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Tribulin and Endogenous MAO-Inhibitory Regulation In Vivo

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Abstract

Tribulin is the name given to a family of endogenous nonpeptide substances which inhibit monoamine oxidase (MAO) and benzodiazepine binding. It is widely distributed in mammalian tissues and body fluids, and exhibit some species and tissue variations. Several components selectively inhibiting MAO A, MAO B, central and peripheral benzodiazepine binding (tribulins A, B, BZc and BZp, respectively) have been recognised. Tribulin A represents some tissue-specific metabolites of trace amines, whereas isatin is the major component of tribulin B. Tribulin content increases in brain under conditions of stress and anxiety and is reduced under sedation. Changes in tribulin content in the brain are accompanied by corresponding changes in the content of monoamines and their acidic metabolites, and also by altered susceptibility of MAO to specific mechanism-based inhibitors. This suggests that tribulin is involved in MAO inhibitory regulation in vivo.

Keywords: MAO; Endogenous regulation; Tribulin; Isatin

INTRODUCTION

Endogenous monoamine oxidase (MAO)-inhibitory activity, which was named tribulin by Merton Sandler (Sandler, 1982), was originally discovered in human urine (Glover, 1993; Glover et al., 1980). Attempts to develop a simple method for the evaluation of urinary tyramine excretion based on the competitive inhibition of MAO activity with tyramine as a substrate led to the discovery of the intriguing fact that human urine exhibited potent inhibition of MAO in test systems even without loading of volunteers with tyramine (Glover, 1993). Major and minor components of urine (including monoamine metabolites) did not inhibit MAO (Glover et al., 1980). Tribulin was readily extractable into ethyl acetate and had molecular mass about 180 Da (Glover et al., 1980).

Subsequent studies in man and animals revealed the presence of tribulin in various tissues and body fluids (see for review Glover, 1993, 1998; Glover and Sandler, 1993; Glover et al., 1988; Medvedev, 1996). Increased tribulin output was associated with conditions of stress and anxiety and tribulin was considered as an endocoid marker of stress (Bhattacharya et al., 1991, 1993, 1995, 1996a,b, 2000; Glover, 1993).

Originally, tribulin content was evaluated by inhibition of MAO in test systems containing tyramine (Armando et al., 1989; Bhattacharya et al., 1991; Clow et al., 1983, 1988, 1989; Glover et al., 1980; Lemoine et al., 1990), which is as a common substrate for MAO A and MAO B. The employment of test systems for separate analysis of MAO A and MAO B-inhibitory activity of tribulin revealed different time-course of accumulation of MAO A and MAO B-inhibitory components of brain tribulin in rats with different intensity of audiogenic seizures (Medvedev et al., 1992). Subsequent studies by several groups revealed different responses of the MAO A and MAO B-inhibitory components of tribulin under various stress conditions, with the MAO A component being selectively elevated (Clow et al., 1997; Doyle et al., 1996; Oxenkrug et al., 2000).

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STRUCTURAL COMPONENTS OF TRIBULIN

Functionally, a crude tribulin fraction inhibits not only MAO A and MAO B but also central and peripheral benzodiazepine receptor binding (Armando et al., 1986; Clow et al., 1983; Hucklebridge et al., 1998). The latter suggests that tribulin is a family of nonpeptide molecules, possessing a range of biological activities (Glover, 1998). However, only some of the MAO A and MAO B components have been isolated, purified and identified (Glover et al., 1988; Medvedev et al., 1995a,b). Isatin is a selective inhibitor of MAO B ($K_i \sim 3$–20 μM); it inhibits MAO A with a K_i of about 60–70 μM (Glover et al., 1988; Medvedev et al., 1995c). Several selective MAO A inhibitors have been isolated from crude urinary and brain tribulin. The selective MAO A inhibitors isolated from human urinary tribulin were all esters of tryptamine and tyramine metabolites. They selectively inhibited MAO A (with IC_{50} values ranged from 40 to 120 μM), whereas IC_{50} for MAO B inhibition was >1 mM. Although good evidence exists that these esters were not formed artificially during purification procedure (Medvedev et al., 1995a) they were not detected in pig brain tribulin fraction, where 4-hydroxyphenylethanol seems to be a selective MAO A inhibitor (Medvedev et al., 1995b). The K_i value for 4-hydroxyphenylethanol is rather high (1.4 mM). Perhaps there is some tissue specificity in the distribution of chemical components underlying MAO A-inhibitory activity of tribulin. The latter may be attributed to various preferential pathways of the trace amine metabolism in particular tissue. For example, 4-hydroxyphenyl acetic aldehyde formed during MAO-dependent oxidative deamination of tyramine may be further converted into 4-hydroxyphenylethanol or 4-hydroxyhenylacetic acid or involved into ester formation. It is also possible that several small molecules fit to the substrate/inhibitor binding site of MAO (A) and thus exhibit MAO A inhibition. In the latter case, the steric requirements might represent the only precondition for such type of inhibition (see also the paper by Veselovsky et al., this issue).

