Pergamon

Biochemical Pharmacology, Vol. 49, No. 8, pp. 1057–1062, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00064-X

INACTIVATION OF ADRENAL CYTOCHROMES P450 BY 1-AMINOBENZOTRIAZOLE

DIVERGENCE OF *IN VIVO* AND *IN VITRO* ACTIONS

HOWARD D. COLBY,*† BRIAN ABBOTT,* MICHAEL CACHOVIC,* KRISTINE M. DEBOLT* and BRUCE A. MICO‡

*Department of Pharmacology and Toxicology, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104; and ‡Department of Drug Metabolism and Pharmacokinetics, Hoffmann-LaRoche, Inc., Nutley, NJ 07110, U.S.A.

(*Received* 21 *September* 1994; *accepted* 5 *December* 1994)

Abstract—Recent investigations demonstrated that administration of 1-aminobenzotriazole (ABT) to rats caused adrenal gland enlargement. Studies were done to pursue the mechanism(s) involved. Preliminary experiments revealed that the adrenal enlargement caused by ABT was associated with a decline in plasma corticosterone concentrations, suggesting inhibition of adrenal steroidogenesis. Indeed, a single injection of ABT (25 or 50 mg/kg body weight) to rats caused concentration-dependent declines (60–80%) in adrenal mitochondrial and microsomal cytochrome P450 (P450) concentrations. The decreases in adrenal P450 levels exceeded those in hepatic microsomes. Accompanying the declines in adrenal P450 concentrations were decreases in steroid hydroxylase activities. Mitochondrial 11β-hydroxylase and cholesterol side-chain cleavage activities and microsomal 21-hydroxylase activity were diminished markedly (60–90%) by ABT treatment. In contrast, activity of adrenal 3β-hydroxysteroid dehydrogenase-isomerase was not affected by ABT, indicating specificity for P450-dependent reactions. Incubation of adrenal microsomes or mitochondria *in vitro* with ABT plus an NADPH-generating system had no effect on P450 concentrations or on steroid hydroxylase activities. Similar incubations with hepatic microsomes caused declines in P450 levels and in the rates of P450-mediated xenobiotic metabolism. The results demonstrate that ABT is a potent inhibitor of adrenal steroid hydroxylases *in vivo*, but the *in vitro* studies indicate that the mechanism of action differs from that on other P450 isozymes. The absence of inhibitor effects *in vitro* suggests that an extra-adrenal metabolite of ABT is responsible for the *in vivo* inactivation of steroidogenic enzymes.

Key words: 1-aminobenzotriazole; adrenal gland; cytochromes P450; steroid hydroxylases; steroidogenesis

ABT§ is a mechanism-based inhibitor (suicide substrate) of various P450 isozymes [1–7]. Inactivation of P450 isozymes by ABT has been demonstrated in several organs including liver, lung, and kidney [1–7]. As a suicide substrate, ABT undergoes a P450-catalyzed oxidation, resulting in the formation of benzyne [1–3]. The prosthetic heme group of P450 is alkylated by the benzyne causing the production of an abnormal porphyrin and the irreversible loss of enzyme activity [1–3].

The results of most prior investigations indicated that ABT is a relatively non-selective inhibitor of P450 isozymes, effecting substantial P450 losses in most organs studied [1–7]. In contrast, we recently reported that the *in vitro* actions of ABT in guinea pig adrenal glands are highly selective [8]. Incubation of adrenal microsomes with ABT plus an NADPH-generating system causes almost complete loss of xenobiotic-metabolizing activity, but has no effect on P450-catalyzed steroid hydroxylation reactions [8]. Similarly, ABT *in vitro* does not affect the activities of mitochondrial steroidogenic P450 isozymes. These observations led us to conclude that ABT is not a suicide substrate for those adrenal P450 isozymes involved in the synthesis of endogenous steroid hormones [8].

