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Cocaine–protein targets in mouse liver

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Abstract

Cocaine has been shown to be hepatotoxic in mice, rats and humans. N-Oxidative metabolism of cocaine is required for this effect, and it has been proposed that binding of cocaine reactive metabolites formed via this pathway might be responsible for cytotoxicity. To explore this hypothesis, cocaine–protein adducts in liver following cocaine treatment in naive ICR mice were examined by Western blot analysis and compared with those formed in mice pretreated with phenobarbital or β-naphthoflavone. Phenobarbital and β-naphthoflavone pretreatments have been shown previously to shift the hepatic necrosis in ICR mice from the midzonal region to periportal and perivenular regions, respectively. Similar patterns of cocaine–protein adduction were detected in naive, phenobarbital-pretreated and β-naphthoflavone-pretreated mice, however, suggesting a consistent set of target proteins regardless where within the lobule toxicity occurs. To confirm that Western blot analysis using anti-cocaine antibody was capable of detecting all of the major cocaine–protein adducts, a separate experiment was conducted in which mice were treated with ^{14}C-labeled cocaine and cocaine–protein adducts were detected fluorographically. This technique detected essentially the same protein adducts as the Western blots. Two of the protein adducts were isolated, subjected to N-terminal sequence analysis, and found to have homology with hsp 60 and transferrin. Western blot analysis using anti-hsp 60 and anti-transferrin antibodies following two-dimension PAGE separation was used to confirm the identity of these protein targets. Impairment of function of either protein could plausibly contribute to cocaine hepatotoxicity, although this remains to be demonstrated.

Keywords: Cocaine; Hepatotoxicity; Protein adducts; hsp 60; Transferrin; Enzyme induction

1. Introduction

Morbidity and mortality from cocaine abuse arises from several of cocaine effects, including dysrhythmias, cardiac ischemia, hypertension, hyperthermia, rhabdomyolysis, and neurologic consequences of profound central nervous system stimulation [1–3]. In some cases, hepatic injury from cocaine is prominent [4–8], occasionally leading to hepatic failure and death. Consistent with these case reports, administration of cocaine to laboratory animals has been shown to produce severe hepatocellular necrosis [9,10]. The mechanism of cocaine hepatotoxicity is not well understood, although it is clear from studies in mice that N-oxidative metabolism of cocaine is required for this effect [11].

One of the consequences of N-oxidative metabolism of cocaine is the formation of reactive metabolites that bind to proteins, and it has been postulated that the formation of these cocaine–protein adducts leads to loss of some critical function(s), resulting in cell death [10,12]. In support of this, it has been shown that experimental manipulations that increase or decrease covalent binding of cocaine metabolites to proteins (e.g. co-treatment with inducers or inhibitors of cocaine metabolism) result in corresponding changes in hepatotoxicity [11,12]. Also, immunohistochemistry studies have shown that within the hepatic lobule of animals treated with cocaine, only hepatocytes with detectable levels of cocaine–protein adducts show signs of toxicity [13]. Despite the strong correlation between formation of cocaine–protein adducts and cocaine hepatotoxicity, additional evidence is required before protein adduct formation can be considered a cause of toxicity.

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Abbreviations: SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TEMED, tetramethylethylenediamine; PVDF, polyvinylidene difluoride; NBT, nitro blue tetrazolium; BCIP, bromo-4-chloro-3-indolyl phosphate; BSA, bovine serum albumin; ALT, alanine aminotransferase; IEF, isoelectric focusing; AP, alkaline phosphatase; ELISA, enzyme-linked immunosorbent assay.

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Specifically, it remains to be demonstrated that the adduction of proteins by cocaine leads to cellular changes that could explain its cytotoxicity. This requires the identification of adducted proteins, determination of whether adduction leads to loss of activity or function, and an assessment as to whether this loss is sufficient to account for toxicity. None of these steps has, as yet, been completed.

Using SDS–PAGE and Western blots with anti-cocaine antibody, we previously found the presence of several discrete cocaine–protein adducts in the liver of mice treated with cocaine [14]. Follow-up studies, reported here, compared the proteins adducted in naive mice treated with cocaine with those from mice pretreated with the cytochrome P450 inducers phenobarbital and β-naphthoflavone. Previous studies have noted that pretreatment of mice with these inducers shifts the site of necrosis within the hepatic lobule. In naive mice, necrosis from cocaine typically occurs in perivenular or midzonal regions, depending upon the mouse strain [9,13,15]. In mice pretreated with phenobarbital, cocaine instead produces periportal hepatic necrosis [10,13,16]. β-Naphthoflavone pretreatment in ICR mice produces a shift of the lesion in the opposite direction, with necrosis occurring primarily in perivenular regions, rather than midzonally as in naive mice [13]. The reason for this shifting lesion has never been explained, but there is evidence that it may occur in humans as well [13]. The objective of these experiments was to determine whether the change in the site of necrosis within the lobule is associated with a change in target proteins for cocaine adduction.

