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Effect of acute cocaine administration on the metabolism of antipyrine *in vivo*

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Cocaine, a potent central nervous system stimulant, has been studied extensively with respect to its psychopharmacology. Only recently, however, has cocaine been found to be acutely hepatotoxic in mice, causing necrosis, elevation in serum transaminase levels, and depression of several hepatic enzyme activities [1–3]. It has been proposed that free radical production and the resulting lipid peroxidation play an important role in the production of cocaine-induced hepatotoxicity [4–6].

To date, all studies involving acute cocaine-induced hepatotoxicity have characterized the hepatic damage in terms of histopathology and biochemical damage. Shuster *et al.* [1] have demonstrated previously that two sequential doses of cocaine to induced mice result in enhanced pentobarbital sleeping times and decreased pentobarbital metabolism *in vivo*. Unfortunately, this study chose to measure *in vivo* pentobarbital metabolism merely by monitoring the disappearance of pentobarbital [2-^{14}C] from the blood; this method does not definitively reflect the cytochrome P-450-mediated metabolism but, rather, includes all types of metabolism centered on the 2-carbon of pentobarbital. Similarly, these investigators correlated an increase in plasma pentobarbital half-life after cocaine administration with enhanced sleeping times: no attempt was made to determine any changes in volume of distribution, a factor that plays an important role in determining barbiturate-induced sleeping times. Freeman and Harbison [7] have also reported that low-dose, chronically administered (several weeks) cocaine to non-induced mice results in enhanced hexobarbital narcosis.

The intent of this study was to determine a physiological, *in vivo* correlate to the existing biochemical data on acute cocaine-induced hepatotoxicity in a non-induced mouse strain. We report that a single injection of cocaine produced a dramatic decrease in hepatic cytochrome P-450 content and a correspondingly marked depression of *in vivo* hepatic antipyrine metabolism in non-induced mice. It is suggested that cocaine administration may likewise alter the hepatic metabolism of a wide variety of xenobiotics and, in doing so, may alter the therapeutics and toxicity of these drugs.

Materials and Methods. DBA/2Ha male mice, 10 weeks of age, were obtained from Health Research Laboratories (West Seneca, NY) and were housed on corn cob bedding. Mice were given food (Purina Chow No. 5001) and tap water *ad lib*. Cocaine hydrochloride (Merck) was dissolved in a saline solution immediately prior to 60 mg/kg i.p. injection; control mice received only the vehicle solution. Cocaine was injected 24 hr prior to antipyrine administration, since we have noted maximal hepatic damage during this time period [3]. Serum glutamic-pyruvic transaminase was measured as described previously [8]. Antipyrine (25 mg/kg) was administered by intracardiac injection.

Heparinized blood was obtained by cardiac puncture at various times thereafter; plasma was obtained through centrifugation. Serum was collected from blood that had been allowed to clot at room temperature for 45 min. Washed microsomes were prepared by the method of Schenkman and Cinti [9]. Cytochrome P-450 content was determined by the method of Omura and Sato [10]. Microsomal protein was analyzed according to Bradford [11]. The plasma samples were analyzed for antipyrine by a micro-adaptation of the high pressure liquid chromatographic (HPLC) method of Danhof *et al.* [12], using a Constametric III pump and a Spectro Monitor III variable u.v. detector (Linear Diagnostics Corp.) set at 244 nm, a Rheodyne model 7125 injector, and a Heath model SR-205 recorder. A 25 cm Zorbax CN column (Dupont) was used with a mobile phase of 20 mM phosphate buffer, pH 4.0–acetonitrile–triethylamine (86.5:13:0.5) at a flow rate of 2.0 ml/min. Antipyrine concentrations were determined by the peak height ratio method. Standard curves were generated using blank plasma samples which had been spiked with known amounts of antipyrine. A semi-logarithmic plot of plasma antipyrine levels versus time was used to calculate the apparent elimination rate constant, plasma half-life, and apparent volume of distribution values for each group by standard methods. Results were analyzed by Student's *t*-test and analysis of variance as applied to regression [13] where appropriate.

Results and Discussion. As can be seen in Table 1, an acute dose of 60 mg/kg cocaine to mice produced a severe depression of hepatic microsomal cytochrome P-450 and a drastic elevation in serum glutamic-pyruvic transaminase, in addition to causing extensive hepatic centrilobular necrosis (data not shown). This cocaine-induced hepatotoxicity has been shown previously to be both time and dose dependent [3].

This drastic drop in cytochrome P-450 content prompted us to investigate the corresponding effects, if any, of cocaine administration on *in vivo* hepatic drug metabolism. Antipyrine was chosen as a prototype drug that is metabolized by hepatic cytochrome P-450. Antipyrine distributes to total body water, with negligible binding to plasma or tissue proteins [14–16], and its rate of *in vivo* metabolism is affected only very slightly by changes in hepatic blood flow [17]. Its metabolism *in vivo* has been shown to correlate directly with the activity level of the cytochrome P-450 system, and since the introduction of the "antipyrine test" in 1968 [18, 19], antipyrine has become a popular pharmacologic tool for the *in vivo* assessment of cytochrome P-450 function in both humans and laboratory animals [20, 21].

Table 1. Effect of cocaine administration on hepatic cytochrome P-450 and serum glutamic-pyruvic transaminase*

	Cyt P-450 (nmoles/mg protein)	SGPT (units/l)
Control	0.502 ± 0.050	63.5 ± 10.5
Cocaine-treated	0.201 ± 0.020†	1760 ± 197†

* Animals were injected i.p. with 60 mg/kg cocaine HCl and killed 24 hr later. Controls received only saline. Abbreviations: Cyt P-450, cytochrome P-450; and SGPT, serum glutamic-pyruvic transaminase. Values are expressed as mean ± S.E., N = 6.

