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Return of γ-aminobutyrate transaminase activity in mouse brain after inhibition by aminooxyacetic acid: Chemical and histochemical observations*

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VIRTUALLY all the data in the literature are consistent with the interpretation that the steady-state levels of γ-aminobutyric acid (γABA) in the central nervous system (CNS) *normally* are governed by the glutamic acid decarboxylase activity (GAD) and not by the γABA-α-ketoglutarate transaminase activity (γABA-T).[1] It was shown upon administration to animals of hydroxylamine or aminooxyacetic acid (AOAA), substances which are potent inhibitors of both the GAD and γABA-T *in vitro*, that only the γABA-T was inhibited and that there were marked elevations of γABA content in the brains of the treated animals.[2] This suggested that the first two enzymes of the "γABA shunt" are not present in the same location in the CNS. Studies with cell-fractionation procedures have given results consistent with the interpretation that γABA-T is strictly a mitochondrial enzyme found largely at postsynaptic sites and that at least 40–50 per cent of the GAD activity is located in presynaptic nerve endings.[3–5] Much of the available data could be explained if it were assumed that γABA is a presynaptically liberated inhibitory transmitter by some neurons in various areas of the CNS and that after transport into postsynaptic neuronal sites it is metabolized in mitochondria by a sequence of reactions beginning with γABA-T. A histochemical procedure has been developed for the visualization of the γABA-T-succinic semialdehyde dehydrogenase sequence (γABA-T-S), the endpoint of which is the deposition of an insoluble formazan derivative from a tetrazolium salt (NitroBT), presumably at or close to the site of γABA-T activity.[6, 7] The purpose of the present study was to attempt to correlate the enzymatically measured values for γABA-T with the visually observed formazan deposition in sections of mouse brain, with a view to ascertaining whether or not the histochemical procedure has validity as a semiquantitative monitoring method for γABA-T activity in various loci in the CNS. The depletion and repletion of γABA-T activity in mouse brain after i.p. administration of AOAA was followed by both methods.

Fasted Swiss mice of both sexes, 22–25 g in weight, were injected i.p. with a neutral solution of AOAA (25 mg/kg), and animals were sacrificed by cervical dislocation at various times after the injection. The brains were removed quickly and frozen in dry ice. Ten 10-mμ sections were cut in a cryostat through the cerebrum at the same level in each brain and were placed in 1·0 ml of cold water. The next five sections were placed on slides for histochemical study. Another ten sections of

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10 mμ each then were cut and added to the sections for chemical analysis, and the twenty pooled sections were homogenized. Portions of the homogenate were used for the isotopic assay for γABA-T activity[8, 9] and for determination of protein content.[10]

There was a rapid decrease in γABA-T activity in brains of mice after administration of AOAA (Fig. 1). Within 5 min after the injection of AOAA there was a 78 per cent reduction in γABA-T activity of brain homogenates, and there was much less γABA-T-S activity in the brain sections, as shown by the greatly decreased staining in the histochemical procedure. There was only a slow return of γABA-T to normal levels, 40 per cent of the control values being attained within 1 day: and only after 20 days after AOAA administration did the γABA-T activity come within the normal range. The quantity of formazan precipitate on the slides showed a gradual increase up to 14 days after AOAA, in a manner well correlated with the quantitative estimations. However, after 14 days the amount of formazan precipitate was visually indistinguishable from the control levels, although there still was a 20 per cent reduction of γABA-T activity from the control values, as measured by assays of homogenates.

Immediately after injection of AOAA the most rapid depletion of the stainability was found in the superficial gray matter and in white matter, the deeper nuclear structures retaining the γABA-T-S activity to a much greater extent (Fig. 1). At 6 hr the γABA-T-S system fell below the level of detection in most of the nuclear structures, with only the substantia nigra and other extrapyramidal and hypothalamic structures showing the stain. However, even at 6 hr there appeared to be some increases from the 5-min and 1-hr levels in observable γABA-T-S activity in the superficial gray matter and white matter. In a study of increases in γABA levels in brains of mice receiving the same dose of AOAA,[11] it was found that a plateau in γABA levels had been attained between 3 and 4 hr and that a secondary rise took place between 4 and 6 hr. The biphasic curve of γABA increase may be correlated with the double wave of inhibition of γABA-T-S activity observed on the slides. At subsequent times the superficial layers showed a progressive return of γABA-T-S activity in a diffuse manner; the rate of recovery in the deep nuclei appeared to lag somewhat behind. The slow return to normal after AOAA of chemically assayed γABA-T activity has been noted before.[2]

The present findings indicate that the intensity of staining by the histochemical procedure has a reasonable degree of correlation with the chemical procedure and, thus, may be useful in showing regional differences in distribution of γABA-T-S activity, provided that comparisons are not made between too heavily stained regions. Useful information also may be obtained about the differential rates of disappearance and reappearance of γABA-T after treatment with substances that may influence its activity. The possibility that the histochemical procedure may be used to reveal the times of appearance of this enzyme activity in various brain regions during development is being investigated.

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Reversal by methoxyethylmercury intoxication of NAD induced activation of glutamate dehydrogenase from rat liver

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SOME organic mercury compounds change *in vitro* the effect of allosteric ligands[1] on glutamate dehydrogenase (L-glutamate: NAD(P) oxidoreductase (deaminating), EC 1.4.1.3.).[2] NAD is a coenzyme for this enzyme, but may also act as an allosteric activator.[3–5] As we were interested in mercury effects on the subcellular level *in vivo*, we investigated the activating effect of NAD on the glutamate dehydrogenase acitvity in a liver homogenate from methoxyethylmercury*-intoxicated rats.

The rats were given daily subcutaneous injections of MeEHg, the last about 24 hr before being killed. Ultrasonically-treated suspensions of liver mitochondria in 0·05 M phosphate buffer pH 7·5, passed through a Sephadex G 25 column, were used as the enzyme. The mercury content of the suspensions was determined as mercury dizionate by a method developed at our institute.† The biuret method was used for protein determinations. The glutamate dehydrogenase activity was measured as change in extinction at 340 mμ. A Zeiss selfrecording spectrophotometer RPQ 20 was employed. The reaction mixtures appear in Fig. 1.

The NAD activation of the enzyme is reflected in the deviation from linearity in the Lineweaver–Burk plot.[3] As is seen from Fig. 1, there is no activation following MeEHg treatment of the enzyme *in vitro*, and a slight inhibition seems to be present. The same effects are found without adding mercury *in vitro* when an enzyme from a mercury intoxicated rat is tested.

We do not claim that the present findings explain the symptoms of MeEHg intoxication. Nevertheless they show that the inhibitory effect on the enzymes caused by a change in the sulfhydryl groups does not represent the only possible mechanism whereby mercury may alter the metabolic activity of the cells. The altered reaction to allosteric ligands may well be a common factor as regards

* The seed dressing agent methoxyethylmercury acetate (MeEHg) was a gift from A/B Casco, Stockholm, Sweden.

† Karl Wülfert, personal communication (1966).