A second objective of the study was to attempt detection of cocaine–protein adducts by fluorography, and to compare the results with those obtained using anti-cocaine antibody and Western blots. With immunochemical detection of a variety of protein adducts, there is always the possibility that some of the adducts may not possess the epitope for antibody binding and therefore be missed. Fluorographic detection of cocaine–protein adducts after a radiolabelled cocaine dose allows a potentially more complete assessment, as well as the opportunity to assess the specificity of the immunochemical approach. A third objective of the study was to begin to isolate and identify the target proteins for cocaine adduction. Two adducted proteins were identified by N-terminal sequencing. Their identities were confirmed by SDS–PAGE and Western blots using specific antibodies for these proteins.

2. Materials and methods

2.1. Chemicals and reagents

A rabbit polyclonal anti-cocaine antibody against norcocaine-BSA was used for Western blot analysis. Characterization of this antibody and its ability to detect cocaine–protein binding by ELISA, immunohistochemistry, and Western blot has been described previously [14]. Radioactive cocaine (benzene ring ^{14}C, specific activity 91.5 mCi/mmol, 99.0% purity) was custom-synthesized by Dupont New England Nuclear. Cocaine hydrochloride, goat anti-rabbit IgG alkaline phosphatase conjugate, and molecular weight standards for SDS–PAGE were purchased from Sigma Chemical Co. TEMED was obtained from Biorad Laboratories. PVDF membrane (Immobilon-PSQ, 0.45 μm) was obtained from Millipore Corporation. Ampholine was purchased from Pharmacia Biotech. NBT, BCIP toluidine salt, acrylamide, *N*,*N*-methylene-bis-acrylamide, ammonium persulfate and sodium dodecyl sulfate (SDS) were obtained from Fisher Scientific. Anti-hsp 60 mouse monoclonal antibody (clone LK-1) was purchased from Stressgen Biotechnologies Corp. Rabbit anti-transferrin antibody was purchased from Inter-Cell Technologies. Reagents for SDS–PAGE were of electrophoretic grade and other routine chemicals were of reagent grade.

2.2. Animals and treatments

Male ICR mice (Harlan Sprague–Dawley, Indianapolis, IN), 32–34 g body weight were used in this study. Mice were housed in cages (five mice per cage) in a room with a 12-hr light/dark cycle (08:00–20:00 light), and with controlled temperature and humidity. The animals had free access to water and animal chow (Teklad Rodent Diet 8604, Harlan Sprague–Dawley). Mice were injected with either cocaine (50 mg/kg, i.p.) or saline vehicle, with or without pretreatment with phenobarbital sodium (4 days, 80 mg/kg per day i.p.) or β-naphthoflavone (3 days, 40 mg/kg per day i.p.), and euthanized by carbon dioxide asphyxiation at 6 hr post-injection. In a separate experiment, ICR male mice (28 g body weight) were injected with cocaine (60 mg/kg containing 10 μCi ^{14}C-cocaine) and killed 1 hr after treatment. All experiments were approved by the Institutional Animal Care and Use Committee, and all animals received humane care according to criteria in the NIH "Guide for the Care and Use of Laboratory Animals."

2.3. Liver cell fractionation

Livers from animals in each group were placed in ice-cold preparation buffer (10 mM Tris–HCl, 0.25 M sucrose, 1 mM $MgCl_2$, pH 7.4), homogenized, and used immediately for the isolation of mitochondria, microsomes, and cytosol as described previously [14]. All manipulations were carried out at 4° and samples were stored at −80° until needed.

2.4. Biochemical analyses

Total protein in homogenate and fractions was determined by the method of Bradford using the Micro protein kit (Sigma Chemical Co.), with BSA as standard. Serum ALT activities were measured according to the method of

Bergmeyer *et al.* [17] using a commercial kit from Sigma Diagnostics Inc.

2.5. SDS–PAGE and Western blot analysis for cocaine binding

Liver fractions were diluted with solubilization buffer (2% SDS, 5% 2-mercaptoethanol, 20% glycerol, 0.025% bromophenol blue, 0.1 M Tris, pH 7.0), and proteins (50 μg per well) were separated by one-dimensional (1D) PAGE. Proteins were transferred to PVDF membrane and probed with anti-cocaine antibody as previously described [14]. Immunoreactive proteins were determined by aligning each Western blot with its corresponding Coomassie-stained replicate blot.

2.6. Fluorography of liver homogenate and cell fractions

Cell fractions of liver collected from mice 1 hr after ^{14}C-cocaine injection were separated by 1D-PAGE (80 μg per lane). The gel was stained with Coomassie blue, de-stained, impregnated with 1 M sodium salicylate for 30 min, dried, and exposed to X-ray film at −80°. For two-dimensional (2D) PAGE, proteins in whole liver homogenate were separated according to their isoelectric points by IEF (100,000 cpm per tube gel), followed by separation on a slab gel according to their molecular weights. The gels were stained with Coomassie blue, impregnated with 1 M sodium salicylate, dried, and exposed to X-ray film at −80° for 8 weeks. The films were developed to detect binding signals.

