Biochemical Pharmacology, Vol. 42, No. 3, pp. 699–702, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Dose- and time-dependent effect of an acute 3-amino-1,2,4-triazole injection on rat brain catalase activity

(*Received* 7 *August* 1990; *accepted* 12 *March* 1991)

3-Amino-1,2,4-triazole (AT) is a widely used inhibitor of catalase activity *in vivo* and *in vitro*. Several groups of investigators [1, 2] had established that this reaction requires the presence of hydrogen peroxide. AT produces an irreversibly inhibited enzyme by reaction with "catalase compound I" [catalase–H_2O_2] [2, 3]. Administration or addition of substances which can be oxidized by this catalase-hydrogen peroxide complex I, such as ethanol and formate, prevents the occurrence of this inhibition but can not reverse it once it has taken place [1, 2].

Since the original observation [3] that AT reduces catalase activity in the liver and kidney following its injection in rats, many papers have appeared confirming and extending these findings [1, 2, 4–6]. However, at present, this phenomenon has been systematically studied most frequently *in vivo* in liver [4, 5, 7]. Before 1980, only a single study, in which a survey of various tissues was undertaken, mentioned the apparent catalase activity of rat brain and its inhibition by AT [8]. More recently, however, AT inhibition of brain catalase has been confirmed *in vivo* by several authors [9–13].

The observation that inhibition of brain catalase by AT *in vivo* could be prevented by prior ethanol administration to rats constitutes further indirect evidence of ethanol oxidation by the living brain via the peroxidatic activity of catalase [11]. The neurobiological significance of this phenomenon has already been noted. For example, it has been reported that rats pretreated with AT, and therefore functionally devoid of brain catalase activity, exhibit a significant attenuation or blockade of several known ethanol-induced effects, such as narcosis [13], motor depression [10], lethality [13], corticosterone release [14] and conditioned taste aversion [9].

Although the above observations and possible consequences are of great interest for alcohol research, to our knowledge no attempt has been made to study the inhibition of brain catalase *in vivo* by AT in a more systematic fashion. The experiments in the current report were carried out to evaluate the effect of i.p. administration of AT on rat brain catalase activity.

Materials and Methods

Animals. Male Long Evans rats were obtained from Charles River Breeding Farms (Canada), and maintained on a standard rat chow diet (Agway Inc., Syracuse, NY) and water *ad lib.* until used. Animals (weighing 200–300 g) were fasted overnight before use. 3-Amino-1,2,4-triazole (Sigma Chemical Co.) was administered intraperitoneally, and the rats were killed by exsanguination under ether anesthesia at the times indicated in the text. Approximately 1.0 mL of whole blood was taken by open chest cardiac puncture and used for catalase and AT measurement, after which the organs were perfused *in situ* by whole body perfusion using heparinized isotonic saline [10, 15]. Brains and livers were removed rapidly, weighed, and then placed into the homogenization medium.

Catalase assays. Brain homogenates (10%) were prepared with 0.1% Triton X-100 in 10 mM potassium phosphate buffer, pH 7.0. The liver homogenate (25%) was prepared with 0.25 M sucrose, 0.1 mM EDTA, pH 7.5. All homogenates were stored at 0° and were assayed for catalase activity on the same day. Tissue catalase activity was determined using a Yellow Springs oxygen monitor equipped with a Clark style oxygen electrode as described [10, 15]. The reaction cell was temperature controlled and maintained at 25°. Brain and liver catalase activity are expressed in units of nanomoles O_2 formed per minute per microgram protein. Erythrocyte catalase activity is expressed in units of nanomoles O_2 formed per minute per microgram hemoglobin.

Tissue levels of aminotriazole. Thirty percent brain homogenates and 10% liver homogenates were prepared with 0.1 M perchloric acid. Plasma was diluted 30-fold with 0.1 M perchloric acid. Levels of AT were determined by a colorimetric method as described [12].

Dose–response curves. The equations for the hyperbolic plots of measured brain catalase activity versus aminotriazole dose were solved by an iterative least squares method [16]. The actual curves given for response vs log dose (see Fig. 1) represent these solutions.

