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SHORT COMMUNICATIONS

Increase of brain endogenous monoamine oxidase inhibitory activity (tribulin) in experimental audiogenic seizures in rats: evidence for a monoamine oxidase A inhibiting component of tribulin

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Abstract—Brain tribulin activity in rats with an inherited predisposition to audiogenic epilepsy was studied after seizures of different intensity were induced by an electric bell. Weak seizures (from 0 to 2 arbitrary units) did not produce any changes in endogenous inhibitory activity towards either monoamine oxidase (MAO) A or B. Moderate seizures were characterized by increases in both MAO A and MAO B inhibitory activity (up to 1.9-fold). Complete tonic epileptiform seizures with total areflexia (4 arbitrary units) induced further augmentation (up to 2.5-fold) of MAO A but not of MAO B inhibitory activity. This dissociation between the two inhibitory activities points to the existence of a separate MAO A-inhibiting component of brain tribulin which is different from isatin.

Tribulin is a low molecular mass endogenous inhibitor(s) of monoamine oxidase (MAO) and benzodiazepine receptor binding, which is extractable into ethyl acetate [1]. It is widely distributed in mammalian biological fluids and tissues [2–5]. In man, increased output of urinary activity of this kind has been observed in a variety of conditions of stress and anxiety [6–10]. Augmentation of tribulin activity in brain and other rat tissues has been demonstrated during cold-restraint stress [11, 12], electroconvulsive shock [13] and footshock [14]. Administration of the anxiogenic agent, pentylenetetrazole, which provokes epileptiform seizures in animals at high doses, also causes a marked increase of MAO inhibitory activity in rat [15] and rabbit brains [5]. A major component of urinary tribulin is isatin, a selective MAO B inhibitor [16, 17]. In this communication, we have investigated the effect of audiogenic epileptiform seizures in rats on brain tribulin activity. In previous studies, tribulin was measured by assessing the inhibition of rat liver MAO activity, using tyramine as substrate, an approach which does not distinguish between effects on MAO A and B. In the present study, we have used placental MAO A and platelet MAO B to assess any differential action on the two forms of the enzyme.

Materials and Methods

Wistar rats and rats of Krushninskii–Molodkina strain (150–250 g), selected at Moscow State University for high incidence of audiogenic epilepsy, were used. Generalized seizures were induced by the sound of an electric bell (110–120 dB for 90 sec). Their intensity was scored using the following arbitrary units: 0, absence of any response during 90 sec; 1, locomotor excitement; 2, locomotor excitement with jumps and preferred recumbent position "on the belly" (after seizure); 2.5, two wave excitation: locomotor excitement with jumps interrupted for short intervals (5–30 sec), with subsequent manifestation of a second wave of locomotor excitement; 3, clonic seizures and preferred recumbent position "on the side" (after seizure); 4, tonic seizures with total areflexia. rats were killed within 1–2 min after termination of seizure. After decapitation, brains were removed, weighed and frozen at −20° until analysis. Individual brains were then homogenized (20% w/v) in cold 2 M HCl and centrifuged in the cold (0–2°) for 15 min at 3000 *g* to give a clear supernatant which was extracted with 2 vol. of redistilled ethyl acetate. After centrifugation, the organic layer was removed and dried down under nitrogen. The residue was reconstituted in 0.5 mL 100 mM phosphate buffer (pH 7.4) and washed once with 1 mL redistilled heptane. Following removal of the heptane, the aqueous phase was extracted into 2 mL redistilled ethyl acetate. The organic phase was evaporated again and the residue dissolved in 250 μL 100 mM phosphate buffer, pH 7.4. Blanks, containing volumes of 2 M HCl equal to those used for tissue homogenization, were also extracted into ethyl acetate and carried through the whole procedure.

Tribulin activity of brain extracts was quantified either by their effect on human placental MAO A homogenates or on MAO B from freeze thawed suspensions of human platelets, both prepared as described previously [18]. Briefly, 100 μL of final extract, dissolved in buffer, was incubated with 20 μL of MAO preparation and 10 μL substrate. Activity of MAO A was assayed using 170 μM [^{14}C]5-hydroxytryptamine and that of MAO B with 5 μM [^{14}C]phenylethylamine, as described previously [2]. Statistical evaluation was by unpaired Student's *t*-test.