2.7. Characterization of adducted proteins by N-terminal amino acid sequence analysis

Following identification of the microsomal fraction as the major site of target formation, proteins in whole liver homogenate and microsomes were separated by 2D-PAGE and stained with Coomassie blue. Replicate blots were probed with anti-cocaine antibody. Protein spots on Coomassie-stained blots corresponding to the binding signals on replicate Western blots were excised and subjected to N-terminal amino acid sequence analysis. Two target proteins, a 64 kDa (pI 5.5) protein in the homogenate, and 70–80 kDa (pI 7.2) protein in the microsomes, were sequenced by Edman degradation (Applied Biosystems Procise Sequencer, Model 494) at the Protein Core of the Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida. The BLAST software program was used to examine homology of N-terminal sequence to the GenBank and protein data bases.

2.8. Western blot analysis for hsp 60 and transferrin

Following sequence identification of the 64 kDa target as hsp 60, and the 70–80 kDa target as transferrin, Western blot analysis was performed using anti-hsp 60 (1:1000 dilution) or anti-transferrin (1:500 dilution) antibodies, respectively, for confirmation. Proteins in the samples were separated by 2D-SDS–PAGE, transferred to membrane, and probed with the respective antibody. Goat anti-mouse IgG conjugated to alkaline phosphatase (for hsp 60, 1:3,000 dilution; from Sigma Chemical Co.) or rabbit anti-mouse IgG-AP (for transferrin, 1:15,000; from Sigma Chemical Co.) were used as the second antibody. The rest of the protocol was similar to that described for Western blot analysis with anti-cocaine antibody [14].

3. Results

3.1. Effects of cytochrome P450 inducers on protein adduction

Previous studies have detected cocaine–protein adducts in mitochondrial and microsomal fractions of mice treated with cocaine [14]. In experiments reported here, the pattern of cocaine–protein adducts in mitochondrial and microsomal fractions was compared between naive mice and mice pretreated with the cytochrome P450 inducers phenobarbital or β-naphthoflavone. Hepatic proteins were separated using 1D-SDS–PAGE, and cocaine-adducted proteins were detected by Western blot analysis using anti-cocaine antibody (Fig. 1). In the mitochondrial fractions, cocaine

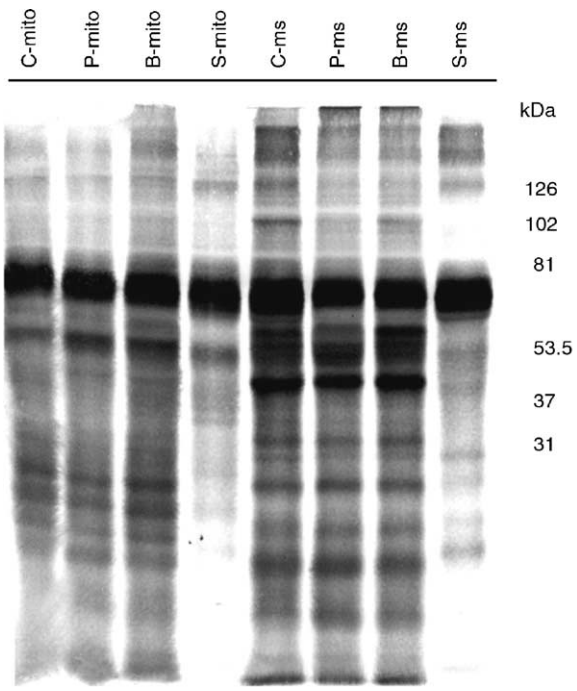

Fig. 1. Effects of P450 inducers on adduct formation following cocaine treatment (50 mg/kg i.p.). Liver was collected 6 hr after cocaine treatment and fractionated. Proteins in fractions were separated by 1D-SDS–PAGE (50 μg per lane), transferred to membrane, and probed with anti-cocaine antibody as described in Section 2. P: phenobarbital + cocaine, B: β-naphthoflavone + cocaine, C: cocaine only, S: saline only.

treatment was associated with antibody binding to a protein with an approximate molecular mass of 64 kDa, and to three or four proteins with molecular masses <30 kDa. In the microsomal fractions, cocaine treatment resulted in strong antibody binding signals with 100, 52–54, and 44 kDa proteins, as well as a number of proteins ≤32 kDa. The number and molecular masses of the adducted proteins were essentially the same for naive mice and animals pretreated with phenobarbital or β-naphthoflavone.

