrogenase carry out the following successive reactions (JAKOBY and SCOTT, 1959):

(1) GABA + α-ketoglutarate $\rightarrow$ succinate semialdehyde + glutamate

(2) succinate semialdehyde + $NADP^+$ + H_2O $\rightarrow$ succinate + NADPH + H^+.

Excess $NADP^+$ is destroyed by heating in weak NaOH. NADPH then catalyses the following cycling reactions in the presence of glutamate dehydrogenase and glucose-6-P dehydrogenase (LOWRY *et al.*, 1961):

(3) NADPH + α-ketoglutarate + NH_4^+ $\rightarrow$ $NADP^+$ + glutamate

(4) $NADP^+$ + glucose-6-P $\rightarrow$ NADPH + 6-P-gluconate + H^+.

The formed 6-P-gluconate reduces $NADP^+$ under the action of 6-P-gluconate dehydrogenase:

(5) 6-P-gluconate + $NADP^+$ $\rightarrow$ ribose-5-P + NADPH + H^+ + CO_2.

The native fluorescence of NADPH is then measured.

Analytical procedure. A 0·13 μl volume of reaction mixture containing 0·22 M-tris–HCl buffer (pH 8·0), 2·2 mM-α-ketoglutarate, 30 μM-$NADP^+$, 8 mM-β-mercaptoethanol, 0–6 μM-GABA and 0·2–0·3 mg protein/ml bacterial enzymes (GABA–glutamate transminase and succinate semialdehyde dehydrogenase) was placed in a 0·1 ml tube, whose lower part of about 5 μl in volume was separated from the upper part by a stopper as shown in Fig. 1B. This type of stopper was simple and quite efficient in preventing evaporation. The reaction mixture was incubated at 38°C for 30 min, and then 1 μl of 0·17 N-NaOH was added. Tubes were again capped as shown in Fig. 1C and heated to 60°C for 15 min.

To each sample in a 0·1 ml tube in ice was added 20 μl of the cycling reagent containing 0·2 M-tris–HCl buffer (pH 7·8), 1 mM-glucose-6-P, 10 mM-α-ketoglutarate, 0·03 M-ammonium acetate, 0·5 mg/ml bovine plasma albumin, 0·2 mM-EDTA, 0·2 mM-ADP, 0·3 mg/ml glutamate dehydrogenase and 0·06 mg/ml glucose-6-P dehydrogenase. The tubes were capped with Parafilm and incubated at 38°C in a water bath for 90 min, and thereafter heated to 100°C for 5 min. Each sample was transferred quantitatively to a 1 ml fluorometer tube containing 1 ml of 0·02 M-tris–HCl buffer (pH 8·0), 0·02 mM-$NADP^+$ and 0·1 mM-EDTA. The fluorescence of each sample was read in Farrand fluorometer, Model A-3, with a Corning 5860 primary filter (365 nm) and a Farrand interference filter (460 nm) as the secondary filter. To each tube, 5 μl of 6-P-gluconate dehydrogenase suspension (2 mg/ml in 2·8 M-ammonium sulphate) was added and, after 30 min at room temperature, the fluorescence of each sample was again measured. The NADPH produced was determined by the difference between the reading of fluorescence before adding 6-P-gluconate dehydrogenase and that 30 min after

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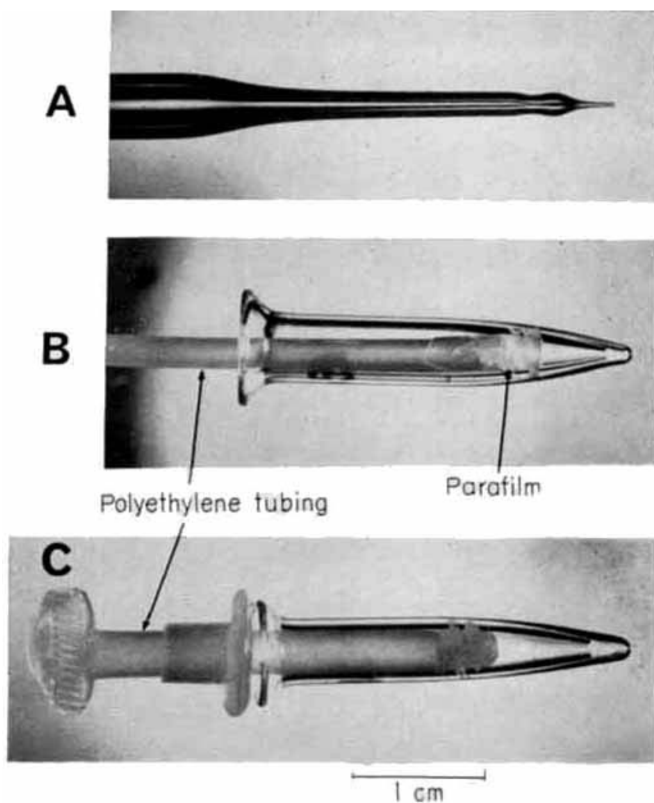

