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GABAergic Basket-Pyramidal and Basket-Granular Systems of the Hippocampal Formation in the Cat

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The localization of GABA transaminase, the marker enzyme of axon terminals of GABAergic neurons, was studied in slices of cat hippocampus by the histological method. By its specific staining, the reaction product was consistently localized in synaptic basket terminals on granular cells of the dentate fascia and of pyramidal neurons of hippocampal areas CA1-CA3.

Key Words: *hippocampal formation; GABA transaminase; epilepsy*

A nerve impulse reaching the entorhinal cortex is capable of activating four excitation routes in succession: the perforant (from the entorhinal cortex to the dentate fascia), mossy fibers (from the dentate fascia to area CA3), Schaffer's collaterals (from the CA3 to the CA1 area), and, finally, the fibers passing from area CA1 to the entorinal cortex through the subiculum [3]. Granular cells of the dentate fascia and pyramids of the CA1-CA3 areas are the main effectors in this trisynaptic circulation; they synthesize glutamate and utilize it in the transmission of a nerve impulse, thus maintaining a high neurotransmitter balance of one of the main stimulating transmitters in the central nervous system [2,6].

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The hippocampal formation possesses an inhibitory apparatus which is one of the most potent in the brain [5,12] and which realizes through GABAergic axoaxonal and axosomatic terminals a retrogressive inhibition of the pyramidal and granular cells and selective depression of the presynaptic release of glutamate in them.

Some authorities [12] believe that impaired functioning of the inhibitory GABAergic mechanisms in the hippocampal formation is the key cause of epilepsy.

In this connection, mapping of GABAergic systems in this region of the brain is vital for the creation of histochemical models to investigate the effects of various drugs in studies of not only convulsive states, but the mechanisms of spatial memory, which is believed to be the principal function of the hippocampus [3].

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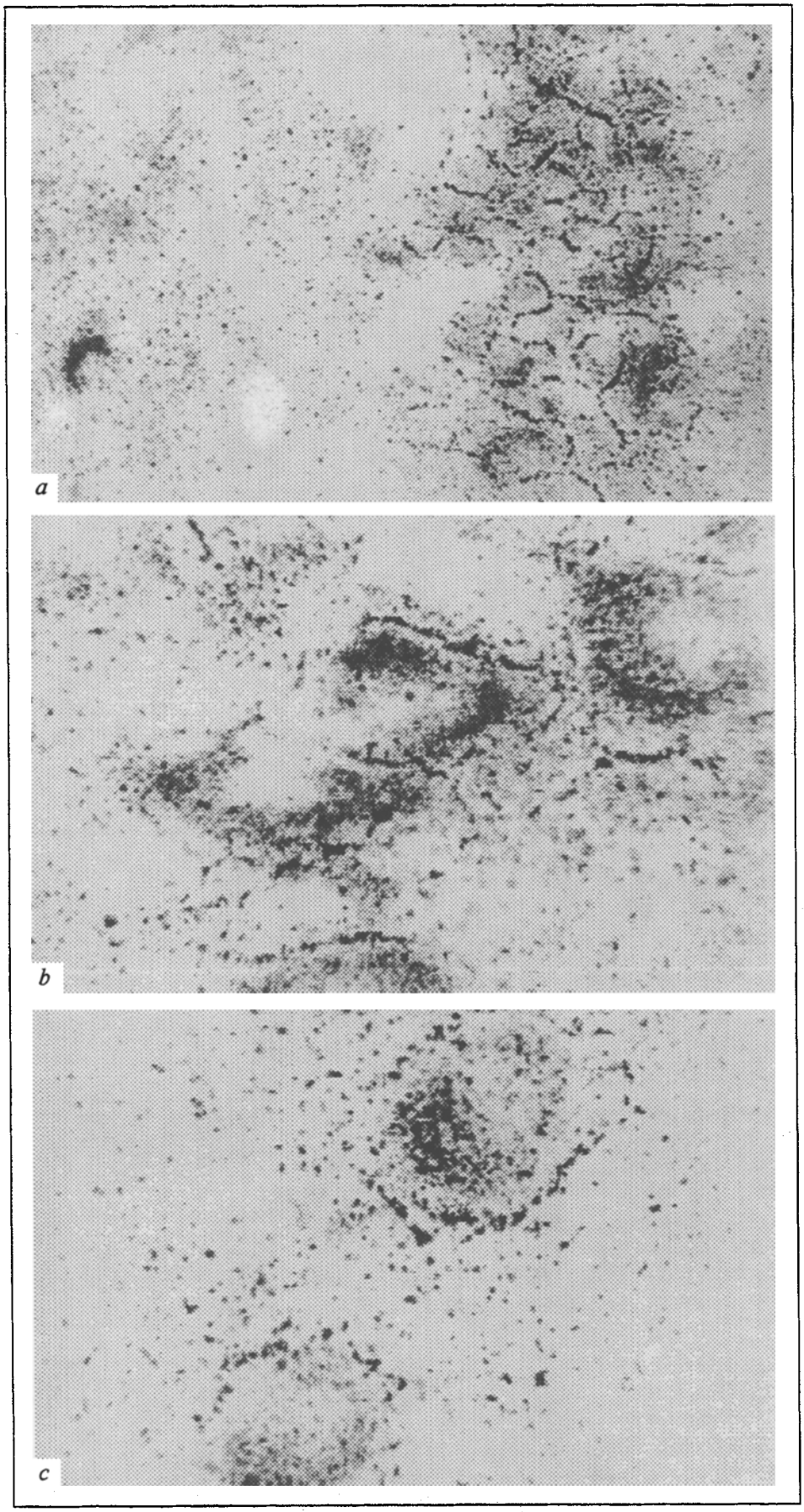