3.2. Cocaine–protein adducts identified by fluorography

For fluorographic detection of adducts, naive mice were administered a radiolabeled dose of cocaine (60 mg/kg cocaine, i.p., containing 10 μCi ^{14}C-cocaine) and euthanized 1 hr after the dose. Cytosolic, mitochondrial, and microsomal fractions of liver were isolated and proteins separated using 1D-SDS–PAGE. Coomassie-stained gels revealed several proteins present in each of the fractions (Fig. 2A). Radiolabeling of protein bands, indicative of cocaine–protein adduct formation, was observed in lanes containing microsomes or mitochondria from cocaine-treated mice (Fig. 2B). Five targets with approximate molecular masses of 100, 64, 52–54, 44, and approximately 30 kDa were present in the microsomes (Fig. 2B). The 44 kDa target protein had the strongest binding signal. The presence of the 64 kDa adducted protein in the microsomal fraction suggests that there may have been some mitochondrial contamination. In other experiments, this target protein was found only in the mitochondrial fraction (see Fig. 1 and [14]). One major binding signal (about 44 kDa), and two lesser signals (about 64 and <30 kDa) were observed in the mitochondria. Binding signals were not observed in the lane containing cytosol from cocaine-treated mice, nor in any of the cell fractions from liver of mice treated with saline (Fig. 2B).

3.3. Sequence analysis of adducted proteins

To isolate adducted proteins for sequence analysis, hepatic proteins from cocaine-treated mice and untreated controls were separated by 2D-SDS–PAGE and cocaine–protein adducts were detected by Western blot analysis. Initially, whole liver homogenate was used, and results were essentially consistent with observations from 1D separation of mitochondrial and microsomal fractions. That is, liver from cocaine-treated mice had immunoreactive proteins at approximately 64, 52–54, 44, and 30 kDa molecular masses, with some weaker binding signals for proteins <30 kDa (Fig. 3A). Two distinct immunoreactive proteins with molecular masses of approximately 44 kDa were revealed as a result of 2D separation. As shown in Fig. 3B, these binding signals were absent in liver homogenate from saline-treated mice.

Among the adducted proteins, the most abundant (although not necessarily the most intensely adducted)

