The Distribution of a Particulate Trypsin Inhibitor in Brain[1] (35136)

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Blackwood and Mandl reported the inhibition of the tryptic hydrolysis of benzoyl-DL-arginine-β-naphthylamide (BANA) by homogenates of rat liver and rat kidney, and noted the heat stability of such inhibitors (1). Similar observations have also been made with normal human and bovine brain homogenates (2). Upon fractionation of the brain homogenates by differential centrifugation (3), inhibitory activity is found in such particulate fractions as nuclei, mitochondria, microsomes, and endoplasmic reticulum, but is not seen in the soluble (90,000g supernatant) fraction (2). These data suggest that the inhibition of tryptic activity may result from the binding of the enzyme to the membranous material (outer membrane of nuclei, mitochondria, and endoplasmic reticulum) of the cell. In view of the morphological and functional differences in brain areas, it was considered desirable to investigate the level of inhibitory activity in such fibrous areas of the brain as corpus callosum and to compare it with that of nuclear areas such as thalamus and cerebral gray matter. This communication reports the results of such an investigation.

Materials and Methods. Fresh human and bovine brain areas such as cerebrum, cerebral gray matter, cerebral white matter, cerebellum, corpus callosum, medulla, thalamus, midbrain, and pons were homogenized with 9 vol of cold 0.32 *M* sucrose in a Teflon mortar and pestle. Inhibition was measured by mixing 0.1 ml of trypsin solution (100 μg/ml in 0.001 *N* HCl) with 0.3 ml of unbuffered homogenates, or homogenates diluted to 0.3 ml with 0.1 *M* phosphate buffer, pH 7.6. Phosphate buffer was added to the trypsin solution in the absence of inhibitor and served as a control. After standing for 15 min at room temperature, 1.6 ml of BANA solu-

TABLE I. The Effect of Myelin on the Tryptic Hydrolysis of BANA.[a,b]

Additions to 10 μg trypsin	% of control activity
—	100
Myelin (mg)	
0.25	110
0.50	111
1.00	112
First supernatant from myelin[c] (mg)	
0.25	120
0.50	113
1.00	106
First crude precipitate from myelin[d] (mg)	
0.25	113
0.50	112
1.00	101

[a] To 0.1 ml of trypsin solution in 1 m*M* HCl (10 μg) were added suspensions of dialyzed, lyophilized myelin, supernatant, and precipitate preparations in 0.1 *M* phosphate buffer, pH 7.6, to a total volume of 0.4 ml. After standing for 15 min at room temperature, 1.6 ml of BANA solution was added, and tryptic activity was measured as described in the text.

[b] The dialyzed, lyophilized myelin, supernatant, and precipitate preparations were kindly supplied by Dr. Marian W. Kies (4).

[c] The supernatant fraction contains microsomal particles.

[d] The precipitates contain mitochondria, nuclei, and debris.

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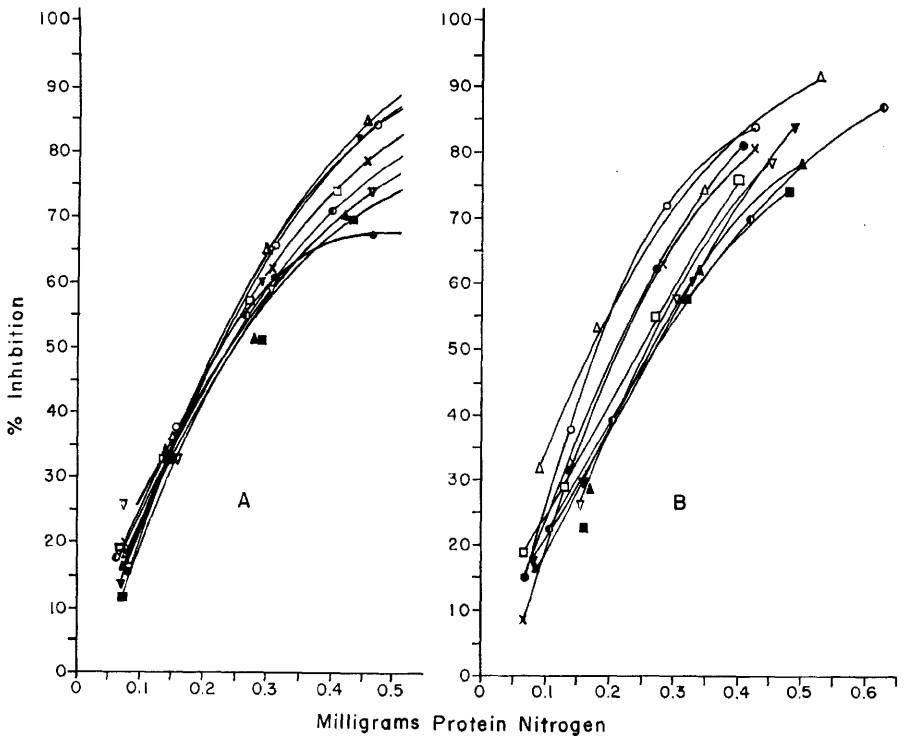

