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.

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*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.

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

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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;

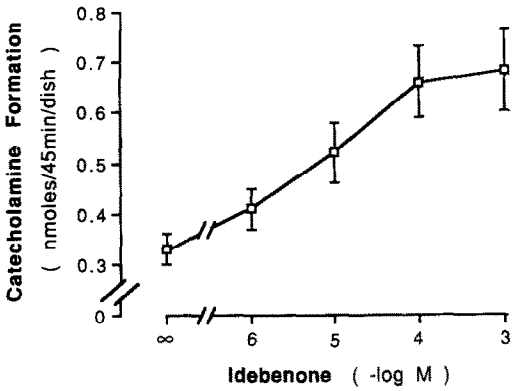

Fig. 1. Concentration–response curve for the effect of idebenone on [^{14}C]CA formation from [^{14}C]tyrosine in cultured bovine adrenal chromaffin cells. The cells were incubated for 45 min with 50 μM [^{14}C]tyrosine (0.5 μCi) in the presence of the indicated concentrations of idebenone. [^{14}C]CA formation was determined as described in Materials and Methods. Values are means ± SEM for 5–7 experiments.

5.6, KCl; 1.2, $MgSO_4$; 2.2, $CaCl_2$; 10, glucose; and 20, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)/NaOH; pH 7.4], and then incubated with BSS containing [^{14}C]tyrosine (final concentration 10–100 μM, 0.5 μCi) at 37° for 5 to 60 min in the presence or absence of test compounds. After incubation, the dishes were rapidly chilled on ice, the medium was removed, and the cells were washed twice with 1 mL of ice-cold BSS and lysed by adding 1 mL of 0.4 N perchloric acid followed by freeze–thawing. Radioactivity in the acid extract was counted in a liquid scintillation spectrometer to determine the amount of [^{14}C]tyrosine taken up into the cells. [^{14}C]-CA in the acid extracts was isolated on aluminium hydroxide, and radioactivity eluted from the gel was counted by liquid scintillation spectrometry [8]. In some experiments, L-[^{14}C]DOPA (50 μM, 0.5 μCi) was used as the substrate instead of [^{14}C]tyrosine. [^{14}C]CA formed from ^{14}C-labelled substrates was measured by ion-exchange chromatography on a Duolite C-25 column [8]. [^{14}C]-Dopamine and [^{14}C]norepinephrine plus [^{14}C]epinephrine constituted approx. 75% and 25% of the total labelled CA, respectively. The percentages of these newly synthesized [^{14}C]CA were similar on incubation with and without test compounds. For determination of endogenous CA release, the CA contents of the cells and medium were determined by fluorometric method [8]. The results were expressed as a percentage of the total amount of cellular CA.

Chemicals. The following materials were used: L-[^{14}C]-tyrosine (Amersham, Japan); 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (Idebenone, Takeda Co.); calcium hopantenate (Tanabe Co.); 3,7-dihydro - 3 - methyl - 1 - (5-oxohexyl)-7-propyl-7*H*-purine-2,6-dione (Propentofylline, Hoechst Co.); carbamylcholine and dibutyryl cyclic AMP (Sigma Chemical Co.). Other chemicals were commercial products of reagent grade.

Results and Discussion

Figure 1 shows the effect of idebenone on [^{14}C]CA formation from [^{14}C]tyrosine in cultured bovine adrenal chromaffin cells on incubation for 45 min. Idebenone stimulated the formation of [^{14}C]CA from [^{14}C]tyrosine in a concentration dependent manner. Stimulation by idebenone was detectable at 10^{-6} M and maximal (about 2-fold) at 10^{-4} M. Under these conditions, idebenone (10^{-4} M) did not affect the release of endogenous CA from the cells (control, 3.8 ± 0.2%; idebenone-treated cells, 4.1 ± 0.3%). Moreover, it did not stimulate [^{14}C]CA formation with [^{14}C]DOPA instead of [^{14}C]tyrosine as substrate (control, 5.5 ± 0.4 nmol, idebenone-treated cells, 5.1 ± 0.5 nmol).