Although the results of our *in vitro* studies [8] suggested that ABT was unlikely to directly affect adrenal steroidogenesis, Meschter *et al.* [9] reported recently that prolonged administration of ABT to rats causes adrenal gland enlargement, indicating an increase in the secretion of ACTH, the major adrenal growth-promoting hormone. This effect could be the result of direct stimulation by ABT of pituitary ACTH secretion or, alternately, ABT could affect ACTH output indirectly by inhibiting adrenal corticosteroid synthesis [10] with the resulting decline in steroid negative feedback causing a compensatory increase in pituitary ACTH secretion. The studies presented in this paper were done to determine which of these mechanisms accounts for the actions of ABT on adrenal size. The results indicate that ABT is a potent inhibitor of adrenal steroid hydroxylases *in vivo*, causing a decline in plasma corticosteroid concentrations. The data presented

† Corresponding author: Howard D. Colby, Ph.D., Department of Pharmacology and Toxicology, Philadelphia College of Pharmacy and Science, 600 South 43rd Street, Philadelphia, PA 19104. Tel. (215) 596-8830; FAX (215) 895-1161.

§ Abbreviations: ABT, 1-aminobenzotriazole; P450, cytochrome P450; CSCC, cholesterol side-chain cleavage; and BP-OHase, benzo[*a*]pyrene hydroxylase.

also suggest that the inhibition of adrenal steroidogenesis may be mediated by a metabolite of ABT.

MATERIALS AND METHODS

ABT was supplied by Hoffmann-LaRoche, Inc., Nutley, NJ. The 3β-hydroxysteroid dehydrogenase inhibitor, 4,4-dimethyl-2α-cyano-20-spirox-5-en-3-one, was provided by Merck, Sharp & Dohme Research Laboratories, Rahway, NJ. The antisera used in radioimmunoassays for corticosterone and pregnenolone were obtained from Radioassay Systems Laboratories, Carson, CA. Except where indicated, other reagents were obtained from the Sigma Chemical Co., St. Louis, MO

Female Sprague–Dawley rats weighing approximately 300–325 g were obtained from Ace Animals, Boyertown, PA. Animals were maintained under standardized conditions of light (6:00 a.m.–6:00 p.m.) and temperature (22°) and received food and water *ad lib*. All rats were allowed at least 1 week to become acclimated to the housing conditions prior to use in experiments. For most *in vivo* investigations, ABT was administered between 4:00 and 5:00 p.m. as a single i.p. injection at a dose of 25 or 50 mg/kg body weight in 0.9% saline; controls received the vehicle only. Rats were killed the following day between 8:00 and 9:00 a.m. by CO_2 inhalation. For the time-course studies (see Tables 1 and 2), ABT was given as a single or daily injection between 8:00 and 9:00 a.m. and rats were killed 1, 2, 4, 24, 48, 72 or 96 hr later. At the time of killing, trunk blood was collected in heparinized beakers for subsequent determination of plasma corticosterone concentrations by radioimmunoasay [11], and adrenal glands and livers were removed quickly and placed in cold 0.25 M sucrose containing 0.05 M Tris–HCl (pH 7.4) on ice. Tissues were then trimmed free of fat and connective tissue, weighed, and homogenized in sucrose–Tris buffer. Washed mitochondrial and microsomal fractions were prepared by differential centrifugation as described previously [12, 13].

Incubation conditions for evaluation of the effects of ABT *in vitro* were essentially the same as described previously [13]. Adrenal or hepatic microsomal suspensions (0.5 mg protein/mL) in 1.15% KCl–0.05 M Tris–HCl containing 3 mM $MgCl_2$ were incubated with ABT (0.1 mM) and/or an NADPH-generating system (0.4 mM $NADP^+$, 10 mM sodium isocitrate, 0.3 U/mL isocitrate dehydrogenase) in a total volume of 2.5 mL. Unless otherwise indicated, incubations were for 30 min in 25-mL Erlenmeyer flasks at 37° under air in a Dubnoff Metabolic Incubator. Adrenal mitochondrial incubations were done similarly except that 0.25 M sucrose was used in the medium instead of 1.15% KCl. For each of the incubation conditions employed, one set of flasks served as unincubated or 0-time controls. After the incubations, flasks were placed in an ice bath, and the mitochondria or microsomes in each flask were washed (recentrifuged) once prior to use in the assays described below.