Statistical analysis. Results are expressed as means ± SEM. Each point is the mean ± SEM for a minimum of five animals. Analysis of variance was performed on these means. Pair-wise comparisons using Tukey tests were also carried out. P values of < 0.05 were accepted as significant.

Results and Discussion

The potency of AT as an *in vivo* inhibitor of brain catalase was evaluated using dose–response curves (Fig. 1). The ED_{50} values for aminotriazole were calculated 3 hr (ED_{50} = 0.120 g/kg, i.p.) and 12 hr (ED_{50} = 0.130 g/kg, i.p.) following AT administration. These times were chosen since they coincide with the onset of maximum inhibition and the beginning of recovery (see Fig. 2). The time courses for the inhibition and recovery of brain catalase activity were determined following the administration of AT (Fig. 2). Maximum inhibition occurred within 3–6 hr of AT administration (0.0625, 0.250 and 1.0 g/kg). Catalase activity returned to within 100% of control values 24 hr following administration of the lower dose (0.0625 g/kg). However, more than 48 hr were necessary for the larger doses (0.250 g/kg and 1.0 g/kg) to return to control values. The pattern of inhibition and recovery of brain catalase activity was identical for the three AT doses tested. These results confirmed previous findings [9–13] that the catalase inhibitor 3-amino-1,2,4-triazole inactivates brain catalase activity in the rat. They also show that this inhibition may be dose-dependent at all times tested. These findings suggest that cerebral levels of hydrogen peroxide, necessary for the inactivation processes, are not a limiting factor in this process.

The presence of AT in brain was confirmed over the time period of the observed inhibition of brain catalase. The absorption of AT was complete at 30 min following the administration of 1 g/kg, i.p., to fasted rats. Figure 3 shows the levels of AT in rat brain, liver and blood following an intraperitoneal injection of a dose of 1 g/kg. These observations confirm the presence of AT in the perfused brain over the time period of inhibition of brain catalase. If the injected dose had been distributed homogenously, an average tissue concentration of 11.9 mM would have been achieved. A value close to this

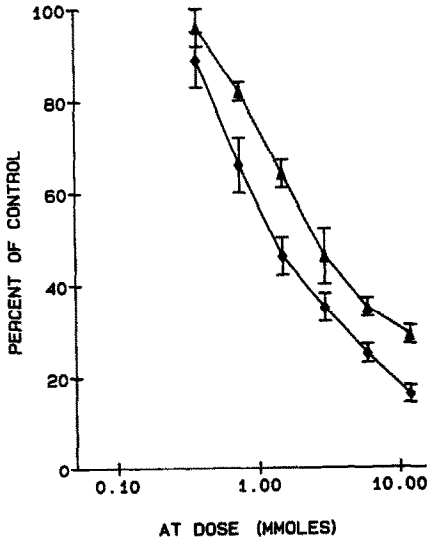

Fig. 1. Log dose vs response curves for the *in vivo* inhibition by 3-amino-1,2,4-triazole of catalase in rat brain. Each animal received AT (0.031, 0.062, 0.125, 0.250, 0.5 or 1 g/kg, i.p.) or isotonic saline (control) 3 hr (◇) or 12 hr (△) before being killed. Each data point is the mean ± SEM for a minimum of five animals. Brain catalase activity plotted as percent of control was calculated from the data in units of nanomoles O_2 formed per minute per microgram protein. Normal values for the saline-treated controls were 0.608 ± 0.018 nmol O_2/min/μg protein.

