Postmortem Increases in GABA Receptor Binding To Membranes of Cat Central Nervous System

GODFREY TUNNICLIFF and G. KEITH MATHESON

SUMMARY: *GABA receptor binding in fifteen regions of cat central nervous system was investigated immediately postmortem and at twelve, twenty-four, and 72 hours postmortem. In each of the supraspinal areas studied, GABA binding increased with time-after-death. Changes after 24 hours occurred in the cerebellum, the visual cortex, the sensorimotor cortex, and the amygdala where more than a twofold increase in binding was observed. Increases were also noted in the thalamus, caudate nucleus, hippocampus, and hypothalamus. The results suggest that caution should be exercised in the interpretation of GABA binding data obtained from human brains that have not been treated in a similar postmortem manner.*

RÉSUMÉ: *Nous avons étudié la capacité de liaison des récepteurs GABA dans 15 régions du SNC du chat, immédiatement après la mort et 12, 24 et 72 heures après le décès. Dans toutes les régions supraspinales étudiées, la capacité de liaison des récepteurs augmentait avec le temps consécutif à la mort. Après 24 heures les changements furent surtout au cervelet, cortex visuel, cortex sensorimoteur et amygdale où une augmentation du simple au double fut observée. Des augmentations furent aussi observées au thalamus, au noyau caudé, à l'hippocampe et à l'hypothalmus. Par contre il n'y eut aucun changement au niveau des membranes de la moelle. Les résultats indiquent qu'il faudra être très circonspect dans l'interprétation des capacités de liaison des récepteurs GABA provenant d'humains dont les cerveaux auront été obtenus et traités de façons différentes.*

From the Evansville Center for Medical Education Indiana University School of Medicine, Evansville, Indiana, U.S.A.

Reprint requests to: G. Keith Matheson, Ph.D., Associate Professor of Anatomy, Evansville Center for Medical Education, Indiana University School of Medicine, PO Box 3287, Evansville, Indiana 47732 U.S.A.

INTRODUCTION

The importance of γ-aminobutyric acid (GABA) in diseases of the nervous system is far from clear. However, evidence is available that levels of this neurotransmitter and/or its synthesizing enzyme glutamic decarboxylase, are below normal in postmortem brains of patients with Huntington's chorea or Parkinson's disease (Bird and Iversen, 1974; McGeer, et al, 1971; Perry, et al., 1973). Furthermore, changes have been observed in GABA receptor binding in certain brain regions of such patients (Enna, et al., 1976a, 1976b; Lloyd, et al., 1977a, 1977b; Rinne, et al., 1978). With postmortem Parkinsonian brains, two groups of workers have reported the substantia nigra GABA binding is decreased compared with controls (Lloyd, et al., 1977b; Rinne, et al., 1978). However, conflicting reports have been made about GABA binding associated with Huntington postmortem brains. Lloyd and colleagues (1977a) found GABA binding considerably increased in the cerebellum but markedly decreased in the striatum. On the other hand, Enna and coworkers (1976a, 1976b) reported GABA binding did not change in the basal ganglia; but they did observe an increase in GABA binding to membranes from the substantia nigra.

In light of the increasing use of GABA binding assays in postmortem brain tissue, and because of the apparent discrepancies noted above, we undertook to determine if changes in receptor binding occur when tissues are sampled at different postmortem times.

METHODS

Adult cats weighing between 2 and 4 kg were anesthetized with α-chlora-

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lose. For the experiments utilizing fresh tissue, the brain and spinal cord were removed, dissected, and weighed. The tissue was then homogenized in 9 vol of ice-cold 0.32 M sucrose containing 5mM HEPES buffer (pH 7.4) as previously described (Tunnicliff, 1979). The membranes were stored at -20° C for at least 24 hours prior to the GABA binding assay. It has been shown that freezing the membranes enhances GABA binding (Zukin et al., 1974). For the postmortem studies, animals were euthanised with α-chloralose and stored in the coldroom. At the appropriate time, the brain and spinal cord were removed and treated as the fresh tissue. A thermister was implanted in the basal ganglia of a cat to monitor the time course of temperature equilibrium. The animal was euthanised and immediately placed in the coldroom.

The binding of 3H GABA (64 curies/m mole; Amersham) to the membranes has been described (Tunnicliff, 1979). To distinguish binding from uptake, binding assays were carried out in a Na^+-free medium at 4° C. Binding is defined as that radioactivity displaced by 1 mM non-radioactive GABA. Protein content was determined by the method of Lowry et al. (1951).

