SEROTONIN-SENSITIVE ARYL ACYLAMIDASE IN RAT BRAIN

Daisaburo Fujimoto

Hamamatsu University School of Medicine
Hamamatsu, Japan

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SUMMARY: Aryl acylamidase (E.C.3.5.1.13) was extracted from rat brain. The enzyme activity was inhibited by low concentrations of serotonin. The inhibition was non-competitive type and K_i value was about 3×10^{-5} M. Tryptamine inhibited the enzyme to a lesser extent. Other amines such as noradrenaline, tyramine and histamine did not affect the enzyme reaction. In contrast, aryl acylamidase from rat liver was insensitive to serotonin.

It is generally accepted that serotonin (5-hydroxytryptamine) plays an important role in mammalian central nervous system and participates in the regulation of diverse biological activities, but little is known about the direct mechanism of action of this amine (for review, see ref.1).

Enzymatic deacylation of aryl acylamides has been known for many years(2) and aryl acylamidase (aryl-acylamide amidohydrolase, E.C.3.5.1.13) has been extracted from various tissues (3,4). However, the enzyme in brain has not been characterized. This paper reports that aryl acylamidase in rat brain is inhibited by low concentrations of serotonin. This enzyme may serve as a model for the study of the action mechanism of serotonin.

METHODS

Rat brain or liver (4g wet weight) was homogenized in a Teflon-glass homogenizer in 20 ml of 0.05M sodium phosphate buffer, pH 7.0, containing 0.5% (v/v) Triton X-100. The homogenate was kept at 0°C for 1 hr and centrifuged at 8,000 x g for 20 min. The proteins in the supernatant was then fractionated by ammonium sulfate precipitation and the fraction precipitating between 33% and 67% saturation was dissolved in 3 ml of 0.05M sodium phosphate buffer, pH 7.0, and dialyzed against the same buffer (500 ml) at 4°C for 16 hr. The solution inside the bag was used as an enzyme source.

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Table 1

EFFECTS OF AMINES ON ENZYMATIC DEACETYLATION OF NITROACETANILIDE

Enzyme source	Amine added	Concentration (mM)	Nitroaniline formed (μmole)	Inhibition (%)
Brain	None	-	0.238	-
Brain	Serotonin	0.033	0.112	53
Brain	Serotonin	0.1	0.079	67
Brain	Serotonin	0.2	0.043	82
Brain	Tryptamine	0.2	0.169	29
Brain	Noradrenaline	0.2	0.229	less than 5
Brain	Tyramine	0.2	0.225	less than 5
Brain	Histamine	0.2	0.238	less than 5
Brain	Aniline	0.2	0.241	less than 5
Liver	None	-	0.230	-
Liver	Serotonin	0.033	0.222	less than 5
Liver	Serotonin	0.33	0.211	8

o-Nitroacetanilide (15 μmoles) was incubated with the brain enzyme (0.88 mg protein) or the liver enzyme (0.31 mg protein) in the presence or absence of amines in 3 ml of 0.01M sodium phosphate buffer, pH 7.0, at 37°C for 120 min and the amount of o-nitroaniline was determined by measuring absorbance at 430 nm. The serotonin sample was a complex with creatinine sulfate but creatinine sulfate alone did not affect the reaction.

Aryl acylamidase activity was assayed by the method proposed by Hoagland and Graf (4) using o-nitroacetanilide as a chromogenic substrate.

RESULTS AND DISCUSSION

The effect of various amines on aryl acylamidase in rat brain and liver are summarized in Table 1. The enzyme in brain was inhibited strongly by serotonin, whereas the enzyme in liver was inhibited by serotonin only very slightly. Tryptamine also inhibited the brain enzyme, but to a lessor extent. Other amines did not affect the reaction.

The mode of serotonin inhibition was examined. The inhibition was non-competitive type and K_i value was about 3×10^{-5} M .

Besides the sensitivity to serotonin, the elution position of the brain enzyme from a Sepharose 6B column was different from that of the liver enzyme (Fig.1). The elution profile suggested the heterogeneity of the brain enzyme but each enzyme fraction was sensitive to serotonin.

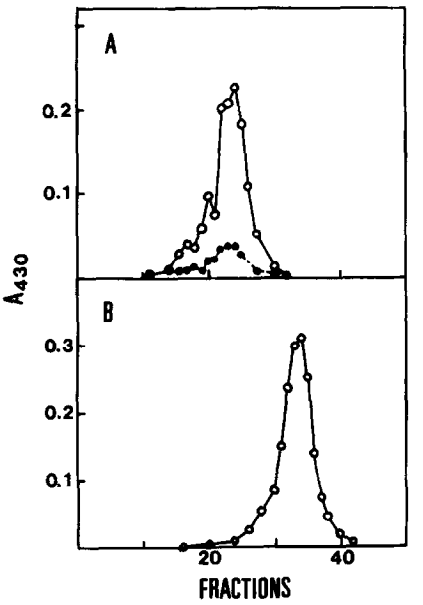

Fig.1. Elution profiles of aryl acylamidases from Sepharose 6B column. A Sepharose 6B column (1.3 x 44 cm) was equilibrated with 0.02M sodium phosphate buffer, pH 7.0. The enzyme sample (about 3 mg protein) was applied to the column and eluted with the same buffer. Fractions (1.8 ml) were collected and assayed for aryl acylamidase activity in the absence(o——o) or in the presence(●---●) of serotonin (final concentration, 10^{-4}M). A: brain enzyme, B: liver enzyme.

Since serotonin has assumed an important role in brain, the occurrence of a serotonin-sensitive enzyme in this organ is of special interest. Physiological substrates for brain aryl acylamidase are not clear at the moment, however, some antipyretic and analgesic drugs are aryl acylamides, which may be hydrolyzed by aryl acylamidase in brain. It may be possible that the level of the drugs in brain is regulated by the level of serotonin. Since the effect of serotonin on the enzyme activity can be measured simply and easily, this enzyme may also serve as useful models for the study of interactions between serotonin and the receptors.

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