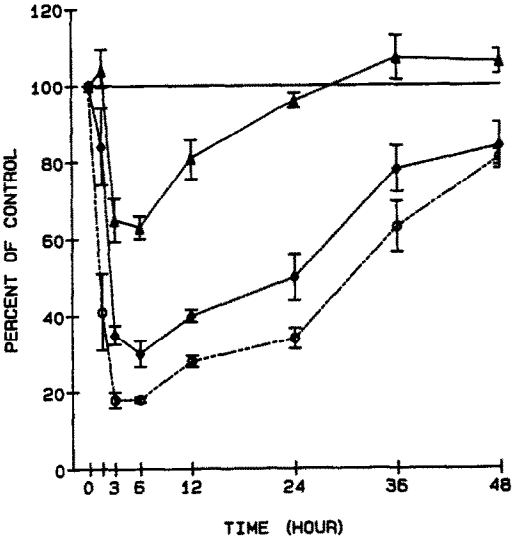

Fig. 2. Time course for the recovery of catalase activity in rat brain following a single dose of 3-amino-1,2,4-triazole. The animals were fasted 16 hr before use. Each animal received an acute dose of AT [0.0625 (△), 0.250 (◇), or 1 g/kg (○)], i.p., or saline and was killed at the time indicated. At 10 hr following AT treatment, the remaining animals were refed. Control and test animals (N = 5) were killed at each time period. Each data point is the mean ± SEM for a minimum of five animals. Catalase activities in homogenates of whole brain of control animals were 0.608 ± 0.018 nmol O_2/min/μg protein.

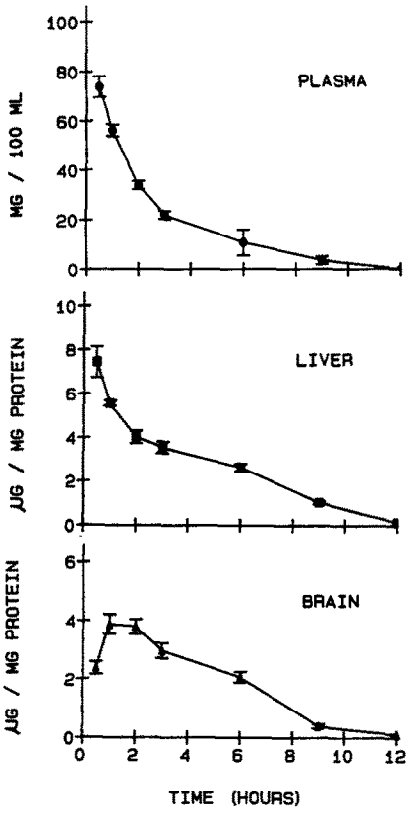

Fig. 3. Concentrations of 3-amino-1,2,4-triazole in brain, liver and blood after intraperitoneal injection of 1 g/kg. Each data point is the mean ± SEM for a minimum of five animals. Values have been corrected for small amounts of endogenous chromogenic material in tissues; the endogenous material in control rats, expressed as AT equivalents was present at 0.073 ± 0.005 μg/mg protein (N = 5) in brain and 0.20 ± 0.10 μg/mg protein (N = 5) in liver.

concentration was observed in liver and blood at 30 min and declined sharply thereafter. Lower values were observed in brain. Maximum cerebral levels were obtained at 1 and 2 hr post-injection.

The time course of inhibition and recovery of catalase activity in homogenates of whole brain and liver and in erythrocyte lysates was determined using an AT dose of 1 g/kg (Fig. 4). A comparison of the time course of inhibition of liver and brain catalase by AT revealed a differential pattern of inhibition in these tissues. While both tissues demonstrated statistically identical levels of inhibition, a differential time pattern between them was observed. Liver catalase levels decreased to 10% (maximum inhibition) of control at 30 min following AT injection. However, brain catalase activity displayed such levels only after 3 hr post-injection. These findings are in agreement with other reports [7, 12]. The return to normal levels in brain was also slower than in liver, although the degree of inhibition was the same for both tissues following an acute dose of 1 g/kg AT. A possible explanation for this variable pattern of inhibition could be the presence of lower levels of AT in brain than in liver for the first hour following AT administration (Fig. 3). This latter observation may suggest that the penetration of AT into the brain and into the catalase compartment may be rate-limiting and,