Adrenal mitochondrial 11β-hydroxylase activity was assayed as the rate of conversion of 11-deoxycorticosterone to corticosterone, as described previously [12]. Corticosterone was measured fluorometrically [14]. CSCC activity was determined as the rate of pregnenolone production by isolated adrenal mitochondria, with endogenous cholesterol as the substrate [12]. Cholesterol metabolism was initiated by the addition of 10 mM sodium isocitrate, and 4,4-dimethyl-2α-cyano-20-spirox-5-en-3-one (50 μM) was included in each flask to prevent the conversion of pregnenolone to progesterone [15]. Pregnenolone was extracted from the incubation flasks with methylene dichloride and measured with a highly specific radioimmunoassay [12].

Adrenal microsomal steroid 21-hydroxylase activity was determined as the rate of conversion of progesterone to 11-deoxycorticosterone. Incubation conditions and HPLC analyses of metabolites were described previously in detail [16]. 3β-Hydroxysteroid dehydrogenase-isomerase activity in adrenal microsomes was measured as the rate of conversion of pregnenolone to progesterone utilizing HPLC for metabolite separation and UV absorbance at 254 nm for quantitation [16]. Hepatic microsomal benzo[*a*]pyrene hydroxylation was determined by the fluorometric method of Nebert and Gelboin [17]. Quinine sulfate was calibrated against authentic 3-hydroxy-benzo[*a*]pyrene and used routinely as the fluorescence standard. For all enzyme assays, conditions were established to ensure linearity of product formation with respect to protein concentrations and incubation times.

P450 was measured as the dithionite-reduced CO complex as described by Omura and Sato [18]. Microsomal and mitochondrial protein concentrations were determined by the method of Lowry *et al.* [19]. Statistical analyses of differences between group means were done with Student's *t*-test or Newman–Keuls multiple-range test, as appropriate. Data are presented as means ± SEM.

RESULTS AND DISCUSSION

When rats were given ABT daily at a dose of 50 mg/kg body weight, there was a significant decline in plasma corticosterone concentrations within 24 hr; the decrease was sustained for at least 96 hr (Table 1). Corticosterone is the major steroid hormone secreted by the rat adrenal cortex. Within 72 hr after initiating ABT treatment, adrenal glands were enlarged significantly compared with controls (Table 1). The ABT-induced increase in adrenal mass was even greater after 96 hr of treatment. The adrenal enlargement caused by ABT confirms the observations of Meschter *et al.* [9].

Taken together, the increase in adrenal mass accompanied by a decrease in plasma corticosterone concentrations suggested that ABT inhibited corticosterone synthesis, and the resulting decline in plasma corticosterone caused a compensatory increase in pituitary gland ACTH secretion and stimulation of adrenal growth. It is well established that ABT is a potent inhibitor of P450 isozymes in several organs [1–7], and the steroid hydroxylation reactions required for adrenal corticosterone synthesis are catalyzed by P450 isozymes [10]. Accordingly, studies

Table 1. Effects of daily ABT administration to rats on plasma corticosterone concentrations and adrenal weights*

Duration of treatment (hr)	Plasma corticosterone (ng/mL)		Adrenal weights (mg/pair)	
	Control	ABT	Control	ABT
24	138 ± 9	67 ± 3†	69.7 ± 4.8	67.6 ± 5.1
48	152 ± 11	49 ± 3†	72.8 ± 3.9	75.9 ± 4.8
72	144 ± 12	45 ± 4†	70.1 ± 4.6	84.3 ± 5.2†
96	157 ± 10	52 ± 7†	74.8 ± 4.1	98.3 ± 6.0†

* ABT was administered at a dose of 50 mg/kg daily for the duration of the treatment indicated. Values are means ± SEM of 4–5 animals per group.
† $P < 0.05$ (vs corresponding control value).