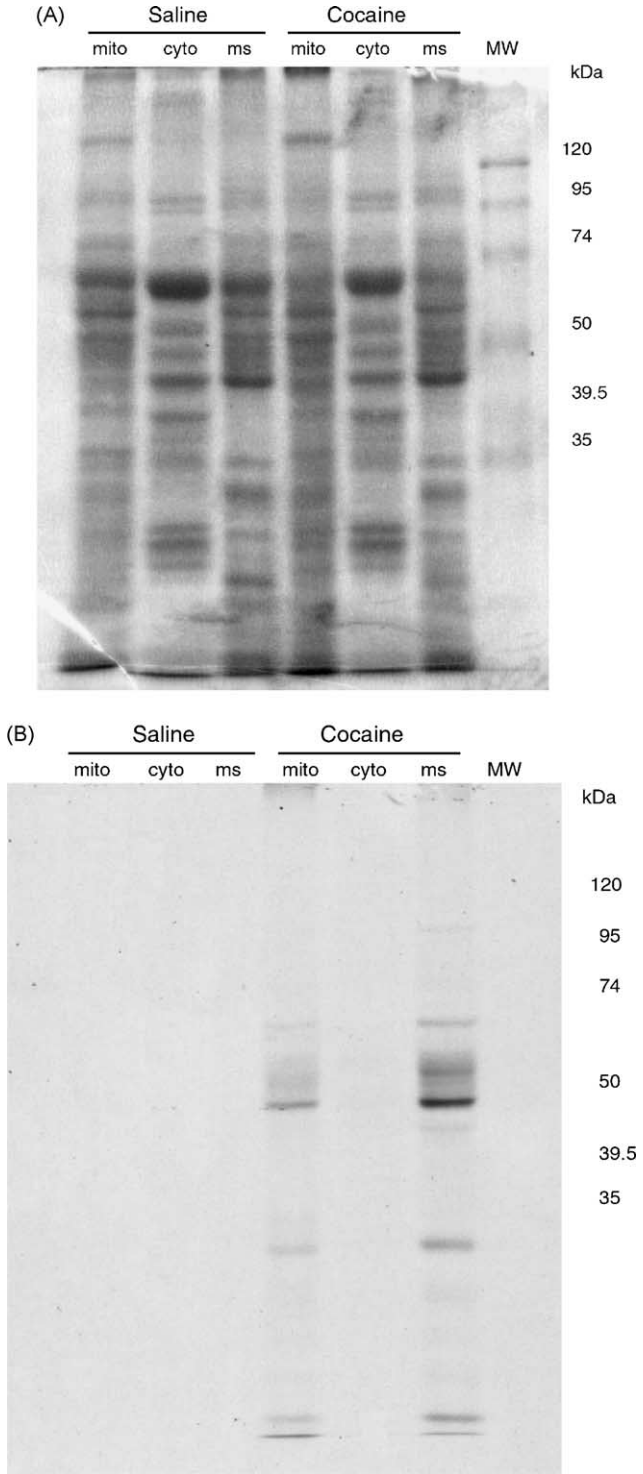

Fig. 2. Radiograph analysis of cocaine–protein adducts in liver. Livers were collected from mice 1 hr after treatment with ^{14}C-cocaine (50 mg/kg, 10 μCi per mouse) or saline, and fractionated. Proteins (80 μg per lane) in cell fractions were separated by 1D-PAGE, stained with Coomassie blue and dried (A). The dried gel was exposed to X-ray film. Binding signals were present only in microsomes and mitochondria from cocaine-treated mice (B). Lanes (1–3) mitochondria, cytosol, and microsomes from saline group; (4–6) mitochondria, cytosol, and microsomes from cocaine group; (7) molecular weight standards.

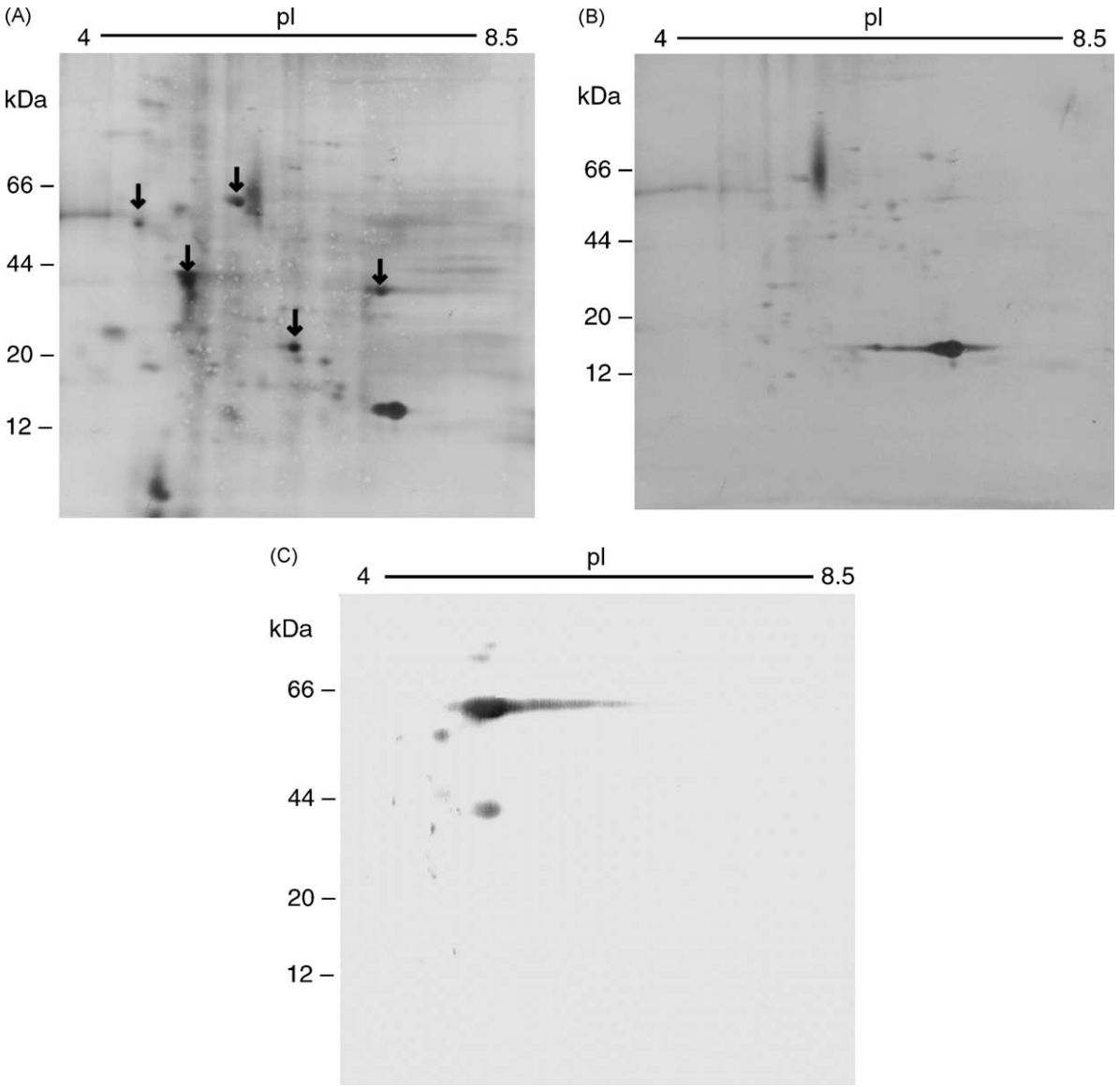

Fig. 3. Western blot analysis of whole liver homogenate from cocaine-treated (A) and control (B) mice. Proteins (200 μg per gel) were separated by 2D-SDS–PAGE, transferred to membrane and probed with anti-cocaine antibody. Western blots were aligned with replicate Coomassie-stained blots to identify immunoreactive proteins. Western blot analysis using anti-hsp 60 antibody showed immunoreactivity only to the 64 kDa target protein, confirming its identity as hsp 60 (C). (Panels (A) and (B) from [14]).

