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COCAINE-INDUCED CHANGES IN PERFUSION PRESSURE AND BILE FLOW IN PERFUSED RAT LIVERS*

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Abstract—Cocaine produces hepatotoxicity. To study the acute effect of cocaine on the liver, we used the isolated, single-pass perfused rat liver. When perfusion pressure was measured in a constant flow system, a 15-min infusion of cocaine (1.47 mM) increased perfusion pressure (136 ± 15%), decreased bile flow (61 ± 5%), and decreased oxygen uptake (82 ± 5%). The vasoconstriction was concentration-dependent and reversible. The pressure increase elicited by cocaine was not inhibited by the alpha-receptor antagonists phentolamine, prazosin, or yohimbine. These antagonists did inhibit phenylephrine-induced increases in perfusion pressure. Neither serotonin at concentrations up to 1 mM nor lidocaine or procaine in concentrations equimolar to cocaine increased the perfusion pressure. Indomethacin (5 μM), SKF-525A, and chloramphenicol also failed to block vasoconstriction induced by cocaine. High concentrations of cocaine were cholestatic, while concentrations lower then 0.6 mM were choleretic. These results indicate that cocaine-induced vasoconstriction in the liver is not mediated by alpha-receptor activation or prostaglandins and does not require metabolic activation of cocaine. The acute effects of cocaine in the perfused liver are vascular (vasoconstriction) and functional (alteration in bile formation).

Cocaine has many actions. In addition to its central nervous system effects, it is also a local anesthetic and produces vasoconstriction. It is widely accepted that cocaine-induced vasoconstriction is due to elevation of sympathomimetic amines resulting from inhibition of re-uptake of these amines [1]. Cocaine also produces hepatotoxicity in mice [2–4]. The liver damage is characterized by accumulation of lipids, necrosis, and release of enzymes including glutamic-oxaloacetic transaminase (GOT)‡. Studies from this laboratory have shown that metabolism of cocaine by the cytochrome P-450 system is necessary for its hepatotoxic effects [5]. The toxic response to cocaine varies with the method of enzyme induction and the sex and strain of mice [4, 6]. Although formation of norcocaine, one of the toxic metabolites of cocaine, has been demonstrated in the rat [7, 8], cocaine failed to produce liver damage in rats [9]. It has been suggested that cocaine is converted rapidly to non-toxic metabolites by the abundant serum esterases in rats. Recently, liver damage from cocaine has been reported in spontaneously hypertensive rats and may be related to strain differences in *N*-demethylase activity [10].

We decided to study the effect of cocaine in isolated perfused rat liver primarily for two reasons: (1) Isolated rat livers perfused with electrolyte solution should be devoid of extracellular esterases and, thus, may allow detection of hepatotoxic effects of cocaine. (2) Most of the studies on the hepatotoxicity of cocaine have been *in vivo*, and release of hepatocellular enzyme (GOT) was used as an indicator of liver damage. Since the release of hepatocellular enzymes represents major damage to plasma membranes, it is likely that such damage may be preceded by other acute effects which can be detected in isolated perfused livers. Preliminary studies with a gravity flow, recirculating perfusion system showed that cocaine (1.47 mM) decreased perfusate flow and bile formation. The decrease in perfusion flow could be due to cocaine-induced vasoconstriction, and the decrease in bile flow could be a direct effect of cocaine or the result of the decrease in perfusion. The present study describes experiments designed to define the mechanism of cocaine-induced changes in perfusion flow. Results show that this effect of cocaine is unique among local anesthetics and is not indirectly mediated by sympathomimetic amines.