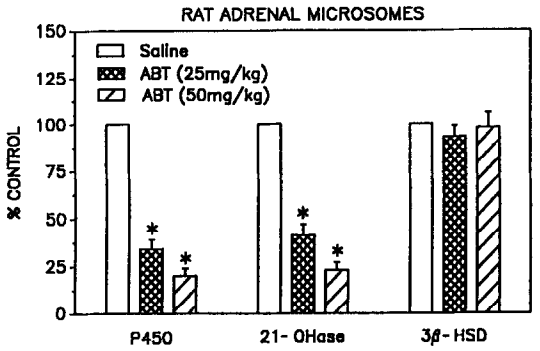

Fig. 1. Effects of ABT administration to rats on adrenal microsomal P450 concentrations and on 21-hydroxylase (21-OHase) and 3β-hydroxysteroid dehydrogenase-isomerase (3β-HSD) activities. Animals were pretreated for approximately 16 hr, and enzyme activities were determined as described in Materials and Methods. Data are expressed as percent of control values and are the means ± SEM of 5–6 animals in each group; 100% is equivalent to: 0.8 ± 0.1 nmol·(mg protein)$^{-1}$, P450; 5.5 ± 0.1 nmol·min^{-1}(mg protein)$^{-1}$, 21-OHase; and 4.2 ± 0.3 nmol·min^{-1}·(mg protein)$^{-1}$, 3β-HSD. Key: (*) $P < 0.05$ (vs controls).

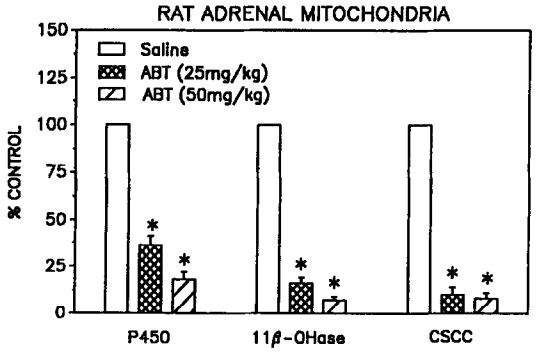

Fig. 2. Effects of ABT administration to rats on adrenal mitochondrial P450 concentrations and on 11β-hydroxylase (11β-OHase) and cholesterol side-chain cleavage (CSCC) activities. Animals were pretreated for approximately 16 hr and enzyme activities were determined as described in Materials and Methods. Data are expressed as percent of control values and are the means ± SEM of 5–6 animals in each group; 100% is equivalent to: 1.1 ± 0.2 nmol·(mg protein)$^{-1}$, P450; 10.7 ± 0.8 nmol·min^{-1}·(mg protein)$^{-1}$, 11β-OHase; and 0.41 ± 0.06 nmol·min^{-1}·(mg protein)$^{-1}$, CSCC. Key: (*) $P < 0.05$ (vs controls).

were done to test the hypothesis that ABT was an inhibitor of adrenal steroidogenic enzymes.

Administration of ABT to rats at doses of 25 or 50 mg/kg body weight caused marked declines in adrenal P450 concentrations and in P450-catalyzed steroidogenic enzyme activities (Figs. 1 and 2). Adrenal steroid hydroxylases are localized in both microsomal and mitochondrial membranes [10], and ABT had similar effects on enzymes in both subcellular fractions. The decreases in microsomal P450 levels and in 21-hydroxylase activities caused by ABT were proportionately similar (Fig. 1). The latter was not surprising since 21-hydroxylase (P450C21) is the major P450 isozyme in rat adrenal microsomes. In contrast, ABT treatment had no effect on microsomal 3β-hydroxysteroid dehydrogenase-isomerase activity, a non-P450 dependent reaction involved in steroid hormone biosynthesis [10]. The latter suggests some degree of specificity for P450-catalyzed reactions by ABT.

ABT pretreatment caused dose-dependent decreases in mitochondrial P450 concentrations (Fig. 2) that were of a magnitude similar to those in microsomes. There were corresponding decreases in the activities of the P450-dependent mitochondrial enzymes, 11β-hydroxylase and CSCC. The higher dose of ABT lowered both 11β-hydroxylase and CSCC activities to approximately 10% of control values. Since the latter represents the rate-limiting reaction in adrenal corticosteroid synthesis [10], decreases in CSCC activity would be expected to decrease the rates of steroid hormone synthesis and secretion. The extent of inhibition of adrenal P450 isozymes by ABT was as great as or greater than the decreases in hepatic P450 content and in hepatic BP-OHase activities (Fig. 3).