Fig. 1. Localization of GABA transaminase in synaptic basket terminals. *a*) in the granular layer of the dentate fascia, ×800; *b*) in the pyramidal layer of the CA3 area, ×5000; *c*) around the pyramids in the CA1 area, ×5000.

Our research aimed at elucidating the localization and distribution of GABAergic terminals in the granular and pyramidal layers of the hippocampal formation of the cat.

MATERIALS AND METHODS

The histochemical method for GABA transaminase (GABA-T; EC. 2.6.1.19), first proposed by Van Gelder [13] and modified in recent years [1,4,7,9, 14], was used in this study. The main advantage of this method over the immunocytochemical method for glutamate decarboxylase is that it permits a more selective detection of GABAergic synaptic terminals [4]. We investigated the hippocampal formation of the brain in 12 adult cats. The animals were anesthetized by intravenous phenobarbital. The brain was removed and put on glass, the corpus callosum was dissected, and each hemisphere was divided into two approximately equal parts. The fragments were frozen in a cryostat, where 25 μ frontal slices were prepared which were then placed on slides and dried in a flow of cold air from a ventilator for 2 min. Dried slices were placed in polypropylene cuvettes and 1 ml of incubation medium containing 0.1 M Tris-HCl buffer, 50 mg/ml α-ketoglutarate, 10 mg/ml nicotinamide adenine dinucleotide (NAD), 72 mg/ml NaCl, 5 mg/ml malonate, 10.2 mg/ml magnesium chloride, 0.5 mg/ml potassium cyanide, 50 mg/ml GABA, and 10 mg/ml nitroblue tetrazolium, pH 8.6. The samples were incubated at 37°C for 20 min, then washed 3 times in 0.1 M phosphate buffer, pH 7.4, dehydrated in alcohols, and embedded in balsam. Control samples were incubated in medium without substrate (GABA or α-ketoglutarate) or in the presence of 10 M aminooxyacetate, a specific GABA-T inhibitor [9]. A negative histochemical reaction was observed in all cases. Tris-buffer, α-ketoglutarate, GABA, aminooxyacetate, and NAD were from Serva, Sigma, and Calbiochem.

RESULTS

Specific staining of hippocampal slices for GABA-T detection revealed that the cytoplasm of neurons in the granular layer of the dentate fascia and in the pyramidal layer of areas CA1-CA3 did not react with GABA or α-ketoglutarate. The product of the histochemical reaction is localized mainly in the synaptic terminals (Fig. 1, *a-c*), which are clearly seen on the granular cells and pyramids, as well as in the neuropil along the proximal parts of the apical dendrites.

A sediment of bluish-black diformazan granules forms in intensively stained synaptic "knobs." The size of these granules varies from 0.5 to 1.5 ×μ. Sometimes they are situated discretely at intervals of 1-3 μ, but more frequently they merge to form clearly discernible bold lines running in semicircles around the borders of granular cells and pyramids.

The GABA-T-positive terminals detected in this study correspond to the localization and distribution of the typical basketlike plexuses described for the hippocampal formation [8]. The sources of GABA-T-positive terminals are GABAergic basket neurons of the pyramidal and subpyramidal layers of Ammon's horn and of the granular and polymorphous layers of the the dentate fascia [9,10]. It is noteworthy that perikaryons of hippocampal GABAergic neurons have been identified by a pharmacohistochemical method for GABA-T [14], but, in contrast to the case with Van Gelder's method [13], the axons and terminals of basket neurons do not stain at all [9].

It is possible that besides basket and axosomatic contacts, GABAergic axoaxonal contacts of chandelier cells may be present in consistently GABA-T-positive plexuses [12]. Together with basket neurons, chandelier cells represent a sort of "safety device" which protects the hippocampal formation from overexcitation "runoff" via the trisynaptic route. It is quite possible that pathological changes in the GABAergic inhibitory apparatus may be the cause of such overexcitation and switch on the triggering mechanisms of the epileptic process in the hippocampus [11,12]. Thus, the method for the detection of GABA-T which we have described here may be valuable for model experiments exploring the mechanisms of epilepsy.

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