MATERIALS AND METHODS

Materials. Cocaine hydrochloride was obtained from Merck and the Research Division of the National Institute on Drug Abuse. Tritiated cocaine, levo-[*benzoyl*-3,4-^{3}H(N)]cocaine (sp. act. 26.2 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Norcocaine was synthesized by the method of Borne *et al.* [11]. Procaine, lidocaine, epinephrine bitartrate, histamine dihydrochloride, indomethacin, yohimbine, heparin, NADH, sodium pyruvate, and the hydrochloride salts of dibucaine, tetracaine, norepinephrine, phenylephrine, papaverine, and serotonin were purchased from Sigma (St Louis, MO). Prazosin was a gift from Pfizer (Groton, CT), phentolamine from CIBA (Suffern, NY), and SKF-525A from Smith, Kline & French (Philadelphia, PA). Tropacocaine was purchased from Aldrich (Milwaukee, WI), and chloramphenicol

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‡ Abbreviations: GOT, glutamic-oxaloacetic transaminase; LDH, lactic acid dehydrogenase; and SKF-525A, 2-diethylaminoethyl-2,2-diphenyl-valerate hydrochloride.

from Parke, Davis & Co. (Morris Plains, NJ). All other chemicals were reagent grade.

Liver isolation and perfusion. Livers were obtained from male Wistar rats (≥ 200 g) and perfused by a method modified from that described by Anwer and Hegner [12]. While the rat was under ether anesthesia, the bile duct was cannulated with PE 10 tubing. The rat was heparinized, and the portal vein was cannulated with a 16-gauge intravenous catheter. After exsanguination, the caudal vena cava was cannulated with a 14-gauge catheter. The liver was removed from the body cavity and placed on a covered plexiglass plate above a collecting funnel. A Cole–Parmer peristaltic pump delivered warm (37°) buffer to the portal cannula via a bubble trap. Pressure was monitored continuously by a simple manometer column and a Medex dome pressure transducer connected to the perfusion line just proximal to the liver. Perfusion pressure was recorded on a Grass model 78D physiograph. The liver lobes were positioned to minimize perfusion pressure. The perfusion flow (~ 40 ml/min) was then increased until the hydrostatic pressure was 14–15 cm of water. The perfusate contained 120 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 0.6 mM $MgSO_4$, 5.5 mM glucose, 1.3 mM $CaCl_2$, and 26 mM $NaHCO_3$ and was aerated with 95% O_2:5% CO_2. The pH was maintained within the range of 7.35 to 7.45. In several experiments, oxygen uptake was measured with a YSI Biological Oxygen Monitor (model 53) by inserting the oxygen electrode in the effluent perfusate.

Experimental protocol. A single-pass system with constant flow rate was employed. Cocaine and other substances were infused into the perfusate just proximal to the liver. Following a baseline period, cocaine and [^{3}H]cocaine or other substances were infused for 15 min. Perfusion continued for up to 90 min after the drug infusion ended. Bile was collected and measured gravimetrically at 5-min intervals. Perfusate was collected, and the volume was measured at 5-min intervals. Aliquots of perfusate and bile were counted in order to calculate the hepatic uptake and biliary excretion of cocaine. No attempt was made to separate cocaine from its metabolites. Thus, the values reported for uptake and excretion include cocaine and its metabolites.

In a separate set of experiments, the following protocol was used to determine the effect of inhibitors on the concentration-dependent vasoconstriction produced by cocaine. Because of the variation among individual livers, the same liver was used to compare the effects of cocaine in the presence and absence of the inhibitor. After a 20-min baseline perfusion, a concentration–response curve was produced by a series of 1-min infusions of cocaine. This was followed by infusion of an inhibitor (or buffer for controls), and 30 min later another concentration–response curve was generated in the presence of the inhibitor. Only one inhibitor was tested in each liver.

Lactic acid dehydrogenase (LDH) assay. Aliquots of perfusate were assayed spectrophotometrically for LDH by a method based on the work of Wroblewski and LaDue [13].

HPLC quantitation of cocaine and norcocaine. In two experiments, effluent perfusate samples were assayed for cocaine and norcocaine by the HPLC method of LeDuc *et al.* [14]. The perfusate was diluted in mobile phase, 0.05 M KH_2PO_4 (pH 2.6)/acetonitrile 18% (v/v), containing 50 μl/liter nonylamine. Chromatography utilized a Nova-PAK C_{18} column and a PLRP-S guard cartridge with detection at 234 nm.