The effects of a single administration of ABT on adrenal steroidogenic enzymes were manifested fully within 2 hr after administration, indicating a rapid onset of action, and maximal inhibition was sustained for at least 24 hr (Table 2). After 24 hr, adrenal P450

Table 2. Adrenal P450 concentrations and steroid hydroxylase activities at various times after a single administration of ABT to rats*

	Controls	ABT-treated					
		1 hr	2 hr	4 hr	24 hr	48 hr	72 hr
Mitochondria							
P450 [nmol·(mg protein)$^{-1}$]	1.2 ± 0.2	0.7 ± 0.1†	0.3 ± 0.1†	0.4 ± 0.1†	0.3 ± 0.1†	0.6 ± 0.1†	1.0 ± 0.2
11β-Hydroxylation [nmol·min^{-1}·(mg protein)$^{-1}$]	11.4 ± 1.6	6.7 ± 0.4†	3.8 ± 0.4†	3.0 ± 0.4†	3.1 ± 0.5†	5.9 ± 0.8†	9.8 ± 1.1
Microsomes							
P450 [nmol·(mg protein)$^{-1}$]	0.5 ± 0.1	0.2 ± 0.1†	0.1 ± 0.1†	0.2 ± 0.1†	0.1 ± 0.1†	0.3 ± 0.1	0.6 ± 0.1
21-Hydroxylation [nmol·min^{-1}·(mg protein)$^{-1}$]	4.8 ± 0.7	2.6 ± 0.3†	1.6 ± 0.2†	1.6 ± 0.3†	1.5 ± 0.2†	3.1 ± 0.4†	4.6 ± 0.6

* A single dose of ABT (50 mg/kg) was administered at time zero, and assays were done as described in Materials and Methods. Values are means ± SEM of 3–5 animals per group. Because control values were similar at all time points, they were combined (N = 18).

† $P < 0.05$ (vs control value).

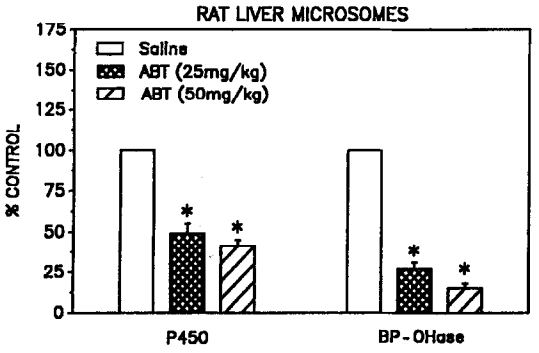

Fig. 3. Effects of ABT administration to rats on hepatic microsomal P450 concentrations and on benzo[*a*]pyrene hydroxylase (BP-OHase) activity. Animals were pretreated for approximately 16 hr and assays were done as described in Materials and Methods. Data are expressed as percent of control values and are the means ± SEM of 5–6 animals in each group; 100% is equivalent to: 0.7 ± 0.1 nmol·(mg protein)$^{-1}$, P450; and 374 pmol·min^{-1}·(mg protein)$^{-1}$, BP-OHase. Key: (*) $P < 0.05$ (vs controls).

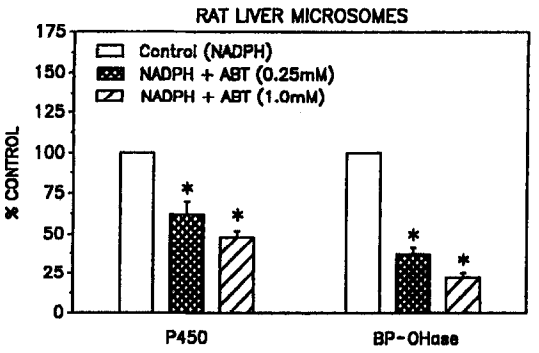

Fig. 4. Effects of incubating hepatic microsomes with ABT and/or NADPH on P450 concentrations and benzo[*a*]pyrene hydroxylase (BP-OHase) activities. Incubations were for 30 min as described in Materials and Methods. Data are expressed as percent of control values and are the means ± SEM of 4 experiments; 100% is equivalent to: 0.7 ± 0.2 nmol·(mg protein)$^{-1}$, P450; and 310 pmol·min^{-1}·(mg protein)$^{-1}$, BP-OHase. Key: (*) $P < 0.05$ (vs controls).