ISATIN

Isatin is the major MAO B-inhibitory component of tribulin. Besides effective competitive inhibition of MAO B in vitro isatin administration to rats protects brain MAO B (but not MAO A) against irreversible inhibition by the mechanism-based inhibitor, phenelzine

Table 1
Isatin content of cerebrospinal fluid (CSF) and MAO B-inhibitory activity of CSF tribulin (Sandler et al., 2000)

Isatin content as assessed by GC–MS	42.0 ± 4.6 ng/ml
Calculated concentration of isatin, as diluted in the in the assay medium of tribulin	1.2 ± 0.2 μM
Inhibition of MAO B by CSF tribulin	23.9 ± 2.9%
MAO B inhibition by	
1 μM isatin	23.0 ± 3.8%
10 μM isatin	57.0 ± 2.0%

Tribulin fraction was isolated from human samples of cerebrospinal fluid with known isatin content. The latter was used for calculation of rough isatin concentration in the assay medium containing tribulin. It was assumed that CSF isatin was completely extracted into the tribulin fraction.

(Panova et al., 1997; Sandler et al., 2000). In some tissues and body fluids, isatin does account for the MAO B-inhibitory component, e.g. in cerebrospinal fluid (Sandler et al., 2000, see also Table 1). Hamaue et al. (1998) found a positive correlation between isatin concentration and tribulin-like activity in both rat urine ($r = 0.924$, $P < 0.001$) and kidney extracts ($r = 0.862$, $P < 0.01$).

In contrast to MAO A-inhibitory components which may be considered as products of MAO-dependent metabolism trace amines tyramine and tryptamine (Medvedev et al., 1995a,b), the origin of isatin remains unclear. One possibility is that isatin may be derived from the amino acid tryptophan; however, the chain of events, leading to isatin formation is unknown (Sandler et al., 2000). In vitro isatin catabolism may occur via oxidase and reductase pathways. The latter involves, isatin reductase, a member of a family of NADPH-dependent carbonyl reductases, which has recently been purified from human kidneys and liver (Usami et al., 2001). The enzyme catalyzes NADPH-dependent reduction of isatin into 3-hydroxy-2-oxindole. The apparent K_m value for isatin (10 μM) is within the upper limits of physiological range of concentrations (Usami et al., 2001). Although nothing was known before about the occurrence of this metabolite in vivo it was detected in the human urine (Usami et al., 2001).

The oxidase pathway in vitro involves superoxide-generating systems like xanthine oxidase + xanthine or glucose oxidase + glucose (Medvedev et al., 1994; Sandler et al., 2000). Urate oxidase, generating hydrogen peroxide (in the presence of uric acid), without the intermediate formation of superoxide is ineffective (Medvedev et al., 1994). The reduction of isatin level in these systems was accompanied by the appearance of anthranilic acid (Sandler et al., 2000; Panova, 2001) (Fig. 1). Although this co-oxygenation reaction of isatin degradation is less specific than isatin reduction,

