4. Gonzalez FJ, Matsunaga T, Nagata K, Meyer UA, Nebert DW, Pastewka J, Kozak CA, Gillette J, Gelboin HV and Hardwick JP, Debrisoquine 4-hydroxylase: characterisation of a new P450 gene subfamily, regulation, chromosomal mapping and molecular analysis of the DA rat polymorphism. *DNA* **17**: 149–161, 1987.
5. Tanaka E, Kobayashi S, Uchida E, Oguchi K and Yasuhara H, Antipyrine metabolism in female Lewis and Dark Agouti strains of rats, which are extensive and poor metabolisers of debrisoquine, respectively. *Jap J Pharmacol* **49**: 433–435, 1989.
6. Daly M, Houston JB, Lennard MS, Smith DA and Tucker GT, Drug metabolising capacity of the dark agouti (DA) rat *in vitro* and *in vivo*. In: *Proceedings of the Eleventh European Workshop on Drug Metabolism, Konstanz, September 1988, Abstract 206*, 1988.
7. Jacqz E, Billante C, Moysan F and Mathieu H, The non-human primate: a possible model for human genetically determined polymorphisms in oxidative drug metabolism. *Mol Pharmacol* **34**: 215–217, 1988.
8. Otton SV, Tyndale RF, Inaba T, Kalow W and Sellers EM, Monkey and human hepatic cytochrome P450db1 are catalytically and immunologically identical. In: *Proceedings of the VIIIth International Symposium on Microsomes and Drug Oxidations, Stockholm, 1990.* Abstract 379, 1990.
9. Tannen RH and Weber WW, Inheritance of acetylator phenotype in mice. *J Pharmacol Exp Ther* **213**: 480–484, 1980.
10. Otterness DM, Keith RA and Weinshilboum RM, Thiopurine methyltransferase: mouse kidney and liver assay conditions, biochemical properties and strain variation. *Biochem Pharmacol* **34**: 3823–3830, 1985.
11. McGourty JC, Silas JH, Lennard MS, Tucker GT and Woods HF, Metoprolol metabolism and debrisoquine oxidation polymorphism—population and family studies. *Br J Clin Pharmacol* **20**: 555–566, 1986.
12. Staats J, Standardised nomenclature for inbred strains of mice. *Cancer Res* **45**: 945–977, 1985.
13. Otton SV, Crewe HK, Lennard MS, Tucker GT and Woods HF, Use of quinidine inhibition to define the role of the sparteine/debrisoquine cytochrome P450 in metoprolol metabolism by human liver microsomes. *J Pharmacol Exp Ther* **247**: 242–247 1988.
14. Cornish-Bowden A, *Fundamentals of Enzyme Kinetics*. p. 26. Butterworths, London, 1979.
15. Lennard MS, Adams M, Otton SV, Tucker GT and Woods HF, Species differences in the sensitivity of metoprolol oxidation to inhibition by the quinidine/quinine isomer pair. *Br J Clin Pharmacol* **30**: 323P, 1990.
16. Kobayashi S, Murray S, Watson D, Sesardic D, Davies DS and Boobis AR, The specificity of inhibition of debrisoquine 4-hydroxylase activity in the rat is the inverse to that in humans. *Biochem Pharmacol* **38**: 2795–2799, 1989.

Biochemical Pharmacology, Vol. 42, No. 4, pp. 949–951, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Metabolism of T-2 toxin by rat brain homogenate

(*Received* 5 *February* 1991; *accepted* 16 *April* 1991)

T-2 toxin is one of the most poisonous trichothecenes, produced by *Fusarium* species [1]. Its metabolism has been studied in the liver, in various internal organs [2, 3] and in whole blood and its components [4]. Ohta *et al.* [5] included rat brain in their experiments. Microsomal preparations of this organ converted T-2 toxin into HT-2 toxin in 30 min, but further metabolic changes were not studied, in spite of the extensive metabolic reactions found with liver. Furthermore, it was not stated whether the brain was used with its physiological blood content.