FIG. 1.—Constriction pipette of 0·13 μl (A) and stoppers for microtubes (B and C). Microtubes contained 0·13 μl (B) and 1 μl (C) of water. For the use of these stoppers see text.

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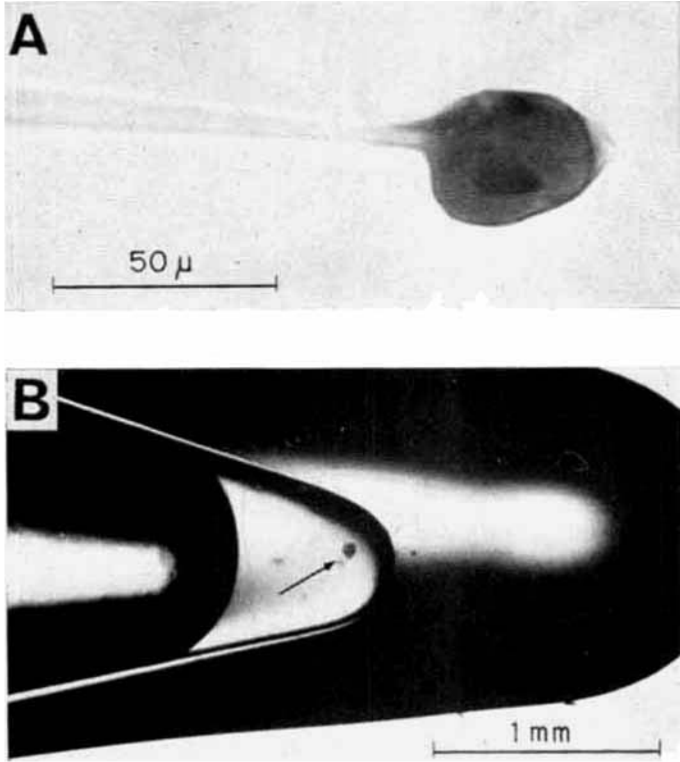

FIG. 3.—A: single Deiters' cell body attached to the tip of a dissecting glass needle and placed in xylene. B: a single Deiters' cell (arrow) was transferred into 0·13 µl of 0·1 N-HCl at the bottom of a microtube.

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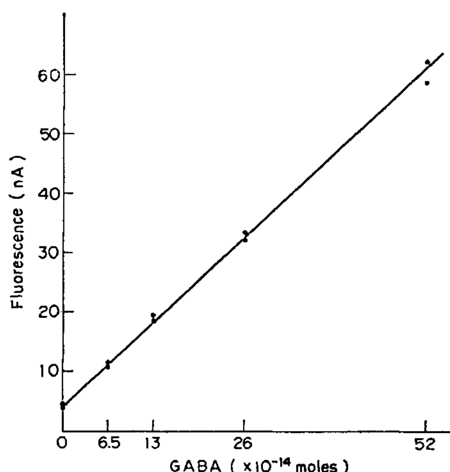

FIG. 2.—Standard curve for GABA obtained by enzymic cycling method. Ordinate records the difference between the fluorescence reading before adding 6-P-gluconate dehydrogenase and the reading 30 min after the addition of the enzyme. For details see text. Fluorescence units are galvanometer readings of the fluorometer and duplicates are recorded individually.

addition of the enzyme. An example of the standard curve is shown in Fig. 2. Reproducible measurements were obtained with samples ranging from 2–60 $\times$ 10^{-14} mol. Overall amplification, i.e., the molar ratio of added GABA to 6-P-gluconate produced, was 5000–10,000.