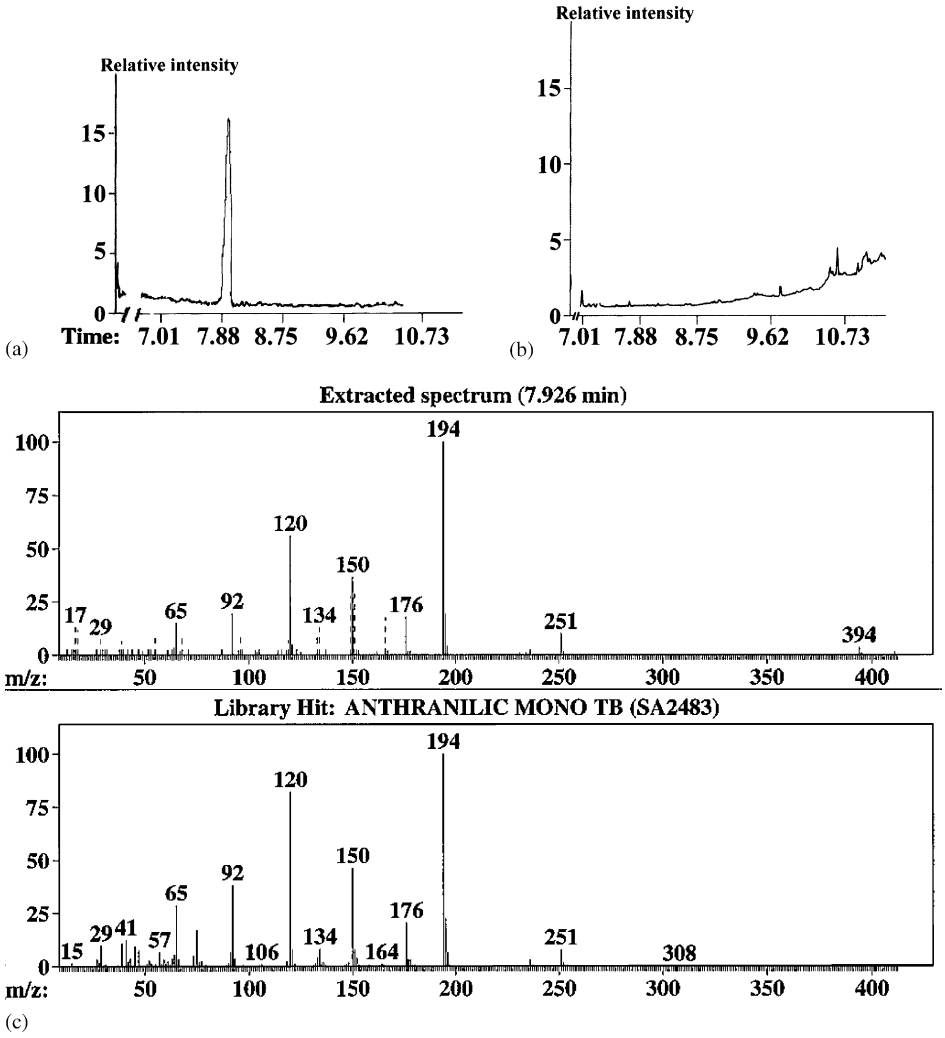

Fig. 1. Anthranilic acid formation during oxidative degradation of isatin by xanthine + xanthine oxidase system (adapted from Panova, 2001): (a) incubation of isatin in the presence of xanthine + xanthine oxidase; (b) incubation of isatin in the presence of xanthine + xanthine + HCl; (c) identification of anthranilic acid.

(any superoxide generating system may be potentially involved in isatin degradation), the resultant anthranilic acid may link isatin catabolism with the general pathway of tryptophan metabolism.

