BINDING OF γ-AMINOBUTYRIC ACID BY BRAIN PREPARATIONS *

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γ-Aminobutyric acid (GABA), a substance which has a unique occurrence in the central nervous system, has been shown to have physiological effects in a number of biological test systems (see references in Roberts and Eidelberg, 1960). Most of the observations have shown GABA to be inhibitory, a greater amount of a given type of stimulation being required to elicit the same response from a particular neuronal element after exogenous application or endogenous elevation of GABA than before. GABA and a variety of other ninhydrin-reactive constituents can be extracted quantitatively from homogenized brain tissue by mild procedures which involve denaturation of proteins and possibly other high molecular weight constituents, but not cleavage of covalent bonds. These include extraction with 80% alcohol and cold, dilute aqueous picric, trichloracetic, or perchloric acids, and heat coagulation followed by dialysis. Although studies in our laboratory of extracts of particulates prepared from liver and kidney in a variety of aqueous media invariably have shown a virtually complete liberation of the easily extractable amino acids into the suspending media (Roberts and Simonsen, 1960), recently sediments prepared from brain homogenates in various saline mixtures have been found to retain approximately 60% of the total GABA (Elliott and van Gelder, 1960).

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These findings suggested that a specific binding mechanism for GABA might exist in tissue of the central nervous system.

The results of typical experiments indicate a binding of GABA in brain which is apparently non-enzymatic (Table I). The experiments were performed at 0-4° C. under conditions which were shown in separate experiments not to permit metabolism of the added GABA-1-C^{14} to take place. To weighed portions of freshly dissected rat brain, lung, heart, spleen, kidney, and liver were added 9 volumes of incubation medium containing GABA-1-C^{14}. The tissues were homogenized, incubated at 0° C. with shaking for 50 minutes and then centrifuged for 30 minutes at 23,000 x g. at 0°. The supernatant fluid was poured off, the residue weighed and resuspended in the original volume of fresh medium not containing isotope, and suitable aliquots of the original homogenate and resuspended residue were counted in a scintillation counter. The results show that GABA-1-C^{14} is bound to the brain sediment but not to that of other tissues (Exp. 1) or to brain acetone powder (Exp. 3). The radioactivity in sediment from heart homogenate (Exp. 2) was washed out by resuspension in the medium but most of that in the original sediment from brain remained through two washings.

Results similar to those above were obtained in experiments employing equilibrium dialysis, in which 2 ml. aliquots of 10% tissue homogenates were dialyzed against an equal volume of suspending medium and aliquots of the external fluid counted after 4 hours of equilibration at 4° C. Subsequent experiments showed that in mouse brain homogenates binding of GABA-1-C^{14} could take place optimally in 0.05M TRIS buffer, pH 7.5, containing 0.5M NaCl. Virtually no binding took place when the NaCl was omitted. The GABA binding capacity was shown to reside entirely in the low-speed residue in experiments in which the resuspended residue was compared with the supernatant fluid. To date all attempts to solubilize the GABA binding material have failed. Clarification of brain homogenates by treatment with Tween-20, desoxycholate, or by

TABLE I

Uptake of GABA-1-C^{14} into Sediments of Homogenates of Brain and Other Tissues

Exp. No.	Tissue	% of total volume in sediment * (A)	% of total counts in sediment (B)	(B)/(A)
1	Brain	10.0	26.0	2.60
	Liver	9.0	5.9	0.65
	Heart	10.4	7.4	0.71
	Lung	7.4	5.9	0.80
	Spleen	8.6	5.7	0.66
	Kidney	9.9	7.9	0.80
2	Brain			
	a. Original sediment	10.1	37.8	3.74
	b. 1x washed "		29.9	2.96
	c. 2x washed "		32.3	3.20
	Heart			
	a. Original sediment	9.8	8.3	0.85
	b. 1x washed "		1.0	0.10
	c. 2x washed "		0.8	0.09
3	Mouse brain acetone powder +	15.7	12.7	0.81

* Derived from residue weight assuming a density of unity for both the sediment and suspending medium. The suspending medium had the following composition: 0.154 M NaCl, 10.4 parts; 0.154 M $MgSO_4$, 0.1; 0.25 M glucose, 0.3; 0.11 sodium phosphate buffer, pH 7.2, 1.2. 0.1 mg of GABA-1-C^{14} (2.7 mC/mM) was employed in 10 ml of the above medium. The sediments in Exp. 2 were resuspended in isotope-free medium.

\+ 100 mg of acetone powder containing high levels of L-glutamic acid decarboxylase and γ-aminobutyrate-α-ketoglutarate transaminase activities was suspended in 2.5 ml of medium and incubated for 70 minutes.

ultrasonication resulted in loss of activity. It is likely that the binding of GABA-1-C^{14} found in the above experiments was minimal, since there was considerable GABA present in the brain preparations employed.

More accurate quantitative and kinetic experiments await the removal of endogenous GABA and purification of the binding material. The binding of GABA which has been observed cannot yet be related to the known physiological effects of GABA in the central nervous system.

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