concentrations and enzyme activities gradually recovered. Restoration to control levels occurred within 72 hr after ABT administration, indicating that the effects were ultimately reversible (Table 2).

These observations indicate that ABT *in vivo* is a potent inhibitor of adrenal steroidogenic enzymes and support our explanation for the adrenal enlargement and decline in circulating corticosterone concentrations in ABT-treated rats (Table 1). The inactivation of adrenal steroid hydroxylases by ABT decreases the capacity for steroid synthesis, which is probably responsible for the decline in plasma corticosterone levels. A fall in plasma corticosterone concentrations would be expected to cause a compensatory increase in pituitary ACTH secretion, but because of the magnitude of the ABT-induced decline in steroidogenic enzyme activities, restoration of corticosterone production is unlikely to occur. However, the elevated levels of ACTH should stimulate adrenal growth, resulting in enlarged glands. A similar sequence of events has been found to occur with long-term administration of other inhibitors of adrenal steroidogenesis [20].

To determine if the mechanism of action of ABT on adrenal steroid hydroxylases is similar to that on other P450 isozymes, a series of *in vitro* investigations was initiated. As demonstrated previously by other investigators [2, 4, 7], incubation of liver microsomes with ABT plus an NADPH-generating system caused rapid concentration-dependent declines in P450 concentrations and in BP-OHase activities (Fig. 4). Neither ABT nor NADPH alone had any effect on P450 content or on BP-OHase activity (data not shown). These results are expected for a mechanism-based P450 inhibitor [21] and are similar to those obtained by others [1–7]. However, when adrenal

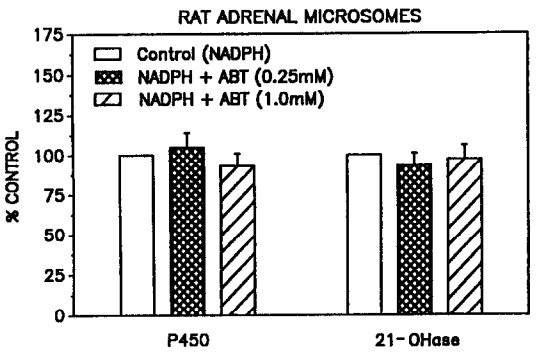

Fig. 5. Effects of incubating adrenal microsomes with ABT and/or NADPH on P450 concentrations and 21-hydroxylase (21-OHase) activities. Incubations were for 30 min as described in Materials and Methods. Data are expressed as percent of control values and are the means ± SEM of 5 experiments; 100% is equivalent to: 0.5 ± 0.1 nmol·(mg protein)$^{-1}$, P450; and 7.2 ± 0.4 nmol·min^{-1}·(mg protein)$^{-1}$, 21-OHase.

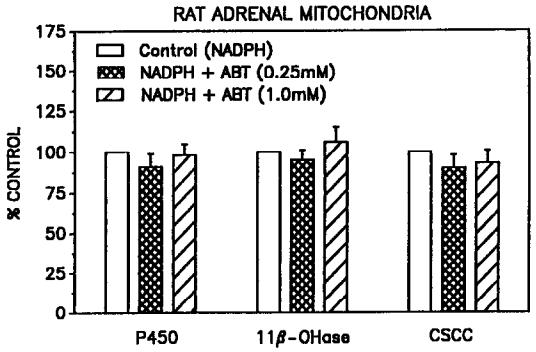

Fig. 6. Effects of incubating adrenal mitochondria with ABT and/or NADPH on P450 concentrations and on steroid 11β-hydroxylase (11β-OHase) and cholesterol side-chain cleavage (CSCC) activities. Data are expressed as percent of control values and are the means ± SEM of 5 or 6 experiments; 100% is equivalent to: 1.3 ± 0.2 nmol·(mg protein)$^{-1}$, P450; 10.7 ± 0.8 nmol·min^{-1}·(mg protein)$^{-1}$, 11β-OHase; and 0.26 ± 0.04 nmol·min^{-1}·(mg protein)$^{-1}$, CSCC.