Administration of isatin over a dose range of 10–300 mg/kg increases the concentration of monoamines such as dopamine, 5-HT and noradrenaline in the rat brain (see Table 2). The nature of the effect depends on both the dose and the time of analysis after isatin injection. It is also possible that sensitivity to isatin varies between different rat strains. At higher concentrations, both MAO A and B are inhibited and this has to be considered as a partial explanation for the observed increased levels of noradrenaline and 5-HT, both of which are mainly metabolised by MAO A in rat brain. However, the effects are quite complex and variable at higher dosage and depend on the brain region and monoamine being measured (Table 2). In most studies, an increase in 5-HT concentration was not accompanied by a corresponding decrease in 5-hydroxyindoleacetic acid (5-HIAA), which would have been expected if MAO were inhibited functionally (McIntyre and Norman, 1990; Bhattacharya and Acharya, 1993; Hamaue et al., 1994): in fact, 5-HIAA concentration was essentially unchanged. As MAO A is more important than MAO B for metabolising the major neurotransmitter monoamines in the brain, most of the pharmacological effects described seem likely to stem from some other mechanism, independent of MAO inhibition (Glover et al., 1988). This remains to be identified.

Isatin readily crosses the blood–brain barrier and accumulates in the brain (Bhattacharya et al., 1991). Isatin transport into some type of cells involves the serotonin transporter as it is sensitive to serotonin reuptake inhibitor, fluoxetine (Medvedev et al., 2002).

Table 2
Effect of isatin administration on monoamine levels in rat brain (adapted from Medvedev et al., 1996)

Amine	Isatin dose (mg/kg)	Time of exposure (min)	Rat strain	Brain area	Effect (%)
Serotonin	80	60	Sprague–Dawley	Hypothalamus	+60
				Frontal cortex	+37
Serotonin	80	120	Fisher 344N	Whole brain	+15
Serotonin	10	30	Charles Foster albino	Whole brain	+33
	10	60		Whole brain	+77
	20	15		Whole brain	+96
	20	60		Whole brain	+97
Dopamine	20	15	Wistar Kyoto	Whole brain	+55
	20	30		Whole brain	+81
	20	60		Whole brain	+60
Serotonin	50	120	Wistar Kyoto	Cortex	+47
	50	120		Hypothalamus	+18
	200	120		Cortex	+329
	200	120		Hypothalamus	+82
Noradrenaline	50	120	Wistar Kyoto	Cortex	+27
	50	120		Hypothalamus	–34
	200	120		Cortex	+428
	200	120		Hypothalamus	–46
Serotonin	300	120	Albino rats	Whole brain	+27
	300	360		Whole brain	+15

Isatin-binding sites have been recognised in the brain and peripheral tissues of rat (Figs. 2 and 3) (Ivanov et al., 2002). Isatin binding predominated in the membrane fraction of brain, liver and heart preparations, whereas in the kidneys the highest binding was observed in the soluble fractions. The distribution of isatin binding sites in the particulate fraction reduced in the following order: brainstem > brain hemispheres = cerebellum > heart > kidneys > liver. In the soluble fraction there was a different rank of isatin binding: kidneys > heart > brainstem = brainhemispheres > liver > cerebellum (Ivanov et al., 2002).

Purification of the rat liver mitochondria by digitonin treatment and isolation of the purified outer mitochondrial membranes (the total content of marker enzyme of the inner mitochondrial membrane was less than 10% of mitochondrial activity) increased specific binding of these preparations to immobilised isatin (Ivanov et al., 2002). Changes in such binding by clorgyline and deprenyl points to an interaction of immobilised isatin with mitochondrial MAO (Ivanov et al., 2002). However, it is clear that besides MAO other targets for isatin also exist. These include atrial natriuretic peptide (ANP) stimulated particulate guanylate cyclase (GC) (Glover et al., 1995; Medvedev et al., 1998; Sandler, 1982) and soluble nitric oxide-stimulated guanylate cyclase (Medvedev et al., 2002). These enzymes are even more sensitive to isatin than MAO B.

Certain evidence exists that isatin antagonises atrial natriuretic peptides in vivo. Isatin administration blocks cyclic GMP excretion under conditions of fluid overload, which recruits endogenous natriuretic peptides (Medvedev et al., 2001). It also inhibited brain natriuretic and C-type natriuretic peptide-induced facilitation of memory consolidation in passive-avoidance learning in rats (Telegdy et al., 2000) and some other natriuretuic peptide-dependent effects (Bhattacharya et al., 1996a,b; Pataki et al., 2002).