There are many reports on the mechanism regulating CA formation from tyrosine in various tissues [9]. Precursor supply (tyrosine uptake by the cells) is considered to be one factor regulating CA formation in the brain [10]. Therefore, we examined whether the increase in formation

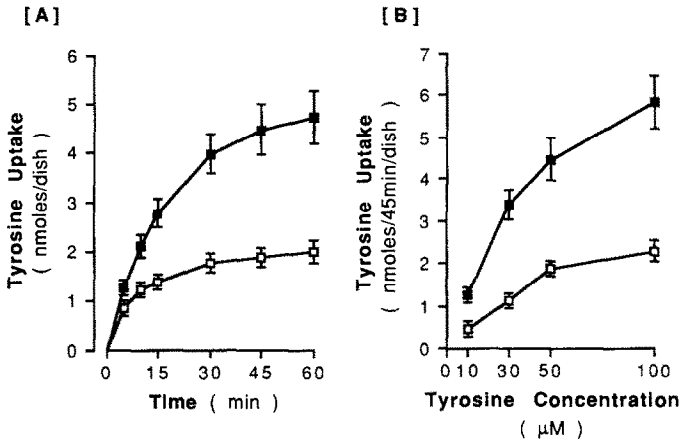

Fig. 2. (A) Time course for the effect of idebenone on [^{14}C]tyrosine uptake by cultured bovine adrenal chromaffin cells. The cells were incubated for the indicated times with 50 μM [^{14}C]tyrosine (0.5 μCi) in the presence (■) or absence (□) of idebenone (10^{-4} M). [^{14}C]Tyrosine taken up by the cells was determined as described in Materials and Methods. Values are the means ± SEM for 5–7 experiments. (B) Effect of idebenone on [^{14}C]tyrosine uptake by cultured bovine adrenal chromaffin cells as a function of tyrosine concentration. The cells were incubated for 45 min with various concentrations of [^{14}C]tyrosine (0.5 μCi) in the presence (■) or absence (□) of idebenone (10^{-4} M). [^{14}C]Tyrosine taken up by the cells was determined as described in Materials and Methods. Values are means ± SEM for 3–5 experiments.

Table 1. [^{14}C]Tyrosine uptake and [^{14}C]CA formation by cultured bovine adrenal chromaffin cells incubated with various agents

Addition	[^{14}C]Tyrosine uptake (nmol/45 min/dish)	[^{14}C]CA formation (nmol/45 min/dish)	Conversion rate (%)
None	1.87 ± 0.19	0.33 ± 0.03	17.6
Idebenone	4.48 ± 0.51*	0.67 ± 0.07*	14.7
Hopantenate	1.79 ± 0.19	0.32 ± 0.03	17.9
Propentofylline	1.97 ± 0.22	0.36 ± 0.04	18.3
Vitamin K_2	1.82 ± 0.19	0.34 ± 0.03	18.7
Carbamylcholine	1.95 ± 0.21	0.64 ± 0.08*	32.8*
DB·cAMP	2.01 ± 0.24	0.59 ± 0.06*	29.4*

The cells were incubated for 45 min with 50 μM [^{14}C]tyrosine (0.5 μCi) in the presence or absence of idebenone (10^{-4} M), hopantenate (10^{-4} M), propentofylline (10^{-4} M), vitamin K_2 (10^{-4} M), carbamylcholine (10^{-5} M) or dibutyryl cyclic AMP (DB·cAMP 10^{-3} M). [^{14}C]Tyrosine uptake and [^{14}C]CA formation were determined as described in Materials and Methods.

Values are means ± SEM for 5–7 experiments (* $P < 0.01$ vs control value). The conversion rate was calculated as the percentage conversion of [^{14}C]tyrosine taken up by the cells to [^{14}C]CA during the incubation period.

of [^{14}C]CA caused by idebenone was due to an increase in [^{14}C]tyrosine uptake by the cells. Figure 2A shows the effect of idebenone on [^{14}C]tyrosine uptake by the cells as a function of time. The results show that idebenone stimulated the uptake of [^{14}C]tyrosine by the cells: its effect was detectable after 5 min and observed during incubation for 60 min. At each incubation time, idebenone induced a 2–2.5-fold increase in [^{14}C]tyrosine uptake.

Next, we examined the effect of idebenone on [^{14}C]-tyrosine uptake by the cells in the presence of various concentrations of tyrosine. As shown in Fig. 2B, stimulation by idebenone of [^{14}C]tyrosine uptake was observed at concentrations of tyrosine of 10–100 μM. The stimulation was clearly observed even when a saturating concentration of tyrosine, 100 μM, was present in the medium. Kinetic analysis of these results showed that idebenone caused an increase in the V_{max} without any change in the K_m value (the V_{max} values of the control and idebenone-stimulated cells were 5.20 ± 0.56 and 11.88 ± 1.32 nmol/dish/45 min and the K_m values were 88.9 ± 9.2 and 83.3 ± 8.6 μM, respectively).

These results indicate that idebenone increased [^{14}C]-tyrosine uptake by the cells, leading to an increase in accumulation of [^{14}C]tyrosine in the cells, and thus resulting in an increase in [^{14}C]CA formation from [^{14}C]tyrosine in the cells. In fact, as shown in Table 1, the rate of conversion of [^{14}C]tyrosine to [^{14}C]CA (expressed as the percentage conversion of [^{14}C]tyrosine taken up by the cells) was not affected by idebenone.