GABA analyses of isolated nerve cell bodies

Tissue slices were stained with 0·1% brilliant blue 6B-Locke solution. For staining nerve cells, methylene blue is more satisfactory than brilliant blue, but methylene blue was unsuitable for the present purpose because the dye prevented the formation of NADPH by GABA conversion in the reaction mixture. Types of nerve cells were identified by their characteristic size and location. Deiters' nucleus was located according to the atlas of BERMAN (1968) with the aid of a stereotaxically placed needle. Under a dissecting microscope, a single nerve cell body was lifted out from the surface of the slice with a hand-held glass needle with a tip diameter of about 1 μm (HYDÉN, 1959; OBATA, 1969). The isolated nerve cell attached to the tip of a glass needle was soaked in xylene (Fig. 3A) and examined under a compound microscope ($\times$200). When the cell was markedly contaminated by the surrounding tissues, it was discarded. The isolated cell body was photographed in xylene and its volume was calculated assuming the cell to be a prolate spheroid. The obtained value was used for the estimation of GABA concentration. Xylene was used during the microscopic observation because GABA is virtually insoluble in it (OTSUKA *et al.*, 1967). The shrinkage of isolated cells in xylene was found to be negligible. The isolated cells were placed in a desiccator and dried *in vacuo*. The collection of cells, usually 10–20 at a time, was performed at room temperature, and it usually took less than 30 min from the time the tissue was removed from the animal until the isolated cells were placed *in vacuo*. In some experiments on Deiters' cells, parts of brain stem, after being removed from the cat, were kept in a cold room (3°C) for less than 2 hr before collection of the cells was started. Preliminary experiments showed that keeping the brain in a cold room did not significantly influence the values of GABA concentration in tissue slices or in isolated nerve cells.

A glass needle with a dried cell at the tip was held in a micromanipulator and under a dissecting microscope the dried cell was transferred into 0·13 μl of 0·1 N-HCl at the bottom of a 0·1 ml microtube (Fig. 3B; LOWRY, ROBERTS and CHANG, 1956). To each microtube with 0·13 μl of 0·1 N-HCl, 1–5 cells were taken, and the sample was kept in a desiccator at 3°C to remove water and HCl. Just before assay, samples were further treated *in vacuo* for 10 min. To each sample, 0·13 μl of enzyme reagent containing GABA-glutamate transaminase and succinate semialdehyde dehydrogenase was added. Samples were incubated at 38°C and the assay was completed as described in the previous section. Blanks and GABA standards were run at the same time. A 0·13 μl volume of 0·1 N-HCl used for the extraction occasionally caused a slight increase in the blank value (corresponding to 0·5–1·0 $\times$ 10^{-14} mol of GABA). In such a case, the obtained values of GABA analysis were corrected. The amount of tissue contained in 0·13 μl of reaction mixture with the bacterial enzymes was 3–500 $\times$ 10^{-12} l. (see Tables 2 and 3). Preliminary experiments showed that brain tissue in an amount of

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500 × 10^{-12} l. per 0·13 μl of the reaction mixture gives a negligible tissue blank reading (amino-oxyacetic acid was added to the enzyme reagent in a concentration of 5 mM) and that the internal standard is 97 per cent in the presence of the tissue.

RESULTS

GABA concentrations in different regions of CNS. In order to compare the values of GABA concentration in isolated nerve cells with those in their background, average GABA concentrations of tissues in which the nerve cells were located, were assayed and the results are shown in Table 1. GABA concentrations in all tissues except spinal ganglia ranged between 1 and 2 μmol/g of fresh tissue. The GABA concentration in spinal ventral grey matter is close to the value obtained by GRAHAM, SHANK, WERMAN and APRISON (1967) in the cat. The values for cerebral and cerebellar cortices as well as cerebellar nuclei are lower than those obtained by LOVELL, ELLIOTT and ELLIOTT (1963) in beef brain.