microsomal preparations from the same animals were incubated under identical conditions with ABT plus NADPH, neither P450 concentrations nor 21-hydroxylase activities were affected (Fig. 5). Incubation of adrenal microsomes with ABT plus NADPH for as long as 90 min was similarly without effect (data not shown). In addition, incubation of ABT with adrenal mitochondria did not influence P450 levels or the activities of the P450-dependent enzymes, 11β-hydroxylase and CSSC (Fig. 6). Even when whole adrenal homogenates were incubated with ABT (1.0 mM) plus NADPH, followed by isolation of mitochondria and microsomes, there was no effect on P450 levels or on steroid hydroxylase activity in either subcellular fraction (data not shown).

The absence of ABT effects *in vitro* on adrenal steroid hydroxylases contrasts with its potent inhibitory actions *in vivo*. However, the results are consistent with our prior studies on guinea pig adrenal glands [8]. The adrenal cortices in guinea pigs are somewhat unusual in that they contain P450 isozymes having high xenobiotic-metabolizing activities in addition to having a full complement of steroid hydroxylases [22–25]. We found that incubation of guinea pig adrenal microsomal preparations with ABT plus NADPH selectively inactivated xenobiotic metabolism without affecting steroid hydroxylase activities [8]. Thus, in both rat and guinea pig adrenals, steroidogenic P450 isozymes are resistant to the actions of ABT *in vitro*, indicating that ABT is not a suicide substrate for these isozymes.

In conclusion, the data presented in this paper demonstrate that ABT is a potent *in vivo* inhibitor of adrenal steroidogenic P450 isozymes. However, the mechanism of action appears to be different from that reported for other isozymes in various organs [1–8]. ABT may be directly responsible for the inactivation of steroid hydroxylases *in vivo*, but via a mechanism other than as a suicide substrate. It is also possible that an extra-adrenal metabolite of ABT mediates the actions of the parent compound on steroidogenic P450 isozymes *in vivo*. Additional investigations are now in progress to consider these as well as other possible mechanisms of action of ABT on adrenal P450 isozymes.

Acknowledgements—These investigations were supported by Hoffmann-LaRoche, Inc. and by USPHS AG-11987.

REFERENCES

1. Ortiz de Montellano PR and Mathews JM, Autocatalytic alkylation of the cytochrome P-450 prosthetic haem group by 1-aminobenzotriazole. *Biochem J* **195**: 761–764, 1981.
2. Ortiz de Montellano PR, Mico BA, Mathews JM, Kunze KL, Miwa GT and Lu AYH, Selective inactivation of cytochrome P-450 isozymes by suicide substrates. *Arch Biochem Biophys* **210**: 717–728, 1981.
3. Ortiz de Montellano PR, Mathews JM and Langry KC, Autocatalytic inactivation of cytochrome P-450 and chloroperoxidase by 1-aminobenzotriazole and other aryne precursors. *Tetrahedron* **40**: 511–519, 1984.
4. Mathews JM, Dostal LA and Bend JR, Inactivation of rabbit pulmonary cytochrome P-450 in microsomes and isolated perfused lungs by the suicide substrate 1-aminobenzotriazole. *J Pharmacol Exp Ther* **235**: 186–190, 1985.
5. Mico BA, Federowicz DA, Ripple MG and Kerns W, *In vivo* inhibition of oxidative drug metabolism by, and acute toxicity of, 1-aminobenzotriazole (ABT): A tool for biochemical toxicology. *Biochem Pharmacol* **37**: 2515–2519, 1988.
6. Woodcroft KJ, Szczepan EW, Knickle LC and Bend JR, Three *N*-aralkylated derivatives of 1-aminobenzotriazole as potent and isozyme-selective, mechanism-based inhibitors of guinea pig pulmonary cytochrome P-450 *in vitro*. *Drug Metab Dispos* **18**: 1031–1037, 1990.
7. Mugford CA, Mortillo M, Mico BA and Tarloff JB, 1-Aminobenzotriazole-induced destruction of hepatic