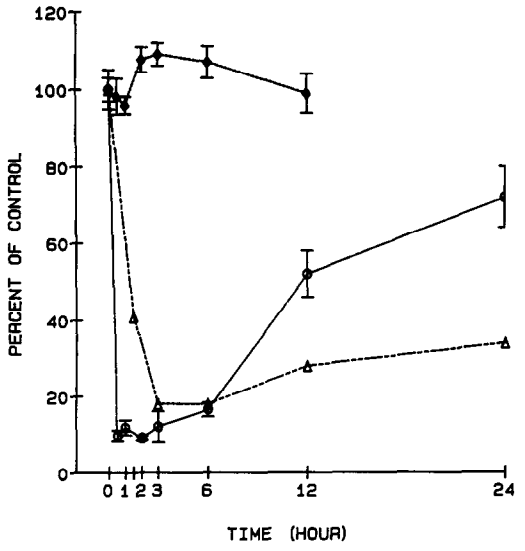

Fig. 4. Time course of the recovery of catalase activity in rat liver (○), brain (△) and blood (◇) following a single dose of 3-amino-1,2,4-triazole (1 g/kg, i.p.). The procedure was as described for Fig. 2. Each data point is the mean ± SEM for a minimum of five animals. Tissue catalase activity is plotted as percent of control. Catalase activities in homogenates of whole brain and liver and in erythrocyte lysates of control animals were 0.608 ± 0.018 nmol O_2/min/μg protein for brain, 115.76 ± 11.57 nmol O_2/min/μg protein for liver, and 31.96 ± 0.76 nmol O_2/min/μg hemoglobin for erythrocyte lysates.

consequently, could be the limiting factor in brain catalase inhibition. Alternatively, the time-dependent relative sensitivity of the brain and liver catalases to AT may simply reflect differential tissue hydrogen peroxide levels with no mechanistic implications. Moreover, it has been suggested that AT does not have a direct effect upon catalase [9, 11] but, rather, undergoes metabolic transformation in the tissues to form an irreversible inactivator of the enzyme [6]. Consequently, the different times at which liver and brain catalase activities proved to be maximal inhibited could also be explained by a possible differential ability of the tissues to transform the compound (AT) into this catalase inactivator. The recovery of the activity of the enzymes in both tissues was associated with the disappearance of AT from brain and liver tissue. Apparently, the inhibition of liver catalase by AT is essentially irreversible [6, 7]. Our data seem to suggest the same processes for brain catalase. However, a slower synthesis of new catalase protein in brain, as compared to liver, seems to be suggested by our data.

Erythrocyte catalase was not inhibited by AT *in vivo*. This finding is similar to those observed by other authors [1, 4, 10]. It has also been reported that cyanamide, another H_2O_2-dependent catalase inhibitor, fails to inhibit erythrocyte catalase activity *in vivo* [15]. However, like cyanamide, AT can inhibit erythrocyte catalase activity *in vitro* in the presence of hydrogen peroxide [1, 15, 17]. Therefore, it has been suggested that a low concentration of hydrogen peroxide in these cells may be the basis for the lack of inhibition by AT [1] and cyanamide [15] *in vivo*. This notion may also account for the findings of the present study.

In summary, the results presented in this study demonstrate a progressive inhibition of rat brain catalase activity by AT *in vivo*. Furthermore, the inhibition of brain catalase by AT demonstrates the presence of hydrogen peroxide in brain [12], since AT inhibits catalase in the presence of this compound [2, 3]. The rate of inhibition of catalase seems to be dependent upon the rate by which H_2O_2 is generated [17]. A time course study showed slower onset of the inhibition of brain as compared to liver catalase, possibly reflecting tissue hydrogen peroxide levels or, alternatively, a rate-limiting penetration of AT into brain and into the catalase compartment. The presence of AT in brain was confirmed over the time period of the observed inhibition of brain catalase. Catalase inhibitors are of particular interest in the study of the physiological role of catalase. This study further supports the use of AT in investigations designed to further understand the role of brain catalase.

Acknowledgements—This study was supported in part by a grant from N.S.E.R.C. of Canada (A0991) to C. M. G. Aragon.