Table 1 also shows the effects of other agents on [^{14}C]-tyrosine uptake and [^{14}C]CA formation by adrenal chromaffin cells. Hopantenate and propentofylline, other agents that improve the cerebral metabolism, had no significant effect on [^{14}C]tyrosine uptake or formation of [^{14}C]CA from [^{14}C]tyrosine. VK_2, a benzoquinone derivative similar to idebenone, also did not affect [^{14}C]-tyrosine uptake or [^{14}C]CA formation by the cells. Carbamylcholine and dibutyryl cyclic AMP (DB·cAMP), which are known to stimulate CA synthesis not only in adrenal medulla [11–15], but also in other tissues [16–20], increased [^{14}C]CA formation from [^{14}C]tyrosine under the experimental conditions, but they did not affect [^{14}C]-tyrosine uptake by the cells. Therefore, the rate of conversion of [^{14}C]tyrosine to [^{14}C]CA was increased on stimulation by carbamylcholine and DB·cAMP. The stimulation of CA formation by carbamylcholine or DB·cAMP is thought to be mediated by Ca^{2+}-calmodulin-dependent or cAMP-dependent protein kinase [9]. Thus, the stimulatory effect of idebenone seemed to be quite different from that of carbamylcholine or DB·cAMP on [^{14}C]CA formation from [^{14}C]tyrosine.

From these results we concluded that idebenone, an agent improving cerebral metabolism, stimulated [^{14}C]-tyrosine uptake and [^{14}C]CA formation by cultured bovine adrenal chromaffin cells and that the increase in [^{14}C]CA formation was mainly due to an increase in [^{14}C]tyrosine uptake (increase in precursor supply) by the cells. The mechanism by which idebenone increased [^{14}C]tyrosine uptake by the cells is unknown, but these findings indicate a new mode of action of idebenone on catecholaminergic neurons in the central nervous system. However, it is not certain whether the concentrations of idebenone used in this study are related to those achieved in the brain *in vivo*. We are now investigating the effect of idebenone on CA formation from tyrosine in guinea pig brain slices and *in vivo*.

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Department of Pharmacology
Tokushima University School of Medicine
3-Kuramoto
Tokushima 770
Japan

HITOSHI HOUCHI
MASANORI YOSHIZUMI
KAZUO MINAKUCHI
MAMI AZUMA
KYOJI MORITA
MOTOO OKA*

REFERENCES

1. Kiyota Y, Hamaji K, Miyamoto M and Nagaoka A, Effect of idebenone (CV-2619) on memory impairment observed in passive avoidance task in rats with cerebral embolization. *Jpn J Pharmacol* **37**: 300–302, 1985.
2. Yamazaki N, Take Y, Nagaoka A and Nagawa Y, Beneficial effect of idenone (CV-2619) on cerebral ischemia-induced amnesia in rats. *Jpn J Pharmacol* **36**: 349–356, 1984.
3. Sugiyama Y and Fujita T, Stimulation of the respiratory and phosphorylating activities in rat brain mitochondria by idebenone (CV-2619), a new agent improving cerebral metabolism. *FEBS Lett* **184**: 48–51, 1985.

* To whom correspondence should be addressed.