TABLE 1.—GABA CONTENT IN REGIONS OF CAT CNS

Region of CNS	GABA (μmol/g wet wt.)
Spinal ganglia (L_6–S_1)	less than 0·06(2)
Ventral grey matter of spinal cord (L_7–S_1)	1·0 ± 0·1(6)
Vestibular nuclei	1·8 ± 0·2(3)
Cerebellar nuclei (lateral, interpositus and medial nuclei)	1·8 ± 0·2(5)
Cerebellar cortex	1·0 ± 0·1(4)
Cerebral cortex (anterior and posterior sigmoid gyri)	1·4 ± 0·1(5)

Figures represent the mean values ±S.E.M. The figures in brackets indicate the number of determinations.

GABA concentrations in isolated nerve cell bodies. Table 2 shows the results of GABA analyses on samples, each consisting of 3–5 isolated nerve cell bodies of the same type. Spinal ganglion cells and spinal motoneurons contained low concentrations of GABA while cerebral Betz cells contained intermediate and cerebellar Purkinje cells and cerebellar nuclei cells contained high concentrations of GABA. Table 3 shows the results of GABA analyses on single isolated nerve cell bodies. All 10 spinal motoneurons contained low concentrations of GABA (0–1·5 mM). Large cells from the dorsal part of Deiters' nucleus, in contrast, showed rather high concentrations of GABA, while large cells from the ventral part of the nucleus showed intermediate levels. In agreement with the results on pooled cells given in Table 2, all Betz cells contained intermediate concentrations of GABA, and many Purkinje cells and cerebellar nuclei cells showed quite high levels. The reliability of GABA

TABLE 2.—GABA ANALYSIS ON POOLED NERVE CELL BODIES*

Type of nerve cells	GABA content (10^{-14} mol)	Volume (10^{-12} l.)	GABA concentration (mM)
Spinal ganglion cells	1·2 ± 0·1	83·5 ± 40·1	0·2 ± 0·1(2)
Spinal motoneurons	2·4 ± 0·5	24·4 ± 2·9	0·9 ± 0·1(6)
Cerebellar nuclei cells	6·0 ± 0·8	8·1 ± 1·8	8·0 ± 1·1(4)
Purkinje cells	2·1 ± 0·3	4·1 ± 0·1	5·5 ± 1·1(5)
Betz cells	1·6 ± 0·2	8·0 ± 0·4	2·0 ± 0·2(3)

* GABA analyses were made on samples, each consisting of 3–5 isolated nerve cell bodies. Each type of cell was collected from the regions of CNS given in Table 1.
Each value is recorded for an isolated cell, and represents the mean ±S.E.M. The figures in brackets indicate the number of determinations.

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TABLE 3.—GABA ANALYSIS ON SINGLE ISOLATED NERVE CELL BODIES

	Spinal motoneurons			Ventral Deiters' cells			Dorsal Deiters' cells		
	GABA content (10^{-14} mol)	Volume (10^{-12} l.)	GABA concentration (mM)	GABA content (10^{-14} mol)	Volume (10^{-12} l.)	GABA concentration (mM)	GABA content (10^{-14} mol)	Volume (10^{-12} l.)	GABA concentration (mM)
	0	32·4	0	15·2	42·4	3·6	45·2	60·6	7·5
	1·1	38·4	0·3	18·1	51·7	3·5	16·7	24·4	6·9
	1·6	16·3	1·0	5·8	22·2	2·6	45·7	67·8	6·7
	4·8	33·6	1·4	6·4	34·8	1·8	63·5	60·8	10·4
	3·6	24·6	1·5	12·6	38·4	3·3	7·6	22·2	3·4
	6·8	50·8	1·3	13·0	51·6	2·5	70·0	88·0	8·0
	2·5	36·8	0·7	12·0	48·9	2·5	15·2	49·6	3·1
	1·8	19·8	0·9	22·5	102·5	2·2	20·9	77·1	2·7
	0·6	26·9	0·2	11·8	57·1	2·1	46·0	64·7	7·1
	5·1	35·3	1·4				31·4	43·0	7·3
Mean ±S.E.M.	2·8 ± 0·7	31·5 ± 3·2	0·9 ± 0·2	13·0 ± 1·7	50·0 ± 7·5	2·7 ± 0·2	36·2 ± 6·7	55·8 ± 6·7	6·3 ± 0·8