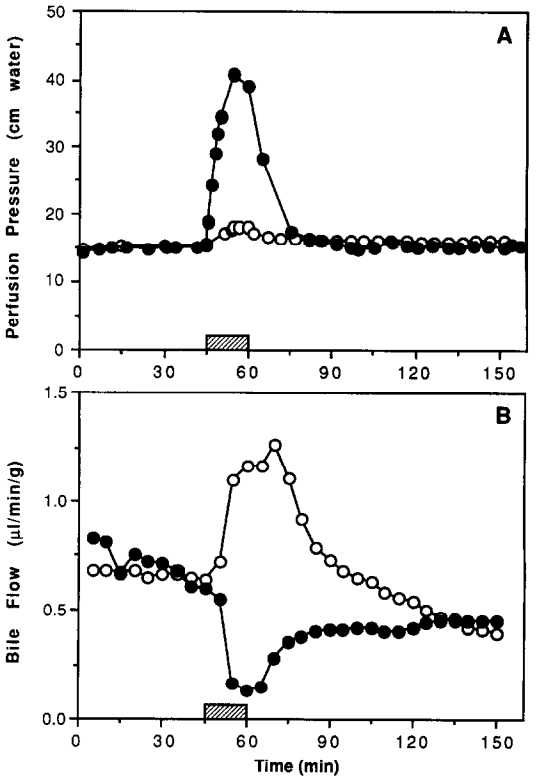

Fig. 1. Comparison of the effects of high and low concentrations of cocaine on perfusion pressure (A) and bile flow (B). Rat livers were isolated and perfused with a bicarbonate-buffered electrolyte solution in a single-pass, constant flow system. Cocaine [0.365 mM (○) or 2.59 mM (●)] was infused from 45 min through 60 min as indicated by the bar. (Representative experiments, N = 3.)

RESULTS

Effect of cocaine on perfusion pressure and bile flow. Infusion of cocaine affected both perfusion pressure and bile flow (Fig. 1). These effects were concentration-dependent and reversible. At low concentrations (0.365 mM) cocaine produced a small increase in perfusion pressure and increased bile flow. At high concentrations (2.59 mM), pressure increased almost 3-fold and bile flow declined from 0.7 to 0.13 μl/min/g liver before returning to basal level after about 1 hr (Fig. 1). Concentration-dependent effects on pressure and bile flow were determined by infusing each liver with a single concentration of cocaine for 15 min. Results of 23 experiments showed that there was a concentration-dependent increase in perfusion pressure despite

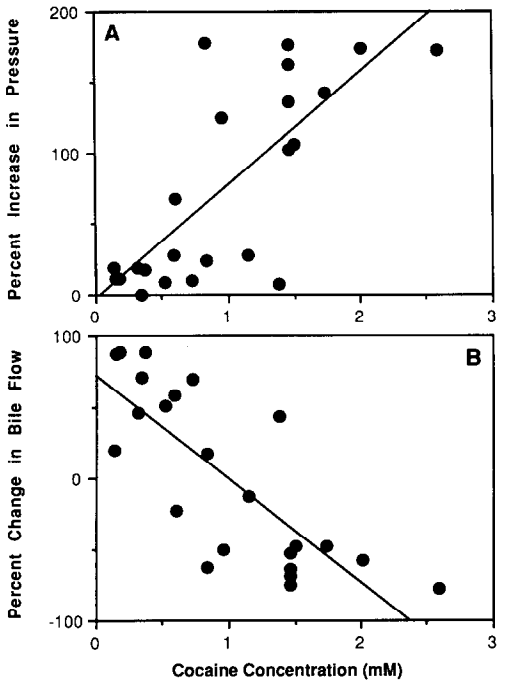

Fig. 2. Correlations of cocaine concentration with pressure and bile flow. Each liver was infused with one concentration of cocaine for 15 min as described in the legend of Fig. 1. (A) Correlation of cocaine concentration and the maximum percent increase in perfusion pressure. $r = 0.763$, $P < 0.001$. (B) Correlation of cocaine concentration and the maximum percent change in bile flow. $r = 0.786$, $P < 0.001$. (N = 23.)