was the 64 kDa protein. Sufficient protein was present such that it could be excised from the gel and subjected to sequence analysis. Sequence data for the 64 kDa protein (32 residues) indicated 100% identity with mitochondrial hsp 60 of the rat, mouse and human (Table 1). Western blot analysis of the 2D gels using anti-hsp 60 antibody confirmed the identity of this protein (Fig. 3C).

In a subsequent experiment, gels were loaded with proteins from the microsomal fraction. Western blot analysis of the 2D gels with anti-cocaine antibody was largely consistent with the previous 1D results, with strong, multiple binding signals present in liver from cocaine-treated mice, particularly for proteins with an approximate mass of 44 kDa (Fig. 4A). These binding signals were not seen in

Table 1
N-Terminal amino acid sequence of the 64-kDa cocaine-binding protein

Protein 64 kDa	1	AKDVKFGADARALMLQGVDLLADAVAVTMGPK	32
Mouse hsp 60	27	AKDVKFGADARALMLQGVDLLADAVAVTMGPK	58
Rat hsp 60	27	AKDVKFGADARALMLQGVDLLADAVAVTMGPK	58
Human hsp 60	27	AKDVKFGADARALMLQGVDLLADAVAVTMGPK	58

N-Terminal sequence has 100% identity to amino acids 27–58 of mitochondrial hsp 60 of the rat, mouse, and human.