It should be noted that ANP antagonises certain monoamine functions (Sandler et al., 2000). It inhibits catecholamine release from adrenal medulla and, in the brain, it is a functional antagonist of angiotensin II which potentiates catecholamine release (Galli and Phillips, 1996; Peng et al., 1996; Matsukawa and Mano, 1996; Wuttke et al., 1992). It is possible that isatin, as the major MAO B-inhibitory component may rise monoamine levels (by MAO-dependent and independent mechanisms) and inhibit natriuretic peptide receptor binding and intracellular signal transduction mechanism. The simultaneous inhibition of a key enzyme of monoamine metabolism (MAO) and of natriuresis may represent a new regulatory mechanism.

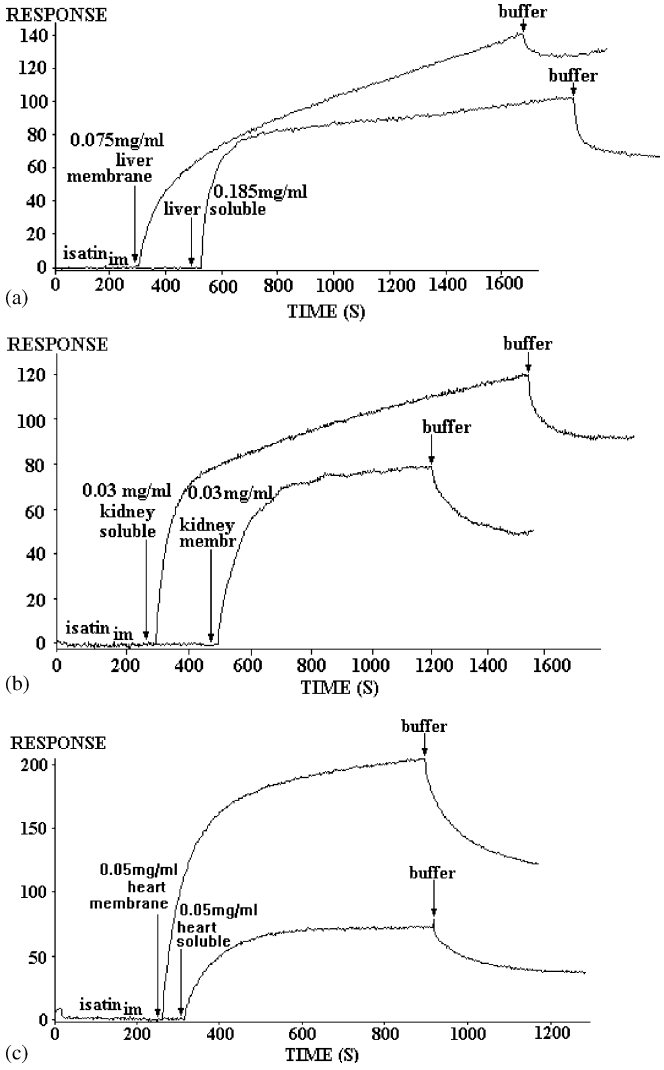

Fig. 2. Isatin binding activity of liver (a), kidney (b), and heart (c) preparations (Ivanov et al., 2002).