Recently we reported that direct administration of T-2 toxin to the rat brain causes death with doses that are only a low percentage of those required for systemic applications [6]. These observations led to the question of whether cerebral T-2 toxin metabolism differs from its metabolism following peripheral administration. This problem was approached by treatment of the toxin with rat brain homogenate. Since whole blood degrades T-2 toxin effectively [4], we have prepared this homogenate by two procedures: either by direct decapitation, extraction of the brain and its homogenization; or first exhaustive saline perfusion to remove all the blood from the brain before decapitation and homogenization.

Materials and Methods

Chemicals (see Fig. 1). The toxin and neosolaniol were isolated from *Fusarium sporotrichioides* [7]. HT-2 toxin, T-2 triol, T-2 tetraol, 4-deacetylneosolaniol (DANS) and 15-monoacetylscirpenol (MAS) were prepared according to the literature [8]. Identification was performed by NMR and mass spectroscopy, purity was checked by GLC. For analysis by GLC with a ^{63}Ni electron capture detector, all trichothecenes were converted into their heptafluorobutyric esters [9]. Heptafluorobutyric (HFB) anhydride was purchased from Aldrich (Milwaukee, WI, U.S.A.) and trimethylamine (TMA) from BDH (Poole, U.K.).

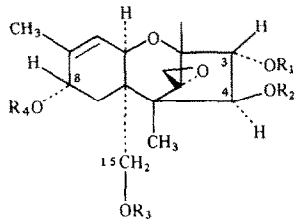
Brain homogenates. Male albino rats of the Hebrew University Sabra strain, of 250 ± 50 g body weight, were used. The animals were anesthetized with pentobarbitone-sodium (40–50 mg/kg i.p. or s.c.) and were perfused via the heart with 37° warm saline, until the perfusate was practically colorless. The skull was rapidly opened, the brain was removed and homogenized with 10 mL of 0.1 M phosphate buffer pH 7.4, per gram of tissue, using an Ultra Turrax homogenizer.

Homogenates were also prepared from whole rat brain without previous perfusion. Such preparations contain about 40 μL of blood per gram of brain [10].

Analysis. The apparatus used for gas chromatography and the conditions of the analytical procedure, including the method of derivatization with HFB-anhydride, have been described previously [9].

Incubation. In a 100 mL Erlenmeyer flask, 20 mL of brain homogenate were thoroughly mixed with 5–10 μg/mL of substrate in dimethyl sulfoxide solution.

Penicillin and streptomycin were added to suppress the growth of airborne microorganisms. The vessels were shaken at 37° for 90 min, and samples of 3 mL were



Compound	R_1	R_2	R_3	R_4
T-2 toxin	H	$COCH_3$	$COCH_3$	$COCH_2CHMe_2$
HT-2 toxin	H	H	$COCH_3$	$COCH_2CHMe_2$
T-2 triol	H	H	H	$COCH_2CHMe_2$
T-2 tetraol	H	H	H	H
Neosolaniol	H	$COCH_3$	$COCH_3$	H
4-Deacetylneosolaniol (DANS)	H	H	$COCH_3$	H

Fig. 1. Chemical structures of 12,13-epoxytrichothecenes.

withdrawn at 0, 15, 30, 60 and 90 min. Each sample was immediately immersed for 1 min in boiling water to stop all enzymatic reactions. Then MAS was added as internal standard.

Extraction procedure. Each sample was mixed with 2 mL of ethyl acetate and stirred in a vortex for 30 sec, followed by centrifugation at 4000 rpm for 20 min. The organic supernatant was taken off, and the whole procedure was repeated a second time. The combined organic layers were brought to dryness *in vacuo*. The solid residue was dissolved in a 1:1 mixture of methylene chloride and methanol. This solution was placed on a small column, filled with 1.5 g of silica gel, which previously had been washed three times with 3 mL methanol. The column was eluted with 2 mL of a mixture of 70% ethyl acetate and 30% methanol. The eluate was brought to dryness under a stream of nitrogen. The residue was derivatized with HFB-anhydride and analysed by GC, as described previously [9]. The metabolites were identified by measurements of GC–mass spectrometry (LKB 2091) (GC–MS), as described previously [9].

Statistics. Each experiment was repeated three to four times. Standard deviations (SD) of the measurements were calculated by ANOVA.