	Cerebellar nuclei cells			Purkinje cells			Betz cells		
	GABA content (10^{-14} mol)	Volume (10^{-12} l.)	GABA concentration (mM)	GABA content (10^{-14} mol)	Volume (10^{-12} l.)	GABA concentration (mM)	GABA content (10^{-14} mol)	Volume (10^{-12} l.)	GABA concentration (mM)
	6·5	5·8	11·2	2·4	4·8	5·0	2·3	8·9	2·6
	6·7	11·2	6·0	2·2	6·0	3·7	2·1	10·4	2·0
	1·8	4.3	4·2	5·5	5·7	9·6	2·7	7·2	3·8
	7·8	8·2	9·5	4·5	6·0	7·5	1·1	5·8	1·9
	1·3	4·9	2·7	3·5	4·4	8·0	1·5	9·2	1·6
	8·7	13·9	6·3	1·7	2·9	5·9	2·1	10·0	2·1
	2·4	5·1	4·7	4·3	4·1	10·5	3·5	11·2	3·1
	4·8	14·2	3·4	0·9	3·9	2·3	2·9	10.9	2·7
Mean ±S.E.M.	5·0 ± 1·0	8·5 ± 1·5	6·0 ± 1·1	3·1 ± 0·6	4·7 ± 0·4	6·6 ± 1·0	2·3 ± 0·3	9·2 ± 0·7	2·5 ± 0·3

Each type of cell was collected from the regions of CNS given in Table 1.

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analyses on single Betz cells and single Purkinje cells is rather low because the GABA content of a single cell was close to the lower limit of the assay. It is to be noted, however, that for each type of neurons the average value of GABA concentration obtained for individual cells (Table 3) is close to the mean value for pooled cells (Table 2).

Effect of denervation on GABA contents of Deiters' cells. Isolated nerve cell preparations assayed in the present study were very probably contaminated by presynaptic nerve terminals attached to the cell bodies. High GABA values in Tables 2 and 3, therefore, may be due to GABA being present at high concentrations in nerve terminals. This possibility was examined in the following experiments. Histological studies of WALBERG and JANSEN (1961) have shown that Purkinje neurons of the cerebellar vermis send their axons to the dorsal part of Deiters' nucleus but they do not send their fibres to the ventral part of the nucleus. Physiological studies showed that these Purkinje axons form inhibitory synapses with the dorsal Deiters' cells (ITO, KAWAI and UDO, 1968; OBATA *et al.*, 1967). On the basis of this information, an attempt was made to eliminate these Purkinje axon terminals from the dorsal Deiters' cells by denervation. In two cats the cerebellar vermis was totally removed (for the procedures of operation, see DUSSER DE BARENNE, 1924) and the animals were kept alive for 9 and 40 days respectively, after which they were killed and Deiters' cells were collected as described in Methods.

GABA concentrations in isolated Deiters' cells from the normal and operated cats are compared in Fig. 4. In normal cats, only one out of 10 dorsal Deiters' cells showed a value lower than 3 mM and the mean was 6·3 mM (see also Table 3). In the operated cats, by contrast, 10 out of 11 dorsal Deiters' cells showed values lower than 3 mM and the mean was 1·7 mM. Removal of the cerebellar vermis did not influence the GABA concentration in isolated Deiters' cells of the ventral part. These results suggest that GABA is present in a quite high concentration within presynaptic terminals attached to the dorsal Deiters' cell bodies. It is probable that these presynaptic terminals originate from Purkinje cells in the cerebellar vermis (see Discussion).

DISCUSSION

The importance of chemical studies of single neurons has been emphasized by many authors (LOWRY *et al.*, 1956; GIACOBINI, 1968; KRAVITZ *et al.*, 1963; KUFFLER and NICHOLLS, 1966). Transmitter substances are certainly among the first substances worth measuring in individual neurons. In the crustacean nervous system, a good correlation was found between the GABA contents of single neurons and their physiological functions, so that a high content of GABA has always designated a cell as inhibitory (KRAVITZ and POTTER, 1965; OTSUKA *et al.*, 1967). In the present study in the mammalian CNS, however, the interpretation of the results of GABA analyses on isolated nerve cells is more complicated. A single cell preparation in the present study contained not only the desired cell body but also presynaptic terminals attached to the cell body and possibly glial cells, small nerve cells and nerve fibres in the immediate vicinity of the cell. So far by light microscopy the amount of contaminating tissues was estimated to be rather small (cf. Fig. 3A). However, an electron-microscopic examination will be needed to further clarify the fine structure of the isolated nerve cells and the adhering tissues (BONDAREFF and HYDÉN, 1969).