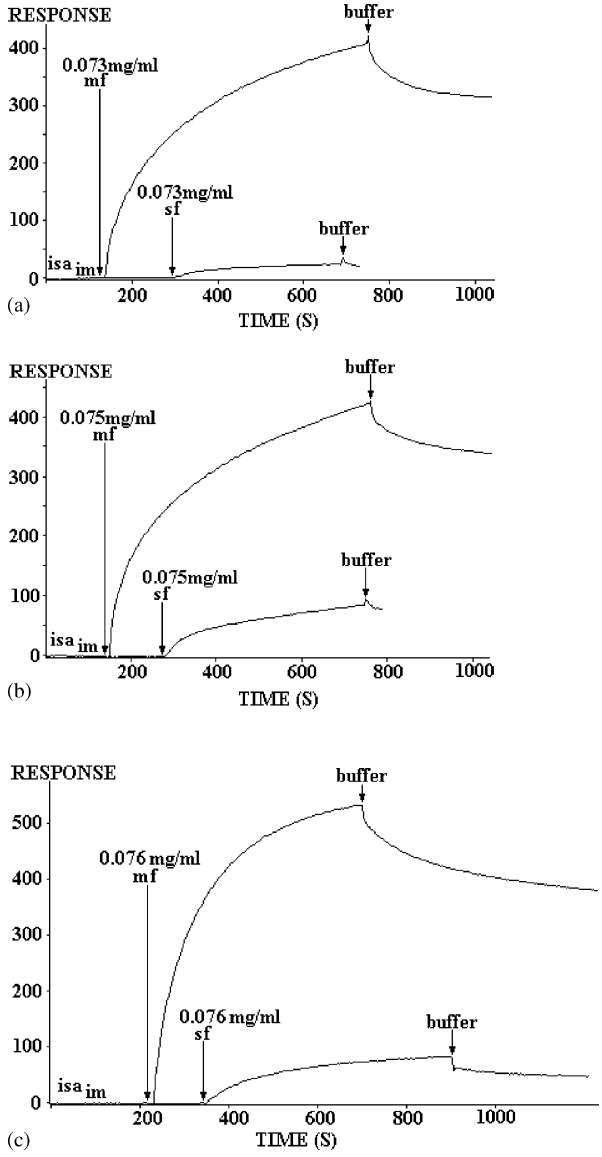

Fig. 3. Isatin binding activity of rat cerebellum (a), hemispheres (b) and brainstem (c).

TRIBULIN A USEFUL CONCEPT?

There have been two interrelated but rather distinct views of tribulin. Some authors have considered tribulin (and its components) as a putative endocoid marker of stress and anxiety (Bhattacharya et al., 2000; Satyan et al., 1995), whereas others believe that the regulation of tribulin level in the brain is itself of biological importance (Clow et al., 1997; Glover, 1993, 1998).

If any compound or a family of related compounds are to be biologically important regulators of some processes (or enzymes) in the cell they obviously should meet several criteria.

(1) There should be certain conditions favouring not only *increased* but also *decreased* formation of such compound(s).
(2) Opposite changes in the level of the compound(s) should be observed under opposite behavioural reactions (e.g. anxiety versus sedation).
(3) Opposite changes in the content of the compound(s) should cause opposite changes in the activity of corresponding enzyme.

Tribulin as the endogenous monoamine oxidase regulator has to be subjected to dual regulation: increase under conditions of stress and anxiety and decrease under sedation. There are several experimental observations that tribulin meets the two first criteria. For example, oral administration of extracts Rhazya stricta leaves, which have both antidepressant and sedative properties caused opposite effects on the

MAO A-inhibitory components of rat brain tribulin (Ali et al., 1998). In an acute study, intermediate doses caused a significant reduction of MAO A-inhibitory activity. Subchronic administration (21 days) caused a significant decrease in MAO A-inhibitory activity, most prominent at low dosage, and an increase in MAO B-inhibitory activity (Ali et al., 1998). Administration of bioactive glycowithanolides from the roots of Withania somnifera or lorazepam also caused reduction of brain tribulin level (Bhattacharya et al., 2000). Chronic alcohol feeding of rats resulted in some decrease of MAO A- and MAO B-inhibitory components of brain tribulin (Panova et al., 2000) whereas the development of drug (or ethanol) withdrawal anxiety in rats was accompanied by a clear increase of brain tribulin components (Bhattacharya et al., 1995). These data provide convincing evidence for the existence of flexible regulation of tribulin level in the brain.