Results and Discussion

Different results were obtained with the two types of homogenate. Homogenate from perfused brain caused hydrolysis of the 4-acetoxy group of T-2 toxin and formation of HT-2 toxin. T-2 triol, neosolaniol, DANS and T-2 tetraol were not formed in amounts determinable by the methods used, i.e. with a lower limit of 1 ng/mL. For quantitative evaluation of the production of HT-2 toxin, recovery rates were determined as follows: T-2 toxin, 65%; HT-2 toxin, 30%. Using these values, Fig. 2 was constructed which shows that the sum of these two compounds remains approximately constant over the period of 90 min. This supports the conclusion that no other metabolite was formed during this period.

The reaction mixture of homogenate from non-perfused brain and T-2 toxin contained at various times HT-2 toxin, T-2 triol, neosolaniol, DANS and T-2 tetraol, which were identified by GC–MS analysis. All these metabolites are formed by the blood [4], except HT-2 toxin which is produced both by blood and by brain tissue. When any of

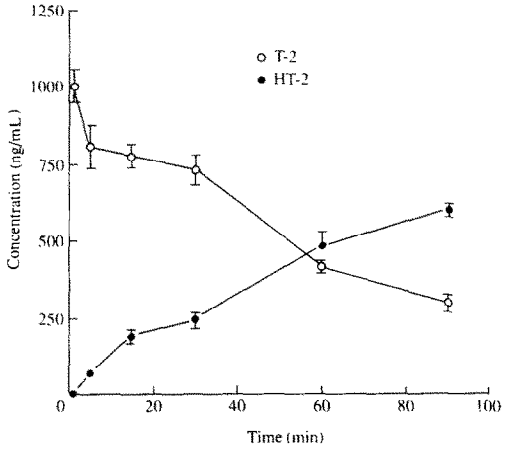


Fig. 2. Enzymatic conversion of T-2 toxin to HT-2 toxin by a homogenate of saline-perfused rat brain. For construction of the points the different recovery factors were used: T-2 toxin, 65%; HT-2 toxin, 30%.

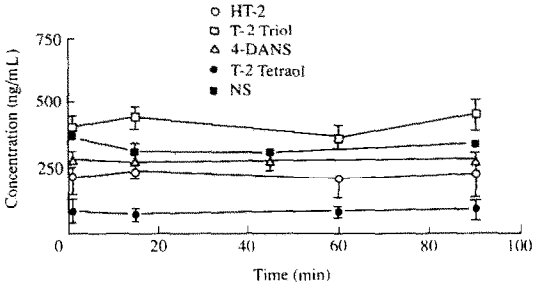


Fig. 3. Stability of trichothecenes upon incubation with a homogenate of saline-perfused rat brain.

these metabolites was incubated with perfused brain homogenate for 90 min or longer, it was recovered unchanged (Fig. 3).

The cerebral hydrolase, responsible for the conversion of T-2 toxin to HT-2 toxin (Fig. 2), exhibits pronounced specificity, since the other ester groups of the T-2 toxin molecule (Fig. 1) are not attacked. In this respect the brain resembles the rat leucocytes. The latter also perform essentially the hydrolysis of the 4-acetoxy group, while other metabolites are either missing or are produced only in traces, which may result from contamination with traces of erythrocytes. Indeed red blood cells yield all the metabolites mentioned above [4].

HT-2 toxin is six times more toxic than T-2 toxin, when applied directly to the rat brain [6]. Formation, stability and accumulation of HT-2 toxin in the brain, upon direct cerebral administration of T-2 toxin, may explain, at least in part, the high cerebral toxicity of the latter.

In summary, HT-2 toxin was the sole metabolite formed when T-2 toxin was treated with homogenate from brain without its blood content. Homogenate from brain with its full blood content produced—besides HT-2 toxin—T-2 triol, neosolaniol, 4-deacetylneosolaniol and T-2 tetraol, i.e. the same metabolites formed by incubation of T-2 toxin with whole rat blood.

Acknowledgement—This research was supported in part by a grant from the Peter Hylston Foundation for Medical Research.