† $P < 0.05$, as compared to control.

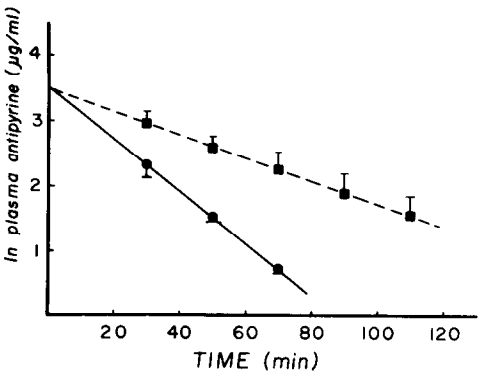

Fig. 1. Effect of acute cocaine on *in vivo* hepatic antipyrine metabolism. Animals received 60 mg/kg cocaine hydrochloride i.p. 24 hr prior to a 25 mg/kg intracardiac injection of antipyrine. Controls received only the vehicle. Animals were killed at various times after antipyrine administration. Results are expressed as mean ± S.E., N ≥ 8. See Table 2 for actual kinetic values. Key: control (●); and cocaine-treated (■).

The effect of cocaine on mouse *in vivo* antipyrine metabolism is shown in Fig. 1 and Table 2. Cocaine administration caused a 133% increase in the plasma half-life of antipyrine, together with a concomitant 55% decrease in the apparent elimination constant. The volume of distribution of antipyrine, presumably a characteristic constant for a drug, remained essentially unchanged between control and cocaine-treated mice.

Thus, it is apparent that the biochemical destruction of cytochrome P-450 through the hepatotoxic action of cocaine results in the impairment of hepatic metabolizing function of at least one drug, antipyrine. Since the hepatic cytochrome P-450 system is responsible for the metabolism, both in the form of bioactivation and detoxification, of a wide variety of xenobiotics, these results suggest that the administration of cocaine, through its hepatotoxic action, may alter significantly the therapeutics and toxicity of a great many xenobiotics. This factor should be taken into account when assessing the potential risks and benefits of therapeutic agents after cocaine administration.

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Table 2. Effect of cocaine administration on kinetics of antipyrine metabolism: Plasma half-life, apparent elimination constant, and volume of distribution*

	Plasma $T_{1/2}$† (min)	k_{app}† (l/min)	V_d‡ (l/kg)
Control (N ≥ 8)	17.0 ± 4.4	0.041 ± 0.008	0.73
Cocaine-treated (N ≥ 8)	39.6 ± 5.3§	0.018 ± 0.003§	0.77

* Animals were injected i.p. with 60 mg/kg cocaine HCl and received a 25 mg/kg intracardiac injection of antipyrine 24 hr later. Controls received only saline. Abbreviations: $T_{1/2}$, half-life; k_{app}, apparent elimination constant; and V_d, volume of distribution.

† Values are means ± S.E.

‡ Values are harmonic means.

§ $P < 0.05$, as compared to control.

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Catecholamine secretion by perfused bovine adrenal medulla in response to nicotinic activation is inhibited by muscarinic receptors

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Increased labelling of phosphatidylinositol and phosphatidic acid with ^{32}P is seen as a response to activation of various cell surface receptors. In the bovine adrenal medulla these phospholipid changes are associated with muscarinic but not nicotinic cholinergic receptors [1, 2]. It is generally agreed that in the bovine medulla catecholamine secretion is mediated by nicotinic receptors but there are conflicting views on the function of the muscarinic receptors. Wilson and Kirshner [3] claimed that the acetylcholine receptors of bovine adrenal medulla are entirely nicotinic. In their hands, the muscarinic drug pilocarpine at concentrations between 10^{-5} and 3×10^{-3} M did not stimulate catecholamine secretion by the perfused gland. Gothert *et al.* [4], on the other hand, obtained a secretory effect dependent upon Ca^{2+} with 1.4×10^{-3} M pilocarpine in similar experiments.

Binding studies show the presence of muscarinic receptors in the bovine gland [5] and studies with isolated chromaffin cells suggest that activation of these receptors inhibits the nicotinic secretion of catecholamine, possibly by increasing c-GMP concentration [6]. Using cultured bovine chromaffin cells, Fisher *et al.* [2] showed that muscarinic receptors do not enhance catecholamine secretion.

It seemed likely then that in the bovine adrenal medulla nicotinic activation promotes catecholamine release while muscarinic activation inhibits this release. The experiments reported below were designed to test this hypothesis using the perfused gland.

Bovine adrenals were removed within 15 min of death and transported to the laboratory on ice. Connective tissue and fat were removed and two incisions made in the cortical tissue without damaging the medulla, so that the perfusion fluid could flow out. Glands were cannulated and perfused in a retrograde manner via the central vein opening at a flow rate of 4 ml/min. The Locke's solution used had the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 6.0 mM $NaHCO_3$ and 10.0 mM glucose. The perfusion fluid temperature was kept at 35° and flow was maintained by a Watson–Marlow MC/10 peristaltic metering pump. The fluid was continuously gassed with O_2–CO_2 (95:5). The perfusion system consisted of two glands, each with its own reservoir.

After perfusion for 20 min in this way, the perfusate was collected in 2 min samples (i.e. from time 0 in Figs. 1 and 2) and these were stored at 4° for analysis. Catecholamines in the perfusate were estimated as described previously [7]. To stimulate secretion, a total of 4 ml 3×10^{-4} M nicotine was injected 1.0 ml at a time into the tube carrying perfusion fluid just before it entered the gland. There was an interval of 30 sec between injections. A further identical nicotine injection was given later as shown in the figures. Both glands received the injections, the first gland being