microsomal fraction was prepared according to a method described previously[5].

The activity of GGTP and the amount of cytochrome P-450 were determined in the microsomal fraction. The activity of GGTP was measured using the method of Adolph[6] and cytochrome P-450 was quantified by the method of Omura and Sato[7]. The protein content was determined by the method of Lowry et al.[8].

Results. Compared to the sham operated controls, hepatic GGTP activity increased significantly after PCS (table), when expressed per microsomal protein as well as per g wet liver weight. 10 days after PCS, the amount of cytochrome P-450 was significantly decreased and remained at this low level for the experimental duration of 30 days.

Discussion. Portacaval shunting leads to an increase of GGTP activity in rat liver as has been shown previously[1,2]. No similar elevation, however, could be observed for cytochrome P-450 but rather a decrease which has been attributed to a deprivation of one or several substances normally supplied by portal blood and contributing to the maintenance of the cytochrome level[9].

From our data and other observations it can therefore be concluded that PCS does not lead to an induction of cytochrome P-450. It is therefore unlikely that the increased activity of GGTP and its histochemical appearance is due to any induction mechanism. Rather, a derepression of a fetal enzyme, as has been put forward as a hypothesis[2], must be the cause of the increased hepatic GGTP activity after PCS.

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A high molecular weight inhibitor of Ca^{2+}-dependent neutral protease in rat brain

I. Nishiura, K. Tanaka and T. Murachi

Department of Clinical Science, Kyoto University Faculty of Medicine, Kyoto 606 (Japan), 24 October 1978

Summary. An endogenous, heat-stable inhibitor of high mol. wt (approximately 3×10^5) was found to be present in rat brain, which inhibited Ca^{2+}-dependent neutral protease specifically but not due to its binding of Ca^{2+} in the medium.

The occurrence of a Ca^{2+}-dependent neutral protease in the brain was first described by Guroff[1]. We have reported the enhancement of the activity of this protease in human malignant glioma tissues, as well as in methylcholanthrene-induced glioma G203 cells in mice[2]. In the course of purification of the Ca^{2+}-dependent neutral protease from rat brain by chromatographies, we often encountered increase in recovery of total activity of this enzyme over 100%, sometimes even more than twice the activity found before the chromatography. This prompted us to search for an inhibitor or inhibitors which might have been present in the starting material and removed during the purification procedures. We have thus found the presence of a high mol. wt protease inhibitor in the soluble fraction of rat brain homogenate, which inhibits the Ca^{2+}-dependent neutral protease specifically but not due to its binding of Ca^{2+} in the medium.

Materials and methods. Wistar strain rats, weighing 120–180 g, were used. Ca^{2+}-dependent neutral protease in the brain was partially purified as previously described[2]. The preparation contained 1 mg protein per ml. Caseinolytic activity at pH 7.5 (Tris-HCl) was assayed by the method of Kunitz[3], using 5 mM $CaCl_2$ and 10 mM cysteine as the activators[2]. The release of acid-soluble peptides was determined spectrophotometrically at 750 nm using Folin-Ciocalteau reagent.

Results and discussion. Brain tissues were quickly removed and homogenized with 3 vol. of 20 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose, 5 mM enthylene glycol bis (β-aminoethyl ether) -N,N,N′,N′,-tetraacetic acid (EGTA), and 5 mM 2-mercaptoethanol. The homogenate was centrifuged at 105,000× g for 1 h, and the soluble fraction thus obtained was concentrated by ultrafiltration with Amicon PM-10 membrane. The concentrate was fractionated on a Sephadex G-200 column. An aliquot of each fraction was preincubated with the partially purified preparation of Ca^{2+}-dependent neutral protease, and then the caseinolytic activity of the mixture was assayed. As shown in figure 1, an enhancement of the protease activity over the level for the pre-existent protease can be seen with

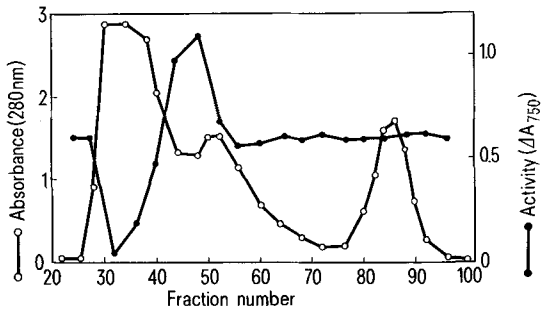

Fig. 1. Resolution of a high mol. wt protease inhibitor and Ca^{2+}-dependent protease in rat brain by gel filtration through Sephadex G-200. The concentrated soluble fraction of rat brain (257 mg protein in 8.0 ml) was applied to a column (3.0× 100 cm) which had been equilibrated with 20 mM EGTA and 5 mM 2-mercaptoethanol in the cold. Elution was carried out with the same buffer, and fractions of 6 ml each were collected at a flow rate of 10 ml/h. A 0.5-ml aliquot of each fraction was preincubated with 50 µl of Ca^{2+}-dependent protease, partially purified from rat brain, at 37 °C for 10 min, and then assayed for caseinolytic activity at 37 °C for 30 min.

fractions between Nos 40 and 55. This position coincided with the position where the Ca^{2+}-dependent neutral protease of rat brain (mol. wt 84,000[1]) should be eluted[2]. By contrast, the fractions at the void volume decreased the preexistent protease activity, giving a large negative profile in figure 1. The results clearly indicated the occurrence of an endogenous, high mol. wt inhibitor(s) in the brain which could be separated from the Ca^{2+}-dependent protease upon gel filtration under the conditions employed.

