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A histochemical demonstration of γ-aminobutyric acid metabolism in spinal cord*

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AT PRESENT there is not sufficient evidence available to suggest a specific function for γ-aminobutyric acid (GABA) in the nervous system of vertebrates, other than that it is probably of an inhibitory nature.[1] Techniques which precisely localize the enzymes concerned with the metabolism of GABA should be of considerable aid in elucidating this function. In this communication a method is presented which appears to be specific for the demonstration of the metabolic pathway that converts GABA to succinic acid; the enzymes involved in this pathway are GABA-α-ketoglutarate transaminase and succinic semialdehyde dehydrogenase.

The method consists of incubating fresh, frozen sections of nervous tissue with a buffered 0·5% agar–saline medium[2] which has the following composition: 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (Nitro BT; Dajac Labs.), 2 mg/ml; NAD, 2 mg/ml; α-ketoglutarate, 5 mg/ml; GABA, 5 mg/ml. The final pH of the medium was 7·4.

Sections were incubated for 30 min at 40° in a covered petri dish containing wet filter paper to prevent evaporation. Control sections were incubated under identical conditions with a medium from which GABA had been omitted.

In the course of incubation NADH is formed as GABA is converted to succinate; NADH in turn causes the reduction of Nitro BT to the insoluble, colored formazan. This occurs spontaneously *in vitro* at 40°, but the participation of succinic dehydrogenase in the reaction cannot be excluded, since it was found that malonate (5 mg/ml) partially inhibits formazan precipitation. However, the distribution of formazan after incubation of the sections with succinate does not parallel that obtained after incubation with GABA, although considerable overlap was noted.

When sections are incubated in a medium containing no GABA a very faint and diffuse formazan production occurs. This may be removed by subsequently placing the sections, after they have dried at room temperature, for 2 min in 100% ethyl alcohol and another 2 min in xylene. Such sections become almost completely transparent and are impossible to photograph. In contrast, strong formazan precipitation occurs in sections incubated in the presence of GABA. Figure 1 is a photograph of a section treated in the manner described above. Although all gray matter shows formazan production, the strongest reaction has occurred in the motor neurons (m) of the ventral horns (V) and the ependymal cells around the central canal (c). This latter finding may provide an explanation for the previous observation that GABA does not get into the central nervous system when injected into the circulation,[3] since it would be metabolized by these cells before it could penetrate further.

The following correlation between GABA transamination and formazan production was noted. The reaction was dependent on the combined presence of NAD, α-ketoglutarate, and GABA; it was inhibited by hydroxylamine (1 mg/ml) and aminooxyacetic acid (1 mg/ml). No reaction occurred when the pH of the solution was changed from 7·4 to 6 even after 1 hr of incubation. GABA stimulated formazan production in liver[4] but not in muscle. Moreover, although excellent localization was obtained in rabbit nervous tissue, the reaction in this tissue occurred at a slower rate and to a lesser extent than in mouse tissue. A similar difference has been noted by Roberts[5] with respect to glutamic acid decarboxylase. Finally, β-alanine, aspartate, glutamate, or glycine did not cause formazan production when substituted for GABA. It is therefore concluded that the reaction is specific for GABA under the present experimental conditions.

Full details of the method and a study comparing mouse and rabbit nervous tissue will be published elsewhere.

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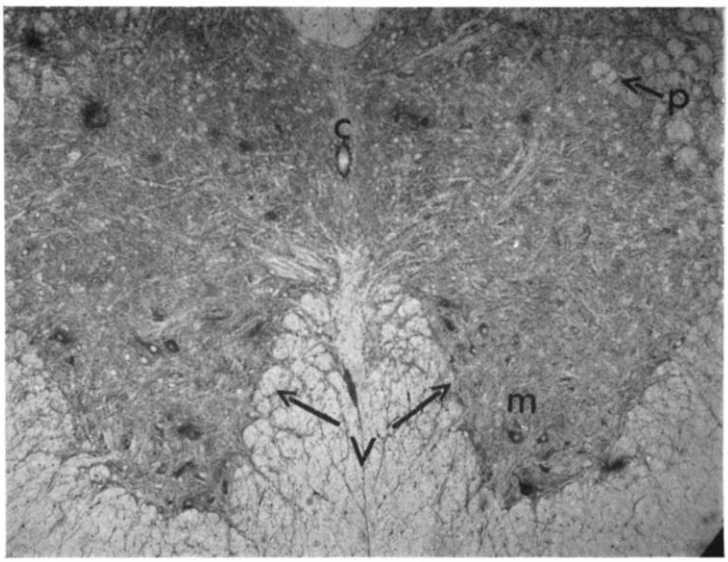

FIG. 1. GABA metabolism is mouse spinal cord, demonstrated by formazan precipitation; V—ventral horns; c—central canal; m—motor neurons; p—posterior column.

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