and renal cytochromes P-450 in male Sprague–Dawley rats. *Fundam Appl Toxicol* **19**: 43–49, 1992.
8. Xu D, Voigt JM, Mico BA, Kominami S, Takemori S and Colby HD, Inhibition of adrenal cytochromes P450 by 1-aminobenzotriazole *in vitro*. Selectivity for xenobiotic metabolism. *Biochem Pharmacol* **48**: 1421–1426, 1994.
9. Meschter CL, Mico BA, Mortillo M, Feldman D, Garland WA, Riley JA and Kaufman LS, A 13-week toxicologic and pathologic evaluation of prolonged cytochromes P450 inhibition by 1-aminobenzotriazole in male rats. *Fundam Appl Toxicol* **22**: 369–381, 1994.
10. Hall PF, Cytochromes P-450 and the regulation of steroid synthesis. *Steroids* **48**: 131–196, 1986.
11. Rourke K and Colby HD, Mechanism of action of spironolactone to inhibit cortisol secretion by guinea pig adrenocortical cells. *Moll Cell Endocrinol* **81**: 127–135, 1991.
12. Colby HD and Eacho PI, Mitochondrial steroid metabolism in the inner and outer zones of the guinea pig adrenal cortex. *J Steroid Biochem* **23**: 477–484, 1985.
13. Sherry JH, O'Donnell JP, Flowers L, LaCagnin LB and Colby HD, Metabolism of spironolactone by adrenocortical and hepatic microsomes: Relationship to cytochrome P-450 destructon. *J Pharmacol Exp Ther* **236**: 675–680, 1986.
14. Mejer LE and Blanchard RC, Fluorometric determination of plasma 11-hydroxycorticosteroids. II. Studies on the specificity of the method. *Clin Chem* **19**: 718–724, 1973.
15. Begue R-J, Gustafsson J-A and Goldman AS, New potent inhbitors of 3β-hydroxy-Δ^5-steroid oxidoreductase with short duration of action. *Endocrinology* **95**: 238–246, 1974.
16. Eacho PI and Colby HD, Differences in microsomal steroid metabolism between the inner and outer zones of the guinea pig adrenal cortex. *Endocrinology* **116**: 536–541, 1985.
17. Nebert DW and Gelboin HV, Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. *J Biol Chem* **243**: 6242–6249, 1968.
18. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* **239**: 2370–2378, 1964.
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
20. Gower DB, Modifiers of steroid-hormone metabolism: A review of their biochemistry and clinical applications. *J Steroid Biochem* **5**: 501–523, 1974.
21. Rando RR, Mechanism-based irreversible enzyme inhibitors. *Methods Enzymol* **46**: 28–41, 1979.
22. Colby HD and Rumbaugh RC, Adrenal drug metabolism. In: *Extrahepatic Metabolism of Drugs and Other Compounds* (Ed. Gram TE), pp. 239–266. Spectrum Publications, Jamaica, NY, 1980.
23. Colby HD and Eacho PI, Chemical-induced adrenal injury: Role of metabolic activation. In: *Endocrine Toxicology* (Eds. Thomas JA, Korach KS and McLachlan JA), pp. 35–66. Raven Press, New York, 1985.
24. Black VH, Barilla JR, Russo JJ and Martin KO. A cytochrome P450 immunochemically related to $P450_{c,d}$ (P450I) localized to the smooth microsomes and inner zone of the guinea pig adrenal. *Endocrinology* **124**: 2480–2493, 1989.
25. Black VH, Immunodetectable cytochromes P450I, II, and III in guinea pig adrenal-hormone responsiveness and relationship to capacity for xenobiotic metabolism. *Endocrinology* **127**: 1153–1159, 1990.