The inhibitor fractions (Nos 26 through 40) were combined and concentrated to 7.4 ml (139 mg of protein) using Amicon PM-10 membrane. When rechromatographed on Sephadex G-200, the inhibitor was eluted near the void volume of the column, giving an approximate mol. wt of 3×10^5. The inhibitor was found to be markedly heat-stable: even after heating at 100 °C for 20 min, the inhibitor retained ist inhibitory potency as well as its large molecular size. This fact excluded the possibility of contamination of the present inhibitor preparation with protease inhibitors in serum such as α_2-macroglobulin and α_1-antitrypsin which are known to be heat-labile. Nevertheless, the inhibitor is of protein nature, since it lost the inhibitory potency by an incubation with one-hundredth ($^1/_{100}$) weight of trypsin at pH 7.5 and 37 °C for 40 min.

The inhibitor did not affect at all the caseinolytic activity of trypsin, chymotrypsin, and papain (data not shown), indicating that it is different from the inhibitor reported to be present in bovine brain and effective on trypsin[4]. The inhibition seemed to be specific to Ca^{2+}-dependent protease, but it was not due to the possible binding of the inhibitor with Ca^{2+} in the medium which is essential for the activity of the protease concerned. The evidence supporting this view is shown in figure 2.

A high mol. wt inhibitor of Ca^{2+}-dependent neutral protease was also found to be present in bovine cardiac muscle[5] and in rat liver[6]. We have recently found that the inhibitor from the liver was also effective on the protease from the brain, and vice versa. Since the biological significance of Ca^{2+}-dependent protease, widely distributed in various tissues, is at least partly understood in connection with cyclic nucleotide-independent activation of protein kinase[7], the present discovery of a unique and endogenous inhibitor must be of prime interest.

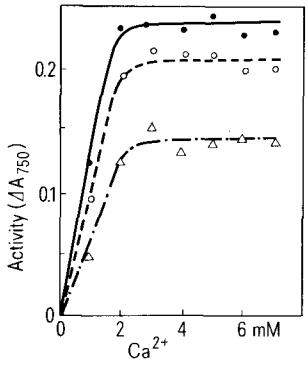

Fig. 2. Inhibition of Ca^{2+}-dependent protease as a function of Ca^{2+} concentration in the medium. The amounts of inhibitor added per tube were: ●, zero; ○, 4.3 µl; △, 13.3 µl. Each tube contained 50 µl of Ca^{2+}-dependent protease from rat brain. The assay was carried out at 37 °C for 30 min.

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Binding capacities of various analogues of S-adenosyl-L-homocysteine to protein methyltransferase II from human erythrocytes[1]

L. Gillet, Y. Looze[2], M. Deconinck and J. Léonis

Laboratoire de Chimie Générale I, Faculté des Sciences, Université libre de Bruxelles, 50, Avenue F. D. Roosevelt, B-1050 Bruxelles (Belgium), 10 November 1978

Summary. A series of analogues of S-adenosyl-L-homocysteine, modified mainly in the amino acid portion of the molecule, have been synthesized. All were found to be competitive inhibitors of protein methyltransferase II from human erythrocytes. S-adenosyl-L-homocysteine remains however by far the most effective inhibitor of the methylase.

5′-isobutylthio-5′-deoxyadenosine (SIBA) has been shown recently to inhibit cell transformation by Rous sarcoma virus[3], mitogen-induced blastogenesis[4], polyoma virus replication[5] and capping of herpes virus mRNA[6]. Furthermore, it possesses an antimalarial activity against *Plasmodium falciparum*[7].

SIBA, as well as other analogues, has been thought to act on the cells as inhibitors of methyltransferases. This remains to be proved, however. Earlier results obtained on various methyltransferases acting on t-RNA[8] or small metabolites[9-14] were not in favour of such an hypothesis. More recent reports have given evidence of a more powerful inhibition by SIBA of the methylation of the 5′-terminal 'cap' of m-RNA, as well as of arginine residues in proteins[6,15].

Protein methyltransferase II (E.C. 2.1.1.24) catalyses the conversion of carboxylic functions in proteins into their methyl esters. These are particularly unstable[16] and the protein substrate is readily regenerated under physiological conditions. The enzyme responsible for this type of methylation was purified and isolated in an homogeneous form from the cytosolic fraction of calf thymus[17] and from equine red blood cells[18]. The latter preparation was well characterized from the molecular point of view. Also, the