TABLE 1

Changes in GABA Binding in Cat Central Nervous System

REGION	HOURS POSTMORTEM			
	0	12	24	72
Cerebellum	263 ± 34	371	594 ± 83	691 ± 75
Visual Cortex	136 ± 19	296	322 ± 41	378 ± 54
Sensorimotor Cortex	130 ± 21	——	314 ± 56	———
Amygdala	141 ± 33	351	323 ± 39	363 ± 42
Caudate Nucleus	110 ± 15	187	166 ± 22	177 ± 18
Hippocampus	80 ± 18	——	131 ± 16	———
Thalamus	72 ± 9	——	118 ± 13	———
Hypothalamus	71 ± 10	120	94 ± 14	140 ± 19
Pons	26 ± 8	——	34 ± 9	———
Spinal Cord				
Cervical, Dorsal	44 ± 6	——	40 ± 8	———
Ventral	25 ± 4	——	32 ± 8	———
Thoracic, Dorsal	15 ± 4	——	42 ± 6	———
Ventral	8 ± 3	——	25 ± 7	———
Lumbosacral, Dorsal	26 ± 7	——	24 ± 7	———
Ventral	27 ± 5	——	27 ± 7	———

These values are the means ±S.E.M. of GABA binding (f moles/mg protein). Each determination was done in triplicate. Three animals were used in each experiment, except for the 12 hour study in which a single animal was used.

RESULTS

Of the fifteen regions studied, by far the greatest binding occurred in the cerebellum (Table 1). The amygdala, visual cortex, and sensorimotor cortex all yielded about half the binding found in the cerebellum. The caudate nucleus, hippocampus, thalamus, and hypothalamus exhibited a third of the binding of the cerebellum. A very small amount of binding was detected in the pons and spinal cord.

The greatest postmortem changes in GABA binding were observed in the cerebellum, visual and sensorimotor cortex, and amygdala (Table 1). It is apparent that at least a two-fold increase has occurred with 24 hours and that binding continued to increase, albeit at a lesser rate, for 72 hours. Augmentation of GABA binding in the other forebrain nuclei ranged from 30 to 80 percent after 24 hours. The pons and areas of the spinal cord that were studied showed no postmortem changes in binding compared with fresh tissue, with the exception, however, of the dorsal and ventral regions of the thoracic cord which showed an increase after 24 hours.

The results of the temperature equilibrium determination are depicted in Figure 1. The rate of temperature decrease during the first six hours was 3.2° C/hour. However, total equilibrium was not reached for 36 hours.

DISCUSSION

The regional distribution of GABA binding in fresh tissue is in good agreement with studies on human brain (Enna, et al., 1977; Lloyd, et al., 1977b) and monkey and rat central nervous system (Beaumont, et al., 1978; Enna, et al., 1975). However, in a regional distribution study of the cat nervous system, Balfagon et al. (1975) reported that the greatest binding occurred in the thalamus, with binding to the cerebral cortex, cerebellum, and hippocampus all slightly lower. However, these workers employed a Na^+-containing buffer to measure GABA binding. Generally, the measurement of the binding of GABA is carried out in the absence of Na^+ in order to reduce the possibility of interference by the binding of GABA to its transporter (Young, et al., 1976). This methodological difference may account for the variation between their data and our data.

Our results demonstrate the existence of a mechanism for the enhancement of GABA binding during postmortem storage. It is worthy of note that there are considerable differences in the degree of postmortem increases in GABA binding between regions. Indeed, no changes were observed in the spinal cord, yet cerebral cortex showed a 2.5-fold increase in binding. The reasons for these differences are unknown. However, they may be related to an endogenous protein inhibitor of GABA binding similar to that recently isolated from fresh membranes by Toffano et al. (1978). The protein they describe is destroyed by freezing, but it is conceivable that other protein inhibitors are present which are denatured by standing at cold room temperatures, yet are not affected by freezing. Such a protein could account for the increased GABA

binding. Variations in the regional distribution of such an inhibitor might also account for the observed postmortem differences in the degree of increase in GABA binding from region to region. Further studies are needed to elucidate the basis of these observations.

Several groups of workers have measured GABA binding to postmortem human brain synaptic membranes, in an attempt to evaluate the role of GABA in Parkinson's and Huntington's diseases (Enna, et al., 1976a; Lloyd, et al., 1977a; Lloyd, et al., 1977b; Rinne, et al., 1978). In Parkinsonian brains, GABA binding appears to be reduced in the substantia nigra and is unchanged elsewhere (Lloyd, et al., 1977b; Rinne, et al., 1978). Yet, there are evident inconsistencies in the literature regarding postmortem GABA binding studies from Huntington brains. Lloyd and coworkers (1977a) found that binding is increased in the cerebellum and is reduced in the caudate nucleus and putamen. Enna and colleagues (1976b) reported that GABA binding significantly increased in the substantia nigra but was unchanged in the putamen and globus pallidus. Our results suggest that some of these apparent increases and decreases in GABA binding may be the result of comparisons of tissues that have not been handled in the same postmortem way. If, for instance, a brain stored for a relatively short time were compared to a brain that had been stored for 24 hours or longer, it would appear that binding in the former brain was considerably lower than in the latter. In this case, obviously, such differences in binding between the two brains will be unrelated to the diseased state of the brain.

We are aware that Enna and coworkers (1976b) claim that there are no postmortem changes in GABA binding. In their rat brain study, these workers allowed their animals to remain for six hours after death at room temperature before brain removal and freezing. Binding assays were carried out at various times on the stored tissue up to four weeks. These conditions, however, did not approach those that may be expected in human brain storage where a delay may occur between death and storage in a hospital or mortuary coldroom. Additional delays could well occur between storage and autopsy and autopsy and preparation of membranes. Even if brains are placed in a coldroom immediately after death (an unlikely situation with human tissue), we have demonstrated (Figure 1) that there is a long delay in the temperature equilibration of cat brain. It could be anticipated that an even greater period of time would be required in the case of a human brain.