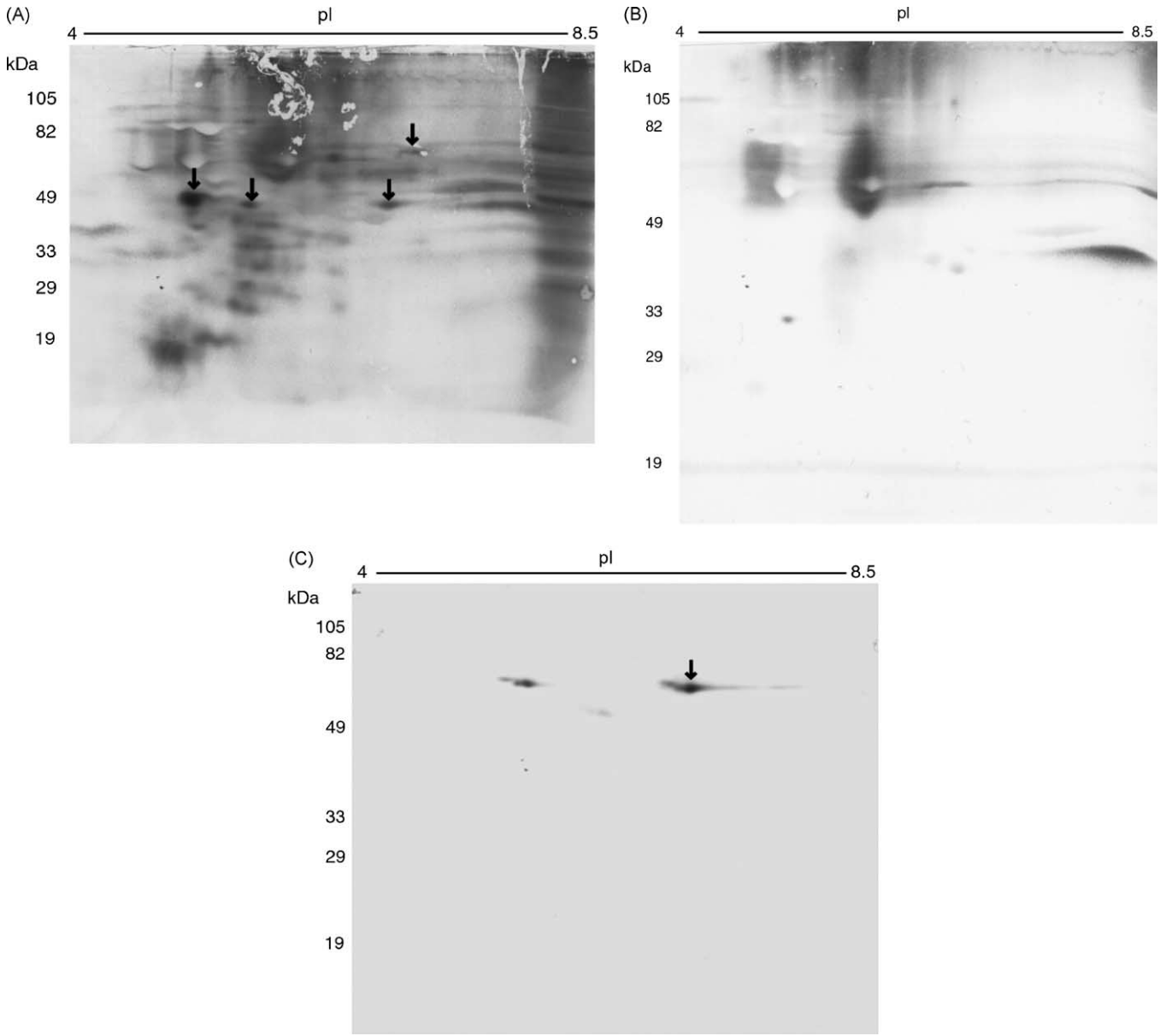

Fig. 4. Western blot analysis of liver microsomes from cocaine-treated (A) and control (B) mice. Proteins (1200 μg per gel) were separated by 2D-SDS–PAGE, transferred to membrane and probed with anti-cocaine antibody. Western blots were aligned with replicate Coomassie-stained blots to identify immunoreactive proteins. Western blot of microsomes using anti-transferrin antibody showed immunobinding to the 70–80 kDa target protein (C).

liver from saline controls (Fig. 4B). Quantities of these proteins in the gels proved insufficient for sequence analysis. Western blots of the 2D gels disclosed the presence of a cocaine–protein adduct not seen in previous 1D experiments, with a relative molecular mass of approximately 70–80 kDa (pI 7.2, Fig. 4A). This protein was excised from the gel, and sequence analysis indicated 85% homology (18 residues) with rat transferrin (Table 2). No secondary sequences that might indicate the presence of contaminating proteins were detected. Western blot analysis of the 2D gels using anti-transferrin antibody was used to confirm the identity of this protein (Fig. 4C).

Table 2
N-Terminal amino acid sequence of the 70–80 kDa cocaine-binding protein

Protein 70–80 kDa	1	VPDKTVKW-AVSEHHNTK-I	20
Rat transferrin	20	VPDKTVKWCAVSEHENTKCI	39

N-Terminal sequence has 85% identity to amino acids 20–39 of rat transferrin.

4. Discussion

It was demonstrated over 20 years ago that the hepatic N-oxidative metabolism of cocaine results in the formation of reactive metabolites that bind to proteins [10]. While it has long been speculated that this binding is responsible for cocaine hepatotoxicity [10,12,18,19] there has been little study of the protein targets for cocaine adduction.

Recently, Western blot analysis using an anti-cocaine antibody has shown that binding of cocaine reactive metabolite(s) occurs rather selectively with specific proteins, and that these proteins are located primarily in the mitochondria and microsomal fractions [14]. Strongest immunoreactivity occurred with proteins with relative molecular masses of 20, 44, 52–54, and 64 kDa.

As an initial step in the further characterization of hepatic cocaine–protein adducts, it was important to determine whether the immunochemical approach (i.e. Western blot analysis using an anti-cocaine antibody) was capable of detecting all of the adducted proteins. The specificity of the anti-cocaine antibody used in these studies has been evaluated by ELISAs, competitive Western blots, and immunohistochemistry, and shown to be adequate for detecting proteins adducted selectively by cocaine metabolites [14]. However, there is always the possibility that the conformation of one or more cocaine–protein adducts may not be immunoreactive, and that these adducts would therefore escape detection by Western blot analysis. To address this, mice were administered a radiolabeled cocaine dose, and cocaine–protein adducts were detected by fluorography. The results of fluorography were comparable with those from Western blot analysis, suggesting that either approach affords a reasonably complete assessment of cocaine–protein adduct formation. The advantage of fluorography is that the signals are likely to be relatively specific and readily interpreted. Its disadvantage is that it involves the use of expensive radiolabeled drug and is time-consuming in that the X-ray film may require months of exposure. Western blot analysis presents some nonspecific binding signals, as observed in the saline-treated mice, and these binding signals could obscure the presence of some cocaine–protein adducts. The principal advantage of Western blot analysis is that it is comparatively inexpensive and rapid.