Demonstration of monoamine oxidase regulation by tribulin in vivo (the third criterion, see above) represents a rather more complex problem, because of the readily reversible tribulin interaction with MAO. This makes hard to detect significant MAO inhibition after tissue preparations in situ. So functional inhibition of MAO in vivo is usually analysed by comparison of the content of monoamines and their metabolites and also by the sensitivity of MAO to specific irreversible mechanism-based inhibitors such as phenelzine, pargyline, clorgyline etc.

Detection of increased monoamine levels accompanied by corresponding decrease of monoamine acidic metabolite(s) under conditions of elevated tribulin output gives a reasonable support for a physiological role for tribulin in MAO regulation in vivo (Clow et al., 1988; McIntyre et al., 1989; Oxenkrug and McIntyre, 1985). However, there is evidence that the acidic metabolites may also be formed in the brain in an MAO-independent manner (Dyck et al., 1993). Nevertheless, the results of several studies have provided convincing evidence for the regulatory role of tribulin as an endogenous MAO inhibitor in vivo. For example, cold-restrained stress increased tribulin content in the rat brain, and the ratio of 5-hydroxytryptamine/5-hydroxyindole acetic acid in the pineal gland (Oxenkrug and McIntyre, 1985; Oxenkrug et al., 2000). This was accompanied by an increase of *N*-acetylserotonin and melatonin content (Oxenkrug and McIntyre, 1985; Oxenkrug et al., 2000). The same changes in pineal *N*-acetylserotonin and melatonin content were also observed after administration of MAO (A) inhibitors (McIntyre et al., 1985; Oxenkrug et al., 1994).

In man, the functional inhibition of MAO under conditions of increased tribulin output is less documented. Nevertheless, it has been demonstrated that lactate-induced panic attacks caused increased output of urinary tribulin, which was accompanied by reduction of urinary excretion of catecholamine metabolites (Clow et al., 1988).

Clow et al. (1989) found that under conditions of increased tribulin output the administration of the antidepressant phenelzine to rats produced smaller MAO inhibition compared to the control. Similar results have been obtained by Lemoine et al. (1990), who found that stress conditions, characterised by increased tribulin output also reduced sensitivity of MAO A to the mechanism-based inhibitor, clorgyline. These data suggest endogenous MAO inhibition by tribulin in vivo which decreases the effect of exogenously administered irreversible inhibitors. However, the latter experimental approach does not take into consideration the possibility of stress-induced oxidative modification of MAO molecules, which is quite possible and which can also account for reduced sensitivity to specific inhibitors (Gorkin, 1983; Medvedev and Tipton, 1997). This problem may be overcome by comparison of in vitro sensitivity of MAOs from control and experimental animals to the inhibitor used. If the sensitivity of MAO to such inhibitor(s) remains unaltered this is the decisive argument, which rules out the possibility of stress-induced modification of MAO. Using this argument Panova et al. (2000) found that chronic alcohol treatment reduced brain tribulin content and increased in the vivo sensitivity of MAO A to the mechanism-based inhibitor, pargyline. Since the in vitro sensitivity of MAO to this inhibitor remained unchanged the increased sensitivity of brain MAO A of alcoholised rats to pargyline may be reasonably attributed to the deficit of brain tribulin.

CONCLUSION

Twenty years ago Merton Sandler named the endogenous monoamine oxidase/benzodiazpine receptor inhibitory activity as tribulin (Sandler, 1982). Now it is clear that tribulin is a complex family of endogenous inhibitors which may be further classified into tribulin A (selectively inhibiting MAO A), tribulin B (selectively inhibiting MAO B), and tribulin BZc and BZp (c and p for central and peripheral benzodazepine inhibitors). These tribulins obviously represent various chemical molecules, which may be different in different tissues. Good evidence now exists that tribulin A and

tribulin B regulate the activity of MAO A and MAO B, respectively. The role of tribulins BZc and BZp as regulatory substances remains to be clarified. Some chemical components as isatin have started their own MAO-independent life and this indicates the existence of various links in the regulatory pathways between MAO, monoamines and other signalling systems.

Thus, we should take the tribulin story into the third millennium and continue to investigate this intriguing family and to characterise its biological and medical role.

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