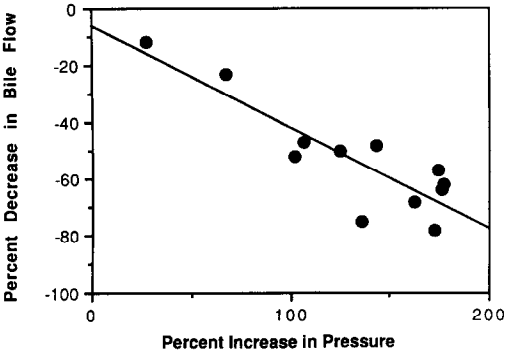

Fig. 3. Correlation between perfusion pressure and bile flow. In experiments where there was no net choleresis, the maximum percent decrease in bile flow was inversely related to the maximum percent increase in perfusion pressure. Each liver was infused with one concentration of cocaine (0.60 to 2.59 mM) for 15 min as described in the legend of Fig. 1. $r = 0.875$, $P < 0.001$ (N = 12.)

wide variations among individual livers (Fig. 2A). The extent of the pressure increase was also dependent on the duration of cocaine infusion. For example, a 15-min infusion of 1.7 mM cocaine produced a 143% increase in pressure, but infusion of 1.7 mM cocaine for 1 min produced only a small change. The effect of cocaine on bile flow was more complex. At low concentrations of cocaine bile flow increased, whereas at high concentrations it decreased (Fig. 2B). Perfusion pressure may play a role in decreasing bile flow. In experiments where there was no net choleresis, bile flow was inversely related to the pressure increase (Fig. 3).

Effect of cocaine on oxygen uptake. Oxygen uptake was recorded in five livers infused for 15 min with 1.47 mM cocaine (Fig. 4). In each case, as the pressure began to increase, there was a brief increase in oxygen uptake of 36.9 ± 4.5% followed by a rapid decrease of 82.4 ± 4.6%. The decrease in bile flow lagged slightly behind the drop in oxygen uptake. After the cocaine infusion, perfusion pressure decreased, oxygen uptake returned to baseline levels, and bile flow began to increase. Again the pressure response was the most rapid, followed by the oxygen uptake and finally the bile flow.

Role of mediators in cocaine-induced increase in perfusion pressure. Different agonists and antagonists were used to determine whether the effect of cocaine was mediated indirectly via release of known mediators. Effects of alpha-adrenoceptor antag-