*Department of Natural Products
The School of Pharmacy and
†Department of Pharmacology
The Hebrew University—
Hadassah Medical School
Jerusalem, Israel

BORIS YAGEN*
FELIX BERGMANN†‡
SHIMON BAREL*
AMNON SINTOV*

REFERENCES

1. Ueno Y, Mode of action of trichothecenes. *Pure Appl Chem* **49**: 1737–1745, 1977.
2. Ellison RA and Kotsonis FN, *In vitro* metabolism of T-2 toxin. *Appl Microbiol* **27**: 423–424, 1974.
3. Pace J, Watts MR, Burrows EP, Dinterman RE, Matson C, Hauer EC and Wannemacher Jr RW, Fate and distribution of [^{3}H] labeled T-2 mycotoxin in guinea pigs. *Toxicol Appl Pharmacol* **80**: 377–385, 1985.
4. Johnsen H, Odden E, Johnsen BA and Fonnum F, Metabolism of T-2 toxin by blood cell carboxylesterases. *Biochem Pharmacol* **37**: 3193–3197, 1988.
5. Ohta M, Ishii K and Ueno Y, Metabolism of trichothecene mycotoxins. I. Microsomal deacetylation of T-2 toxin in animal tissues. *J Biochem* **82**: 1591–1598, 1977.
6. Bergmann F, Yagen B and Soffer D, Toxic and lethal effects of T-2 toxin upon intracerebral administration to rats. *Arch Toxicol* **58**: 40–44, 1985.
7. Yagen B and Joffe AZ, Screening of toxic isolates of *Fusarium poae* and *Fusarium sporotrichioides* involved in causing alimentary toxic aleukia. *Appl Environ Microbiol* **32**: 423–427, 1976.
8. Wei RD, Strong FN, Smalley EB and Schnoes HK, Chemical interconversion of T-2 and HT-2 toxins and related compounds. *Biochem Biophys Res Commun* **45**: 396–401, 1971.
9. Sintov A, Bialer M and Yagen B, Pharmacokinetics of T-2 tetraol, a urinary metabolite of the trichothecene mycotoxin, T-2 toxin, in dog. *Xenobiotics* **17**: 941–950, 1987.
10. Kirk A Frey, Positron emission tomography. In: *Basic Neurochemistry* (Ed. Siegel CJ), Chap. 44, pp. 839–855 (especially p. 843). Raven Press, New York, 1989.

‡ To whom correspondence should be addressed.

Biochemical Pharmacology, Vol. 42, No. 4, pp. 951–954, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Idebenone, an agent improving cerebral metabolism, stimulates [^{14}C]tyrosine uptake and [^{14}C]catecholamine formation by cultured bovine adrenal chromaffin cells

(*Received* 18 *February* 1991; *accepted* 16 *April* 1991)

Idebenone, [6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone] is a therapeutic agent for improving cerebral metabolism. It is reported to prevent or improve neurological disorders, such as impairment of memory retention and locomotor activity, induced by experimental cerebral ischemia [1]. Neurochemical studies showed that idebenone restored the reduction of glucose utilization and the decrease in ATP content in ischemic rat brain [2]. Furthermore, idebenone was shown to increase ATP formation in isolated mitochondria and inhibit lipid peroxidation in mitochondrial membranes [3]. It has also been shown to reverse the decrease in the contents or turnovers of the neurotransmitters acetylcholine and serotonin in the brain of rats with experimental ischemia [4, 5]. However, little is known about the effect of idebenone on catecholaminergic neurons.

In this study, to obtain information on the effect of idebenone on catecholamine (CA) metabolism, we examined whether it affected CA formation from tyrosine in cultured bovine adrenal chromaffin cells. Adrenal chromaffin cells are regarded as a model for catecholaminergic neurons, and are useful for studies on CA biosynthesis as well as CA release.

Materials and Methods

Cell preparation and culture. Bovine adrenal chromaffin cells were dispersed enzymatically [6] and maintained for 3 days in culture in 35-mm tissue cultured dishes at a density of 10^6 cells/dish, in Eagle's basal medium supplemented with 5% heat-inactivated fetal calf serum, 2 mM glutamine, penicillin (100 units/mL), streptomycin (100 μg/mL), gentamicin (40 μg/mL), fungizone (2.5 μg/mL) and 10 μM cytosine arabinoside [7].

Measurement of [^{14}C]*tyrosine uptake and* [^{14}C]*catecholamine formation.* The cultured cells were washed with 1 mL of balanced salt solution [BSS (mM): 135, NaCl;