Results and Discussion

The results are summarized in Table 1. Brain tribulin activity in control Wistar rats showed selective inhibition of MAO B (32% inhibition compared with 20% of MAO A). Slight audiogenic seizures in Krushinskii-Molodkina strain rats did not affect the inhibition. Moderate audiogenic seizures in rats, showing double-wave excitation (with the range of intensity of the second wave of excitation from 2 to 3 arbitrary units) was characterized by a significant increase in endogenous brain MAO inhibitory activity (up to 1.9-fold) occurred to a greater extent than that of MAO B inhibitory activity (up to 1.5-fold) and the increase of the ratio of inhibitory activity, MAO A: MAO B, from 0.52 (in rats with slight epileptiform seizures) to 0.65 (in rats with moderate epileptiform seizures) was statistically significant ($P < 0.02$). The most intense audiogenic seizures in rats (4 arbitrary units) were accompanied by a further increase in the MAO A inhibitory component of tribulin, while that of MAO B remained unchanged.

These results are consistent with previous data indicating that electroshock, giving rise to clonic–tonic convulsions [13], and footshock [14] induce augmentation in rat brain tribulin activity. Using separate test systems for MAO A and MAO B assay, we have also been able to follow the different courses of accumulation of selective MAO A and MAO B inhibitory activities. Moderate and especially strong epileptic seizures caused accelerated accumulation of the MAO A (but not MAO B) inhibitory component of brain tribulin, with a concomitant increase in the ratio of MAO A: MAO B inhibitory activities.

A major component of urinary tribulin has been identified

Table 1. Tribulin activity in brain of rats with inherited audiogenic epilepsy

Experimental group	Intensity of seizures (arbitrary units)	No. of animals	Brain weight (g)	Tribulin activity (% inhibition/g wet weight) MAO A	MAO B	Ratio of inhibition MAO A:MAO B
Control (Wistar)	—	6	1.84 ± 0.08	20 ± 5	32 ± 5	0.57 ± 0.09
Epileptic rats	0–2	8	1.90 ± 0.05	20 ± 1	38 ± 1	0.52 ± 0.03
	2.5–3	4	1.90 ± 0.14	37 ± 2*‡	56 ± 2*‡	0.75 ± 0.03†
	4.0	6	1.85 ± 0.05	50 ± 4*‡§	58 ± 2*‡	0.87 ± 0.15†

Epileptogenic seizures in rats of Krushinskii–Molodkina strain were induced by the sound of an electric bell (110–120 dB) for 90 sec.

* $P < 0.01$ compared with control.

† $P < 0.05$; ‡$P < 0.01$ compared with rats showing weak seizures.

§ $P < 0.05$ compared with rats showing moderate seizures.

as isatin (2,3-dioxoindole) [16, 17]. Isatin has a characteristic and discontinuous distribution in rat tissues [18] and in rat brain, with concentrations in the range of 1–10 μM [19]. At low doses this substance has recently been shown to be axiogenic in rodents [20]. It is possible that it, or some derivative, is also proconvulsant. Isatin is a more potent inhibitor of MAO B (IC_{50}, 3 μM) than MAO A (IC_{50}, 63 μM) [16]. The results of the present experiment show clearly that rat brain possesses selective MAO A inhibitory activity, in addition to isatin, which can be induced by audiogenic seizures. We do not know, at present, what this component might be or whether it is a metabolite of isatin. 5-Hydroxyisatin does have such a selective inhibitory action on MAO A (unpublished observations), but we have no evidence at present that is generated endogenously.

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Institute of Biological and Medical Chemistry
Academy of Medical Sciences
Moscow, Russia
**Biological Faculty of Moscow State University*
Moscow, Russia
†Bernhard Baron Memorial Research Laboratories
Queen Charlotte's and Chelsea Hospital
Goldhawk Road
London W6 0XG, U.K.

A. E. Medvedev
V. Z. Gorkin
I. B. Fedotova*
A. F. Semiokhina*
Vivette Glover†‡
M. Sandler†

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‡ Corresponding author.