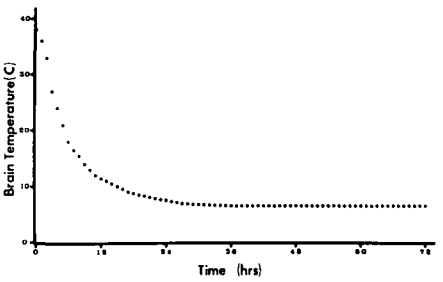

Figure 1 — Change in cat brain temperature with increased time of coldroom storage. Equilibrium was reached by 36 hours.

Although the mechanism for the postmortem increases in GABA binding is unknown, standardisation of the storage of postmortem brains appears to be important and would be a major contribution to the elucidation of the role of GABA in neurological diseases.

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REFERENCES

BALFAGÓN, G., CAMACHO, J.G., GADEA-CIRIA, M., SOMOZA, G., and DEFEUDIS, F.V., 1975. Comparison of GABA binding to synaptosomal fractions of seventeen regions of the feline central nervous system, Expl. Neurol., 48: 383-386.

BEAUMONT, K., CHILTON, W.S., YAMAMURA, H.I., and ENNA, S.J., 1978. Muscimol binding in rat brain: association with synaptic GABA receptors, Brain Res., 148: 153-162.

BIRD, E.D. and IVERSEN, L.L., 1974. Huntington's chorea. Post-mortem measurement of glutamic decarboxylase, choline acetyltransferase and dopamine in basal ganglia, Brain, 97: 457-472.

ENNA, S.J., KUHAR, M.J., and SNYDER, S.H., 1975. Regional distribution of post-synaptic receptor binding for gamma-aminobutyric acid (GABA) in monkey brain, Brain Res., 93: 168-174.

ENNA, S.J., BENNETT, J.P., BYLUND, D.B., SNYDER, S.H., BIRD, E.D., and IVERSEN, L.L., 1976a. Alterations of brain neuro-transmitter receptor binding in Huntington's chorea, Brain Res., 116: 531-537.

ENNA, S.J., BIRD, E.D., BENNETT, J.P., BYLUND, D.B., YAMAMURA, H.I., IVERSEN, L.L., and SNYDER, S.H., 1976b. Huntington's chorea: changes in neurotransmitter receptors in the brain, New Engl. J. Med., 294: 1305-1309.

ENNA, S.J., BENNETT, J.P., BYLUND, D.B., CREESE, I., BURT, D.R., CHARNESS, M.E., YAMAMURA, H.I., SIMATOV, R., and SNYDER, S.H., 1977. Neurotransmitter receptor binding: regional distribution in human brain, Brain Res., 28: 233-236.

LLOYD, K.G., DREKSLER, S., and BIRD, E.D., 1977a. Alterations in ^{3}H-GABA binding in Huntington's chorea, Life Sci., 21: 747-754.

LLOYD, K.G., SHEMAN, L., and HORNYKIEWICZ, O., 1977b. Distribution of high affinity sodium-independent [^{3}H] gamma-aminobutyric acid ([^{3}H]-GABA) binding in the human brain: alterations in Parkinson's disease, Brain Res., 127: 269-278.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L., and RANDALL, R.J., 1951. Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193: 265-275.

MCGEER, P.L., MCGEER, E.G., and WADA, J.A., 1971. Glutamic decarboxylase in Parkinson's disease and epilepsy, Neurology, 21: 1000-1007.

PERRY, T.L., HANSEN, S., and KLOSTER, M., 1973. Huntington's chorea. Deficiency of γ-aminobutyric acid in brain, New Engl. J. Med., 288: 337-342.

RINNE, U.K., KOSKINEN, V., LAAKSONEN, H., LÖNNBERG, P., and SONNINEN, V., 1978. GABA receptor binding in the parkinsonian brain, Life Sci., 22: 2225-2228.

TOFFANO, G., GUIDOTTI, A., and COSTA, E., 1978. Purification of an endogenous protein inhibitor of the high affinity binding of γ-aminobutyric acid to synaptic membranes of rat brain, Proc. Natl. Acad. Sci. (USA), 75: 4024-4028.

TUNNICLIFF, G., 1979. Inhibition by pyridoxal-5^1-phosphate of γ-aminobutyric acid receptor binding to synaptic membranes of cat cerebellum, Biochem. Biophys. Res. Comm., 87: 712-718.

YOUNG, A.B., ENNA, S.J., ZUKIN, S.R., and SNYDER, S.H., 1976. Synaptic GABA receptor in mammalian CNS. In E. Roberts, T. Chase, and D.B. Tower (Eds), GABA in Nervous System Function, Raven Press, New York, pp. 305-317.

ZUKIN, S.R., YOUNG, A.B., and SNYDER, S.H., 1974. Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system, Proc. Natl. Acad. Sci., 71: 4802-4807.