RAT BRAIN DE-ACETYLATING ACTIVITY: STEREOSPECIFIC INHIBITION BY LSD AND SEROTONIN-RELATED COMPOUNDS

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SUMMARY

A de-acetylase (aryl acylamidase, E.C.3.5.1.13) has been isolated and partially characterized from rat brain. Previous studies have shown that this enzymatic activity is inhibited by low concentrations of serotonin. This report examines the effects of closely related tryptamine derivatives and demonstrates that enzymatic activity is stereospecifically inhibited by LSD. Similar enzymatic activity from liver was found to be insensitive to any of the compounds tested. The significance of these findings with regard to well known serotonin-LSD interactions is discussed.

De-acetylation of acylamides by mammalian tissues has been known for many years[1-2]. Although a few investigators have reported significant hydrolysis of n-acetylated arylamines by brain tissue *in vitro*[3-4], the corresponding enzymatic activity has, to our knowledge, not been further characterized. Recently, Fujimoto (5) has described de-acetylating activity of rat brain that is inhibited by low concentrations of serotonin. The presence of a serotonin-sensitive aryl acylamidase (E.C.3.5.1.13) in brain could provide a useful tool for studying the interaction between serotonin and drugs that are structurally and pharmacologically related to serotonin. We therefore decided to further characterize the de-acetylating activity of rat brain by investigating the possible inhibitory effects of closely related tryptamine derivatives.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (150-200 g) were decapitated and their brains (excluding the pineal gland) were quickly removed. The brains were

homogenized with a Teflon-glass homogenizer in 5 volumes of ice cold .05 M sodium phosphate buffer (pH 7.0) containing 0.5% (V/V) Triton X-100. The homogenate was kept at 0°C for 1-2 hours and centrifuged at 8500 g for 20 minutes. The resulting supernatant was subjected to ammonium sulphate precipitation and the fraction precipitating between 40% and 60% saturation was redissolved in 2.5-3.0 mls of .05 M sodium phosphate buffer (pH 7.0) and dialysed overnight at 4°C against the same buffer (500 ml). For comparison, rat liver aryl acylamidase was prepared in an identical manner as the brain enzyme.

Enzymatic activity was assayed with a slight modification of the method of Hoagland and Graf (4). The incubation mixture contained between 1 and 5 mM o-nitroacetanilide as substrate and .25 to 1.0 mg of enzyme protein, in .05 M sodium phosphate buffer at a final incubation volume of 3 ml. Incubation was carried out at 37°C for 60 to 120 minutes. Inhibitors were dissolved and diluted in the same buffer used in the assay and were added just prior to incubation. Blanks included boiled enzyme, buffer, and substrate; buffer and substrate without enzyme; or, buffer, substrate, and enzyme at 0-2°C. Enzymatic activity was terminated by immersion in an ice bath. The extent of de-acetylation was measured by determining the increase of absorbance at 430 nm on a Coleman 124 spectrophotometer, corresponding to the formation of o-nitroaniline (4).

RESULTS AND DISCUSSION

The characteristics of the aryl acylamidase isolated from rat brain will be reported in greater detail elsewhere (Paul et al., submitted for publication). Our initial studies revealed a significant heterogeneity within the total de-acetylating activity of rat brain, but confirmed that this activity is inhibited by low concentrations of serotonin.

It is clear from table 1 that only pharmacologically active tryptamine derivatives are capable of inhibiting enzymatic activity *in vitro*. d-LSD and serotonin were the most potent inhibitors of enzymatic activity, while

TABLE I

EFFECTS OF VARIOUS TRYPTAMINE-RELATED COMPOUNDS (10^{-4}M)

ON RAT BRAIN DE-ACETYLATING ACTIVITY IN VITRO

COMPOUND	O-NITROANILINE FORMED μMoles $mg^{-1}120^{-1}Min$	PERCENT INHIBITION
None	.320 ± .014[a]	---
d-LSD	.062 ± .003[b]	80%
SEROTONIN	.090 ± .004[c]	72%
2-BROMO-LSD	.098 ± .008	70%
METHYSERGIDE	.104 ± .009	68%
BUFOTENINE	.260 ± .007[d]	20%
5-METHOXYTRYPTAMINE	.264 ± .010	20%
N,N,DIMETHYLTRYPTAMINE	.286 ± .010[e]	10%
TRYPTAMINE	.300 ± .011[f]	<5%
1-LSD	.323 ± .012[g]	0%

a vs b $p < .001$, b vs c $p < .001$, c vs d $p < .001$, d vs e $p < .01$
a vs d $p < .01$, a vs e $p < .05$, a vs f $p < .10$, a vs g N.S.

All values are the means plus standard errors of four experiments. Each experiment consisted of triplicate determinations using the same enzyme source (40% to 60% ammonium sulphate precipitate).

tryptamine itself was far less active. The IC50's for d-LSD and serotonin were 2.2×10^{-5}M and 4.0×10^{-5}M respectively. 2-bromo-LSD (BOL) and methysergide, two structurally related LSD congeners, were less potent inhibitors than LSD. Structurally unrelated serotonin antagonists such as cyproheptadine and methiothepin, on the other hand, were ineffective as inhibitors of enzymatic activity. It is also evident that not all tryptamine derivatives inhibit enzymatic activity to the same degree. Bufotenine

(5-hydroxy N, N, dimethyltryptamine) and 5-methoxy-tryptamine were significantly less effective as inhibitors than either BOL or methysergide.

The pharmacologically inactive levo isomer of LSD (l-LSD) was also inactive as an inhibitor of enzymatic activity even at a millimolar concentration. Closely related tryptamine derivatives such as N-acetyl serotonin and melatonin, as well as a number of β-phenylethylamine derivatives, were also without inhibitory effects. Similar inhibition studies with the liver aryl acylamidase revealed that this enzymatic activity was insensitive to any of the above tested indoleamine derivatives. It is unlikely that the hallucinogenic effects of LSD are related to enzyme inhibition since closely related non-hallucinogenic compounds such as BOL are potent inhibitors as well.

Although the physiological significance of the aryl acylamidase isolated from rat brain is unclear at the present time, a number of endogenous N-acetylated compounds[6,7,8], as well as exogenous aryl acylamides (various analgesic-antipyretic drugs), could be potential *in vivo* substrates. Nevertheless, our results demonstrate that the inhibition of rat brain aryl acylamidase occurs only in the presence of pharmacologically active tryptamine derivatives, is stereospecific with respect to its inhibition by lysergic acid diethylamide (LSD), and thus might provide a basis for studying LSD-serotonin-receptor interactions *in vitro*. Finally, this is the first report of enzymatic activity showing inhibition *in vitro* by low concentrations of LSD.

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