The GABA concentrations in isolated dorsal Deiters' cells showed high values,

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and they were markedly reduced after the removal of cerebellar vermis. It may be argued that the reduction of GABA levels in dorsal Deiters' cells after denervation was brought about by a general deterioration of the tissue caused by the operation procedures. However, 9 and 40 days after the operation the regions of Deiters' nucleus from which the cells were collected seemed apparently normal and no sign of circulatory disturbances was seen in these regions. Furthermore, the GABA

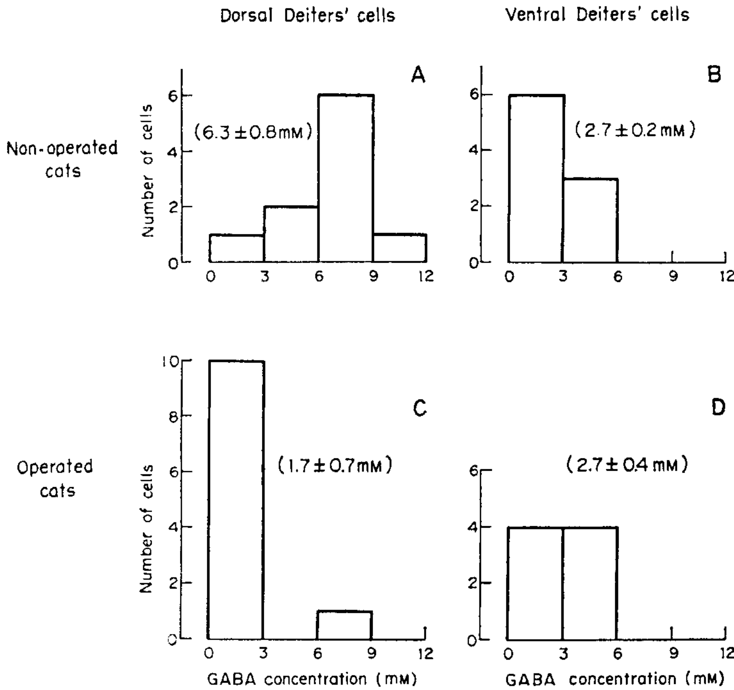

FIG. 4.—Histograms of GABA concentrations in single isolated Deiters' cell bodies from normal and operated (cerebellar vermis removed) cats. A: dorsal Deiters' cells and B: ventral Deiters' cells from normal cats (same experiments as in Table 3). C: dorsal and D: ventral Deiters' cells from operated cats. In each histogram, numbers in brackets represent a mean value of GABA concentration and S.E.M. Average volume of an isolated cell body was $34{\cdot}6 \pm 6{\cdot}0 \times 10^{-12}$ l. and $50{\cdot}6 \pm 10{\cdot}0 \times 10^{-12}$ l. for dorsal and ventral Deiters' cells, respectively, in operated cats. Those in normal cats are given in Table 3.

concentrations in isolated ventral Deiters' cells, collected from the regions neighbouring the dorsal Deiters' nucleus, were not influenced by the removal of the cerebellar vermis (Fig. 4). The most probable explanation for the reduction of GABA concentrations in isolated dorsal Deiters' cells after denervation is that GABA is concentrated in presynaptic terminals attached to the dorsal Deiters' cell bodies. Since the volume occupied by the inhibitory terminals is probably a minor portion of the total volume of an isolated cell preparation, GABA concentration in the nerve terminals may be considerably higher than 6·3 mM assessed for the GABA level of dorsal Deiters' cells in the normal cat.

In the present study each type of nerve cell was identified on the basis of its

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characteristic size and location. Individual nerve cells were arbitrarily sampled among hundreds of neurons of the same type. It is by no means assured that morphologically similar neurons in a given region of CNS are also alike with regard to the transmitter chemistry in their cell bodies and in presynaptic terminals attached to the cells (cf. OTSUKA *et al.*, 1967). So far in the present study, however, GABA contents of isolated cells of each type showed a certain common tendency for low, intermediate or high levels.