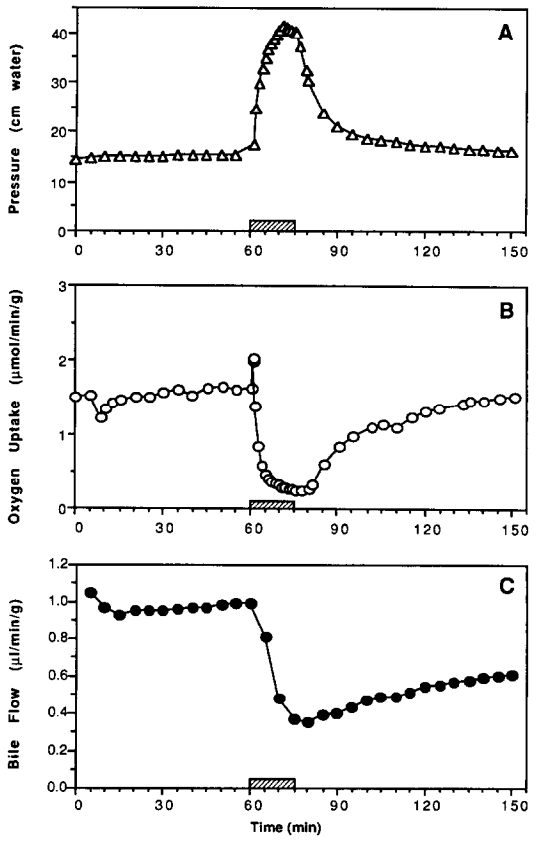

Fig. 4. Effects of cocaine on perfusion pressure (A), oxygen uptake (B) and bile flow (C). Livers were perfused as described in the legend of Fig. 1, and oxygen saturation of the effuent perfusate was measured with a YSI monitor. After a 60-min baseline period, 1.47 mM cocaine was infused for 15 min as indicated by the bar. (Representative experiment, N = 5.)

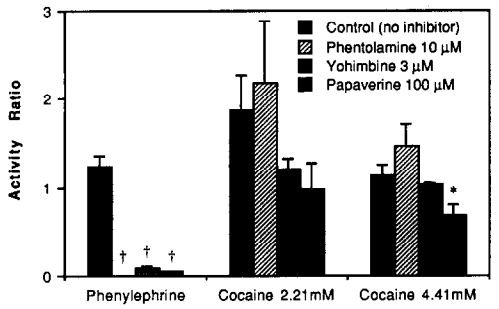

Fig. 5. Effects of antagonists on cocaine-induced vasoconstriction. Experimental protocol in each liver was as follows. A concentration–response to cocaine was determined by a series of 1-min injections. This concentration–response was followed by infusion of an antagonist (or buffer in control experiments) for 30 min. Then another concentration–response to cocaine was determined in the presence of the antagonist. Results are expressed as activity ratios (mean ± SE). Activity ratio = P_a/P_o, where P_a is the pressure increase in the presence of the antagonist (or buffer in control experiments) and P_o is the pressure increase in the absence of the antagonist. An activity ratio of 1.0 would indicate that the response to a given concentration of cocaine was the same in the presence and absence of the antagonist. The effects of antagonists on 5 μM phenylephrine-induced vasoconstriction were determined similarly. The activity ratios determined in the antagonist experiments were compared to the corresponding control value using Student's *t*-test. Key: (*) $P < 0.05$ and (†) $P < 0.001$. (Control, N = 8; phentolamine, N = 5; yohimbine, N = 3; papaverine, N = 5.)

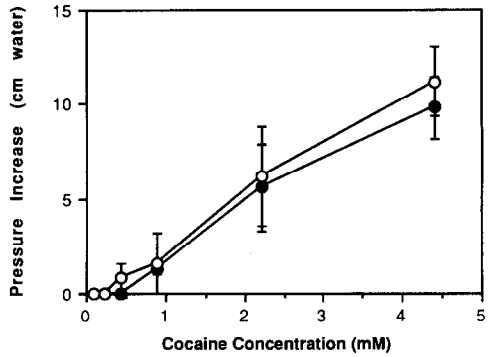

Fig. 6. Effect of indomethacin on cocaine response. Concentration–response curves were produced by 1-min injections of cocaine before (●) and during (○) the infusion of 5 μM indomethacin. Values shown are means ± SE (N = 3).