Center for Studies in Behavioral Neurobiology
Concordia University
Montreal, Quebec
Canada H3G 1M8

CARLOS M. G. ARAGON*
FRANC ROGAN
ZALMAN AMIT

REFERENCES

1. Tephly TR, Mannering GJ and Parks RE, Studies on the mechanism of inhibition of liver and erythrocyte catalase activity by 3-amino-1,2,4-triazole (AT). *J Pharmacol Exp Ther* **134**: 77–82, 1961.
2. Nicholls P, The reaction between aminotriazole and catalase. *Biochim Biophys Acta* **59**: 414–420, 1962.
3. Heim WG, Appleman D and Pyfrom HT, Production of catalase changes in animals with 3-amino-1,2,4-triazole. *Science* **122**: 693, 1955.
4. Heim WG, Appleman D and Pyfrom HT, Effects of 3-amino-1,2,4-triazole (AT) on catalase and other compounds. *Am J Physiol* **186**: 19–23, 1956.
5. Ishii H, Suga T and Niinobe S, Effect of 3-amino-1,2,4-triazole treatment on catalase activity and triglycride level in fatty liver of the rat. *Biochem Pharmacol* **26**: 625–628, 1977.
6. Williams RN, Delamere NA and Paterson CA, Inactivation of catalase with 3-amino-1,2,4-triazole: An indirect irreversible mechanism. *Biochem Pharmacol* **34**: 3386–3389, 1985.
7. Nakamura A, Hara T and Minakami S, Intracellular distribution of hepatic catalase activity in rats treated with aminotriazole and allylisopropylacetamide. *J Biochem (Tokyo)* **73**: 47–53, 1973.
8. Feinstein RN, Berliner S and Green FO, Mechanism of inhibition of catalase by 3-amino-1,2,4-triazole. *Arch Biochem Biophys* **76**: 32–44, 1958.
9. Aragon CMG, Spivak K and Amit Z, Blockade of ethanol induced conditioned taste aversion by 3-amino-1,2,4-triazole: Evidence for catalase mediated synthesis of acetaldehyde in rat brain. *Life Sci* **37**: 2077–2084, 1985.
10. Aragon CMG, Spivak K and Amit Z, Effects of 3-amino-1,2,4-triazole on ethanol induced open field activity: Evidence for brain catalase mediation of ethanol's effects. *Alcoholism Clin Exp Res* **13**: 104–108, 1989.

* Correspondence: Carlos M. G. Aragon, Ph.D., Center for Studies in Behavioral Neurobiology, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, Quebec, Canada H3G 1M8.

11. Cohen G, Sinet PM and Heikkila R, Ethanol oxidation by rat brain *in vivo*. *Alcoholism Clin Exp Res* **4**: 366–370, 1980.
12. Sinet PM, Heikkila RE and Cohen G, Hydrogen peroxide production by rat brain *in vivo*. *J Neurochem* **34**: 1421–1428, 1980.
13. Tampier L, Quintanilla ME, Letelier C and Mardones J, Effect of 3-amino-1,2,4-triazole on narcosis time and lethality of ethanol in UCHA rats. *Alcohol* **5**: 5–8, 1988.
14. Aragon CMG and Amit Z, Genetic variation in ethanol sensitivity in C57BL/6 and DBA/2 mice: A further investigation of the differences in brain catalase activity. *Ann NY Acad Sci* **492**: 398–400, 1987.
15. DeMaster EG, Redfern B, Shirota FN and Nagasawa HT, Differential inhibition of rat tissue catalase by cyanamide. *Biochem Pharmacol* **35**: 2081–2085, 1986.
16. Martin CM, Iterative least squares method. In: *Quantitative Drug Design—A Critical Introduction* (Ed. Grunewald GL), pp. 115–127. Marcel Dekker, New York, 1978.
17. Cohen G, On the generation of hydrogen peroxide in erythrocytes by acetylphenylhydrazine. *Biochem Pharmacol* **15**: 1775–1782, 1966.