FIG. 1. A. Inhibition of the tryptic hydrolysis of BANA by homogenates of bovine brain areas. B. Inhibition of the tryptic hydrolysis of BANA by homogenates of human brain areas. To 0.1 ml of trypsin solution in 1 m*M* HCl (10 μg) were added various volumes of homogenates of brain areas with sufficient 0.1 *M* phosphate buffer, pH 7.6, to a total volume of 0.4 ml. After standing for 15 min at room temperature, 1.6 ml of BANA solution was added, and the tryptic activity was measured as described in the text. x, whole brain homogenate; ○, cerebrum; ●, cerebral gray matter; △, cerebral white matter; ▲, cerebellum; □, corpus callosum; ■, medulla; ▽, thalamus; ▼, midbrain; ◐, pons.

tion (7.5×10^{-4} *M* in 0.1 *M* phosphate buffer, pH 7.6) was added, and the mixture was incubated at 37° for 1 hr. The reaction was stopped by the addition of 1 ml of 40% TCA. After centrifugation at 12,100*g* for 15 min in the Sorvall RC-2 centrifuge, the naphthylamine liberated by trypsin was determined in the supernatant fraction (1). Protein nitrogen was determined by the micro-Kjeldahl procedure.

Results and Discussion. The data in Fig. 1 indicate that homogenates of such human and bovine brain areas as cerebrum, cerebral gray matter, cerebral white matter, cerebellum, corpus callosum, medulla, thalamus, midbrain, and pons inhibit the tryptic hydrolysis of BANA. There appears to be no significant difference in the level of inhibition between areas of white matter, such as medullary and corpus callosum tissue, and those of gray matter such as thalamus and cerebral gray tissue. Furthermore, the level of inhibition of tryptic activity by both human and bovine brain areas is essentially equivalent. Preincubation of trypsin with the homogenates for a period of time is not essential for inhibition. The addition of whole brain homogenate to a mixture of trypsin and BANA results in an almost complete, immediate cessation of hydrolysis of the chromogenic substrate (Fig. 2).

In view of the marked inhibition of the hydrolysis of BANA by trypsin in the presence of white matter of brain and the high content of myelin in white matter, the effect of myelin on tryptic activity was investigated. The results in Table I show that dialyzed, lyophilized preparations of myelin,

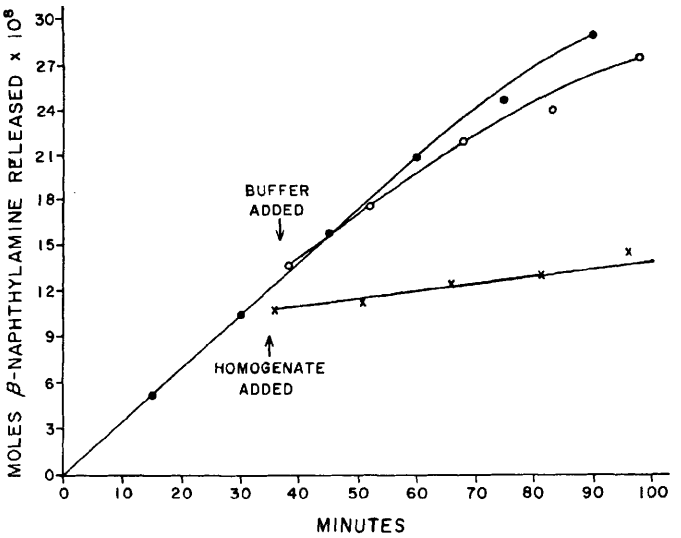

FIG. 2. The effect of bovine brain homogenate on the tryptic hydrolysis of BANA. To 4 ml of trypsin solution (50 μg/ml in 1 m*M* HCl) were added 4 ml of 0.1 *M* phosphate buffer, pH 7.6, and 32 ml of 3.0 m*M* BANA. The mixture was incubated at 37°. After 35 min 10 ml of the incubation mixture was removed and 2 ml of bovine brain homogenate added thereto. An additional 10-ml aliquot was removed at 37 min to which was added 2 ml of phosphate buffer. Two-milliliter aliquots were taken at the times indicated from each of these mixtures for inactivation with 1 ml of 40% TCA and for determination of the β-naphthylamine liberated.

supernatant fractions (microsomal), and sediments (containing mitochondria, nuclei, and debris) do not inhibit the tryptic hydrolysis of BANA. Since it has been reported earlier that particulate preparations in 0.32 *M* sucrose inhibit tryptic activity (2), it is suggested that dialysis and lyophilization of the myelin and cell preparations alter the conformation sufficiently to destroy the structural integrity necessary to bind and inhibit tryptic activity. Myelin will serve as a tryptic substrate (Dr. M. W. Kies, personal communication), although it is not nearly so rapidly hydrolyzed as is casein (Suszkiw and Brecher, unpublished data). Casein also exhibits no inhibition of the tryptic hydrolysis of BANA (2).

Summary. The inhibition of the tryptic hydrolysis of benzoyl-DL-arginine-β-naphthylamide by homogenates of human and bovine cerebrum, cerebrum gray matter, cerebrum white matter, cerebellum, corpus callosum, medulla, thalamus, midbrain, and pons is reported. There is no significant difference between inhibitory levels of primarily gray areas and primarily white areas of brain. Dialyzed, lyophilized myelin does not inhibit tryptic activity. The reaction of brain homogenates with trypsin is rapid.

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