4. Kakihana M, Yamazaki N and Nagaoka A, Effects of idebenone (CV-2619) on the concentrations of acetylcholine and choline in various brain regions of rats with cerebral ischemia. *Jpn J Pharmacol* **36**: 357–363, 1984.
5. Narumi S, Nagai Y, Kakihana M, Yamazaki N, Nagaoka A and Nagawa Y, Effects of idebenone (CV-2619) on metabolism of monoamines, especially serotonin, in the brain of normal rats and rats with cerebral ischemia. *Jpn J Pharmacol* **37**: 235–244, 1985.
6. Oka M, Isosaki M and Yanagihara N, Isolated bovine adrenal medullary cells: studies on regulation of catecholamine synthesis and release. In: *Catecholamines: Basic and Clinical Frontiers* (Eds. Usdin E, Kopin IJ and Barchas J), Vol. 1, pp. 70–72. Pergamon Press, Oxford, 1979.
7. Nakanishi A, Morita K and Oka M, Influence of reduction of cytoplasmic ATP on catecholamine secretion from intact and digitonin-permeabilized adrenal chromaffin cells. *Jpn J Pharmacol* **46**: 109–115, 1988.
8. Matsuoka M, Function and metabolism of catecholamines in the brain. *Jpn J Pharmacol* **14**: 181–193, 1964.
9. Masserano JM, Vulliet PR, Tank AW and Weiner N, The role of tyrosine hydroxylase in the regulation of catecholamine synthesis. In: *Handbook of Experimental Pharmacology. Catecholamines* (Eds. Trendelenburg U and Weiner N), pp. 427–469. Springer, Berlin, 1990.
10. Patrick RL and Barchas JD, Regulation of catecholamine synthesis in rat brain synaptosomes. *J Neurochem* **23**: 7–15, 1974.
11. Oka M and Izumi F, Effects of cyclic adenosine 3′,5′-monophosphate and its dibutyryl derivatives on catecholamine synthesis in bovine adrenal medullary slices. *FEBS Lett* **51**: 253–256, 1975.
12. Haycock JW, Meligeni JA, Bennett WF and Waymire JC, Phosphorylation and activation of tyrosine hydroxylase mediate the acetylcholine-induced increase in catecholamine biosynthesis in adrenal chromaffin cells. *J Biol Chem* **257**: 12641–12648, 1982.
13. Houchi H, Nakagawa H, Oka M, Watanabe J, Isosaki M and Ohuchi T, Increase in cyclic AMP levels and stimulation of catecholamine synthesis in isolated bovine adrenal medullary cells caused by forskolin. *Jpn J Pharmacol* **36**: 97–99, 1984.
14. Houchi H, Nakanishi A, Uddin MM, Ohuchi T and Oka M, Phorbol ester stimulates catecholamine synthesis in isolated bovine adrenal medullary cells. *FEBS Lett* **188**: 205–208, 1985.
15. Houchi H, Oka M, Misbahuddin M, Morita K and Nakanishi A, Stimulation by vasoactive intestinal polypeptide of catecholamine synthesis in isolated bovine adrenal chromaffin cells. *Biochem Pharmacol* **36**: 1551–1554, 1987.
16. Ip NI, Perlman RL and Zigmond RE, Both nicotinic and muscarinic agonists acutely increase tyrosine 3-monooxygenase activity in the superior cervical ganglion. *J Pharmacol Exp Ther* **223**: 280–283, 1982.
17. Horwitz J and Perlman RL, Activation of tyrosine hydroxylase in the superior cervical ganglion by nicotinic and muscarinic agonists. *J Neurochem* **43**: 546–552, 1984.
18. Roskoski R Jr and Roskoski LM, Activation of tyrosine hydroxylase in PC-12 cells by the cyclic GMP and cyclic AMP second messenger systems. *J Neurochem* **48**: 236–242, 1987.
19. Tachikawa E, Tank AW, Weiner DH, Mosimann WF, Yanagihara N and Weiner N, Tyrosine hydroxylase is activated and phosphorylated on different sites in rat pheochromocytoma PC-12 cells treated with phorbol ester and forskolin. *J Neurochem* **48**: 1366–1376, 1987.
20. Houchi H, Masserano JM, Bowyer JF and Weiner N, Regulation of tyrosine hydroxylase activity in pheochromocytoma PC-12 cells by bradykinin. *Mol Pharmacol* **37**: 104–110, 1990.

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Effects of chloroquine and primaquine on rat liver cytosolic *N*-acetyltransferase activity

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N-Acetyltransferase (NAT*) catalyzes the acetylation of a wide number of xenobiotics. The ability to acetylate xenobiotics has been shown to be a significant determinant in the predisposition to toxicity from several agents [1]. Alterations in acetylation, therefore, may have significant consequences.

While genetic factors appear to constitute the primary cause of variability in acetylation capacity, several drugs may also influence the ability to acetylate xenobiotics. It was reported recently that chloroquine may alter hepatic NAT activity in the rat [2]. In particular, single- and multiple-dose pretreatment with chloroquine was reported to reduce the acetylation of isoniazid (INH) and sulphadimidine (SDD) *in vivo*. Moreover, *in vitro* addition of chloroquine reduced the activity of NAT. Since the structure of chloroquine would not lead one to anticipate an interaction with NAT, we attempted to confirm the effect of this antimalarial on NAT activity.

* Abbreviations: INH, isoniazid; NAPA, *N*-acetylprocainamide; NASDD, *N*-acetylsulphadimidine; NAT, *N*-acetyltransferase; PA, procainamide; and SDD, sulphadimidine.

Methods

Chemicals. Procainamide (PA) was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Analytical standards of PA and *N*-acetylprocainamide (NAPA) were gifts from E. R. Squibb & Sons, Inc. (Princeton, NJ). *N*-Proprionylprocainamide, chloroquine, primaquine, acetyl-CoA, acetyl carnitine, and carnitine *O*-acyltransferase (EC 2.3.1.7) were purchased from the Sigma Chemical Co. (St. Louis, MO) and used as received.

Effects of antimalarials on NAT in vitro. The liver was removed from an untreated male Sprague–Dawley rat under ether anesthesia. Liver homogenate was prepared as described [3], and the 100,000 *g* supernatant (cytosol) was used as the source of NAT.