Biochemical Pharmacology, Vol. 42, No. 3, pp. 702–704, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Drug oxidation and N-acetylation in rats pretreated with subtoxic doses of streptolysin O

(*Received* 24 *September* 1990; *accepted* 23 *March* 1991)

The hemolytic, cardiotoxic and lethal streptolysin O (SLO) possesses, when given in low, sublytic doses, notable immunological properties [1–4]. Further, previous studies have shown that SLO increases the acetylation rates of sulfamethazine and p-amino benzoic acid (PABA) in rats and rabbits [5, 6]. The effect of SLO on drug acetylation might be associated with the reticuloendothelial cell system whose primary function is the immunological response and which is involved in the polymorphic drug N-acetylation also [7–10]. There is evidence that morphological and functional changes of this cell system result in altered N-acetylation capacity [9, 11–15]. Whether it also influences the oxidative drug metabolism is not known.

The present study was performed to examine whether SLO when given to rats in sublytic doses would be capable of influencing the activities of selected hepatic microsomal monooxygenases and the cytosolic *N*-acetyltransferase [16].

Materials and Methods

Animals and pretreatment. All experiments with SLO (Imuna, Czechoslovakia) were performed in male Wistar albino rats (200–275 g) purchased from Versuchstierzucht Schönwalde/Berlin. They were housed under controlled conditions in groups of six to eight animals with free access to standard diet and water for at least 14 days prior to the study. After randomization, groups of 12 animals were treated intravenously (tail vein) in shallow ether narcosis with 100 hemolytic units (HU)/kg body weight 1 day (acute pretreatment) or for 5 days (subacute treatment) before being killed, respectively (injection volume 1.0 mL/kg). Controls were administered equivalent volumes of saline at corresponding times. The interval between the last pretreatment dose and the beginning of both studies was generally 24 hr.

Biochemical protocol. After light ether anesthesia of the animals, blood was taken by puncture of the retrobulbar venous plexus for determination of γ-glutamyltransferase (GT), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and creatinine. After decapitation, the abdominal wall was opened to insert a cannula into the portal vein. The liver was quickly removed, freed from connective tissue, weighed and perfused with ice-cold saline. Cytosolic and microsomal fractions of rat livers were prepared by gradient centrifugation. Microsomal cytochrome P450 was determined by the method of Greim [17] and protein levels according to Eggstein and Kreutz [18]. Activities of aminopyrine demethylase and aniline hydroxylase were measured in 1 mL phosphate buffer containing 2 mg/mL microsomal protein, a NADP-regenerating system (3.1 mmol/L glucose-6-phosphate, 0.4 mmol/L NADP, 3 I.U. glucose-6-phosphate dehydrogenase), 13.3 mmol/L niacin, and the substrates (aminopyrine 10 mmol/L and aniline 200 mmol/L, respectively). The pH of the solution was adjusted to 7.4 and 7.8, respectively. Enzyme activities were expressed as amounts of formaldehyde and *p*-aminophenol formed per min and mg microsomal protein [19, 20]. *N*-Acetyltransferase activities were determined with procainamide and *N,S*-diacetylcysteamine as substrates [21]. Aliquots of the cytosolic fractions (about 5 mg protein) were incubated in 1 mL samples (phosphate buffer pH 7.4) with *N,S*-diacetylcysteamine (3.1 mmol/L) and procainamide (4 mmol/l) at 37° for 30 min. The reaction was terminated with NaOH (2.5 mol/L) and the metabolite *N*-acetylprocainamide was measured with a gas chromatographic method [22]. After addition of 100 μL internal standard (0.3 mol/L butyrylprocainamide), the samples were extracted twice with 3 mL ethylacetate. After re-extraction into 1 mL 0.1 mol/L HCl, the samples were washed twice with 0.2 mL 2.5 mol/L NaOH and extracted again with ethylacetate. The organic phase is evaporated to dryness and after that redissolved in 50 μL methanol.

Chromatographic conditions. Apparatus: Hewlett-Packard 5830 A equipped with N-P-FID; column: 4 ft × 2 mm i.d., glass, 3% OV-17 on Chromosorb WAW-DMCS; gases: N_2 30 mL/min, H_2 3 mL/min, air 50 mL/min; temperature: thermostat 280°, injector 300°, detector 300°. ASAT, ALAT, γ-GT and creatinine in serum were