It is interesting to correlate the present results of GABA analyses with the relevant neuronal functions on the basis of the hypothesis that GABA is specifically concentrated in some inhibitory neurons. Low values of GABA concentrations were obtained for the isolated cell bodies of spinal motoneurons and spinal ganglion cells. These results indicate that the GABA concentrations in the cell bodies of these neurons are low, and further suggest that there are not abundant presynaptic terminals with a high GABA content in the close vicinity of these cell bodies. Spinal motoneurons are excitatory, and it has been suggested that the principal transmitter of the postsynaptic inhibition of motoneurons is not GABA but possibly glycine (WERMAN, DAVIDOFF and APRISON, 1968; CURTIS, HÖSLI, JOHNSTON and JOHNSTON, 1968). Spinal ganglion cells are also excitatory and histological observations have shown that their cell bodies are mostly free from synaptic contacts (ROSENBLUTH and PALAY, 1960).

The present results of GABA analyses on Deiters' cells before and after the removal of cerebellar vermis (Fig. 4) may satisfactorily be explained by assuming that GABA is concentrated in Purkinje axon terminals originating from cerebellar vermis and synapsing with dorsal Deiters' cells (see Results). There is a considerable evidence suggesting that GABA is the inhibitory transmitter of Purkinje neurons (OBATA *et al.*, 1967; OBATA and TAKEDA, 1969). The present findings on Deiters' cells are consistent with, and therefore give an additional evidence to, this hypothesis. Deiters' neurons, on the other hand, are known to be excitatory (GRILLNER, HONGO and LUND, 1970) and they may not contain a high concentration of GABA. Studies on the metabolism of GABA in the crustacean nervous system have shown that the activity of glutamate decarboxylase, the enzyme synthesizing GABA, is about 11 times higher in inhibitory axons than in excitatory ones (KRAVITZ, MOLINOFF and HALL, 1965). In this connection the recent observations of FONNUM, STORM-MATHISEN and WALBERG (1970) on this enzyme are quite in parallel with our findings. FONNUM *et al.* (1970) assayed the activity of glutamate decarboxylase in tissue sections from the dorsal and ventral parts of Deiters' nucleus of the cat and found that the activity of the enzyme is 2–3 times higher in the dorsal part than in the ventral part, and further that the enzyme activity in the dorsal part is greatly reduced while that in the ventral part is unaffected by the removal of cerebellar vermis.

GABA concentrations in isolated cerebellar nuclei cells and Purkinje cells also showed high levels, which are considerably higher than their background concentrations determined in tissue slices. Cerebellar nuclei cells are known to be excitatory (ECCLES, ITO and SZENTÁGOTHAI, 1967). Purkinje neurons form inhibitory synapses with cerebellar nuclei cells (ITO, YOSHIDA, OBATA, KAWAI and UDO, 1970). Therefore, as in the case of dorsal Deiters' cells, GABA may be concentrated in Purkinje axon terminals attached to the cell bodies of cerebellar nuclei. As mentioned above, there is evidence to suggest that Purkinje neurons are inhibitory, with GABA as the transmitter (ITO and YOSHIDA, 1966; OBATA *et al.*, 1967). Therefore, the cell bodies

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of Purkinje neurons may contain a high concentration of GABA. However, cerebellar basket cells, which form synapses with Purkinje cell bodies, are also known to be inhibitory (ECCLES *et al.*, 1967). High GABA values obtained for Purkinje cell bodies, therefore, might be due to GABA being concentrated in the axon terminals of basket neurons (KURIYAMA, HABER, SISKEN and ROBERTS, 1966).

Intermediate values of GABA concentration were obtained for cerebral Betz cells, which are known to be excitatory. KRNJEVIĆ and SCHWARTZ (1967) suggested that the inhibitory transmitter of the presynaptic terminals synapsing with cortical pericruciate neurons is probably GABA. The results presented in this paper are altogether consistent with the hypothesis that GABA is specificially concentrated in certain inhibitory neurons of the mammalian CNS. If this is proved, studies of GABA distribution at cellular level will afford a valuable means of mapping certain inhibitory pathways in the mamalian CNS.

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