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SHORT COMMUNICATIONS

Regional distribution of gangliosides and bound acetycholine in beef brain*

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RECENT studies by several laboratories have indicated that gangliosides† are concentrated at nerve endings.[1–5] In addition, Burton *et al.*[4,5] have shown that the isolated synaptic vesicle fraction prepared by the procedure of DeRobertis *et al.*[6] contains 10%–12% gangliosides on a dry weight basis. Since gangliosides are associated with certain subcellular fractions that contain neurohumoral agents (such as acetylcholine), it was thought pertinent to investigate the regional distribution of gangliosides in brain tissue. In addition, these same samples of brain tissue were used to determine the bound acetylcholine‡ content.

TABLE 1. GANGLIOSIDES AND BOUND ACETYLCHOLINE CONTENT OF BEEF BRAIN

Brain tissue region	Gangliosides (μmoles/kg)	Bound acetylcholine (μmoles/kg)	Ratio: gangliosides to bound acetylcholine
I. Gray matter			
Frontal	2,420	23 (31)*	105
Parietal	2,150	20 (19)	107
Occipital	1,770	25 (15)	71
Basal ganglia			
Caudate	2,730	15 (19)	182
Putamen	3,030	(19)	
Globus pallidus	2,510	17 (19)	147
Thalamus	2,850	12 (21)	237
II. White matter			
Corpus callosum	900	13 (14)	69
Fornix	460	13	35
Basis pedunculi	150	0 (1)	
Dorsal column	360	7 (0·3)	52

* Values within parentheses are for dog brain tissue calculated from the table of Paton.[13]

Fresh beef brain and spinal cord were obtained from a meat-processing plant and were rapidly chilled in ice after removal from the skull. The tissue was dissected, and defined neuroanatomical samples were weighed (0·1–1·5 g) in the cold. The tissue samples were homogenized in cold 0·32 M sucrose containing 5×10^{-4} M eserine. Aliquots of the homogenate were removed for the determination of bound acetylcholine by the frog rectus abdominis preparation[7,8] and for the estimation of gangliosides by isolation, followed by hydrolysis and estimation of the N-acetylneuraminic acid released.[9,10] The data so obtained are presented in Table 1.

* Presented in part at the second International Pharmacological Meeting, Prague, 1963: R. M. Burton, S. Baer, R. E. Howard and Y. M. Balfour, *Biochem. Pharmacol.* **12**, Suppl. 161 (1963).

† For definition of ganglioside, proposed structure, and key literature references see Burton.[10]

‡ Bound acetylcholine may be defined as the acetylcholine that can be sedimented with tissue particles, that is not hydrolyzed by cholinesterase (prior to release by suitable treatment), and that fails to produce a response in a suitable biological assay system such as the frog rectus abdominis (prior to release). When the particles are heated (100°) at pH4 the acetylcholine becomes free and cannot be sedimented with the particles, it can be hydrolyzed by choline esterase, and it produces contraction of the frog rectus abdominis.

The data of Table 1 are consistent with reports in the current literature,[11,12] which show that concentration of gangliosides is higher in gray matter than in white matter. Similarly, bound acetylcholine is present in higher concentration in gray than in white matter.[13] Examination of the ratio of the concentration of gangliosides to the concentration of bound acetylcholine (Table 1, column 3) shows a threefold variation in gray matter: from 71 in the occipital region of the cortex to 237 in the thalamus. In the absence of additional data, four possible conclusions must be considered: (a) no relationship may exist between gangliosides and acetylcholine; (b) a relationship does exist, but some bound acetylcholine is lost during trauma involved in killing the steer and post-mortem changes; (c) the high levels of gangliosides in gray matter represent a latent capacity for the binding and storage of acetylcholine (see Burton)[5]; and (d) gangliosides may have other functions in addition to their hypothetical role in the storage and transport of acetylcholine.

The suggestions presented in b, c, and d are compatible with the postulated role for gangliosides in the storage and transport of acetylcholine at presynaptic terminals as suggested by Burton *et al.*;[4,5] these possibilities would be consistent with the known slow turnover of gangliosides in the central nervous system[14] and the rapid synthesis and hydrolysis of acetylcholine. Other functions of gangliosides might involve tetanus toxin receptor sites,[15–18] the maintenance of cerebral activity, and possibly cation transport.[19–21]

Additional work is in progress in an effort to clarify the role of gangliosides in the central nervous system.

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REFERENCES

1. L. S. WOLFE and H. MCILWAIN, *Biochem. J.* **79**, 33 (1961).
2. E. G. TRAMS and C. J. LAUTER, *Biochim. biophys. Acta* **60**, 350 (1962).
3. L. M. SEMINARIO, N. HREN and C. J. GOMEZ, *J. Neurochem.* **11**, 197 (1964).
4. R. M. BURTON, R. E. HOWARD, S. BAER and Y. M. BALFOUR, *Biochim. biophys. Acta.* **84**, 441 (1964).
5. R. M. BURTON, *Int. J. Neuropharmacol.* **3**, 13 (1964).
6. E. DEROBERTIS, G. RODRIGUEZ DE LORES ARNAIZ, L. SALGANICOFF, A. PELLEGRINE DE IRALDI and L. M. ZIEHER, **10**, 225 (1963).
7. J. H. BURN, *Practical Pharmacology*, Blackwell, Oxford (1952).
8. J. H. GADDUM, *Brit. J. Pharmacol.* **8**, 321 (1953).
9. L. WARREN, *J. biol. Chem.* **234**, 1971 (1959).
10. R. M. BURTON, *J. Neurochem.* **10**, 503 (1963).
11. J. FOLCH-PI and F. N. LEBARON, *Metabolism of the Nervous Systems*, D. Richter, Ed., p. 67, Pergamon Press (1957).
12. F. JAMES and K. FOTHERBY, *J. Neurochem.* **10**, 587 (1963).
13. W. D. M. PATON, *Ann. Rev. Physiol.* **20**, 431 (1958).
14. R. M. BURTON, Y. M. BALFOUR and J. M. GIBBONS, *Fed. Proc.* **23**, 230 (1964).
15. W. E. VAN HEYNIGEN, *J. gen. Microbiol.* **20**, 291 (1959).
16. *Ibid.*, p. 301.
17. *Ibid.*, p. 310.
18. *Ibid.*, **31**, 375 (1963).
19. S. BALAKRISHNAN and H. MCILWAIN, *Biochem. J.* **81**, 72 (1961).
20. C. G. THOMPSON and H. MCILWAIN, *Biochem. J.* **79**, 342 (1961).
21. D. H. DEUL and H. MCILWAIN, *J. Neurochem.* **8**, 246 (1961).