In exploring the relationship between cocaine–protein adducts and toxicity, it was important to be cognizant of the shift in the site of cocaine hepatic necrosis associated with pretreatment with certain cytochrome P-450 inducers. In the ICR mouse, hepatic necrosis following a cocaine dose in naive mice occurs primarily in the midzonal region [13]. In phenobarbital-pretreated mice, necrosis occurs in the periportal region, and the perivenular area is spared. Pretreatment with β-naphthoflavone, on the other hand, results in necrosis primarily in the perivenular area, with little or no periportal necrosis except in the case of massive doses. It has been shown by immunohistochemistry that the site of reactive metabolite binding in the liver also shifts, corresponding with the site of necrosis [13]. Since hepatocytes in different regions of the lobule have well described morphological and biochemical differences [20], we hypothesized that the shifts within the lobule in the site of protein binding and toxicity might be associated with changes in target proteins for binding. This was not the case, however. The same pattern of cocaine immunoreactivity was observed in Western blots of liver proteins, separated by 1D-SDS–PAGE, from naive, phenobarbital-pretreated, and β-naphthoflavone-treated mice administered cocaine. It is possible that 2D-SDS–PAGE separation of proteins might reveal some differences, but unlikely that these differences would be substantial. The apparent consistency of cocaine–protein targets in different regions of the lobule confirms the specificity of these proteins as targets for cocaine reactive metabolites.

The Westerns blots did not show a marked increase in the amount of adducted protein in mice pretreated with phenobarbital. Phenobarbital pretreatment induces a number of P450s, including P450 3A, which has been proposed to be important in the bioactivation of cocaine in mice and humans [21]. Based on induction of P450, an increase in the quantity of adducted proteins might be expected. However, the effects of hepatic enzyme inducers on cocaine hepatotoxicity in mice appear to differ greatly among strains and between genders [22], and their effects on the extent of protein adduction likely vary as well. In the ICR mouse, the shift in the site of necrosis with phenobarbital and β-naphthoflavone pretreatment is not accompanied by marked changes in the degree of hepatic injury [13]. Western blots of cocaine–proteins adducts in the present study are consistent with this, showing little change in the overall extent of protein adduction with phenobarbital or β-naphthoflavone pretreatment.

A critical step in the assessment of protein adduction as a mechanism of cocaine hepatotoxicity is the identification of the adducted proteins. Although a number of major protein targets were detected, only the 64- and the 70–80-kDa target proteins were present in amounts large enough for sequence analysis. The 64 kDa protein had 100% amino acid sequence identity to mitochondrial hsp 60. The sequence started from amino acid 27 (alanine) indicating that the mature form of the protein (hsp 60) was adducted because the precursor contains a 26-amino acid mitochondrial targeting sequence at its N-terminal end [23], and Tyr 26 has been identified as the final residue of the mitochondrial transit peptide [24]. Heat shock proteins of the 60 and 70 kDa classes play a role in the recognition of nascent peptides, and ensure proper folding, transport and targeting of proteins [25–27]. In addition, hsp 60 may play a role in preventing denaturation, and/or increased degradation of damaged or abnormal proteins [28]. A study by Soltys and Gupta [29] examined the subcellular distribution of hsp 60 in a variety of mammalian cells, including the rat liver, and showed that 80–85% of hsp 60 is localized in the mitochondria. Thus, cocaine binding to mitochondrial hsp 60 may represent potential adduction of most of the hsp 60 available to the liver cell. If adduction of hsp 60 results in inhibition or loss of hsp 60 function, it is likely that mitochondrial function could be compromised and may eventually lead to cell injury. Ultrastructural studies have shown that membrane disruption and mitochondrial swelling occur in mouse liver 2 hr

after cocaine treatment [30]. Devi and Chan [31] also showed in rats that mitochondrial function is compromised in cocaine toxicity and is accompanied by an increase in reactive oxygen species [32]. The effects of cocaine adduction on hsp 60 function have not been determined, but will be important in evaluating its possible role in cocaine-induced liver damage.

Sequence analysis of the 70–80 kDa microsomal target protein revealed 85% identity with rat transferrin. Transferrin is an iron binding protein that transports iron from sites of absorption and heme degradation to those of storage and utilization. Transferrin can also stimulate cell proliferation and has been regarded as a growth factor [33]. The consequences of transferrin adduction on cellular iron homeostasis and its relationship to cocaine hepatotoxicity can only be speculative at this point. However, free intracellular iron promotes the production of reactive oxygen species that are believed to cause lipid peroxidation of membranes, and have been implicated in cocaine-induced liver damage. Goldlin and Boelsterli [34] have shown that cocaine causes an increase in reactive oxygen species in cultured rat hepatocytes. Pretreatment of hepatocytes with desferoxamine, an iron chelator, significantly reduces production of reactive oxygen species and protects the cells from cocaine cytotoxicity [34].

In conclusion, hepatotoxic doses of cocaine result in the adduction of specific proteins in mouse liver. It appears that the same proteins are adducted regardless where in the hepatic lobule toxicity occurs (i.e. periportal, midzonal, or perivenular regions). Two of the protein targets have been identified—one is mitochondrial hsp 60 and the other is transferrin. Impairment of either protein could plausibly contribute to cocaine hepatotoxicity, although such impairment and its consequences have not yet been demonstrated. The isolation and identification of other cocaine–protein adducts is possible using different approaches (such as preparative scale SDS–PAGE), and will be important in gaining a complete understanding of the potential role of protein adduction in cocaine liver toxicity.

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