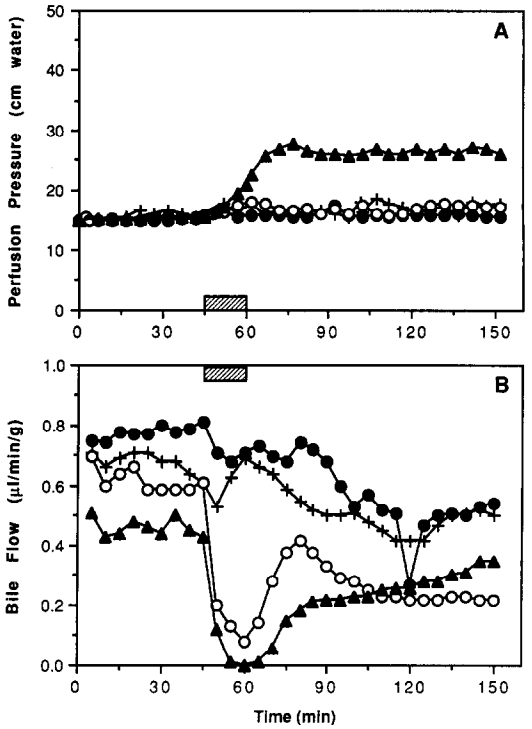

Fig. 7. Effects of local anesthetics on perfusion pressure (A) and bile flow (B). Each local anesthetic was infused for 15 min following a 45-min baseline period. Key: procaine, 1.13 mM (●); lidocaine, 1.85 mM (+); tetracaine, 2.2 mM (○); and dibucaine, 2.31 mM (▲).

onists were tested because alpha-adrenoceptor agonists increase perfusion pressure and because cocaine is known to block the re-uptake of norepinephrine [15]. The alpha-receptor antagonists phentolamine (10 μM), yohimbine (3 μM) (Fig. 5), and prazosin (1 μM) (data not shown) inhibited the increase in perfusion pressure induced by phenylephrine but did not inhibit the pressure increase in response to cocaine. Thus, it is very unlikely that the effect of cocaine is mediated indirectly by the local effects of epinephrine or norepinephrine. Other mediators such as serotonin, acetylcholine and histamine produced only a small change in pressure, indicating that these mediators are not involved. Mediation by prostaglandins also seems unlikely because indomethacin (5 μM) failed to inhibit the effect of cocaine (Fig. 6). Of all the inhibitors and receptor antagonists tested, only papaverine, a nonspecific smooth muscle relaxant, partially inhibited the cocaine effect (Fig. 5).

Role of cocaine metabolism. Since the hepatotoxic effect of cocaine in mice is dependent on the metabolism of cocaine by cytochrome P-450 enzymes [5, 9], the possibility of metabolic activation of cocaine in the rat liver was investigated. Neither chloramphenicol (96 μM) nor SKF-525A (20 μM) inhibited the response to cocaine. The effectiveness of the *in vitro* SKF-525A treatment was verified by measuring the levels of cocaine and norcocaine in the perfusate. For example, a sample of perfusate collected approximately 0.5 min after injection of 4.41 mM cocaine contained 2.85 mM cocaine and 83.01 μM norcocaine. During the infusion of SKF-525A, a similar perfusate sample contained 2.97 mM and 19.04 μM cocaine and norcocaine respectively. Thus, a small percentage of cocaine was converted to norcocaine, and SKF-525A reduced that amount of norcocaine by 77%.

Effect of other local anesthetics. To test whether other local anesthetics produce effects similar to cocaine, livers were perfused for 15 min with procaine, lidocaine, tetracaine or dibucaine (Fig. 7).

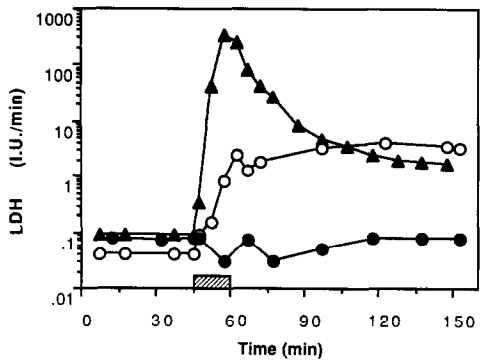

Fig. 8. Effects of local anesthetics and cocaine on LDH release. Livers were infused with 2.59 mM cocaine (●), 2.21 mM tetracaine (○), or 2.31 mM dibucaine (▲) as described in the legend of Fig. 1. LDH is expressed as I.U. released per minute of perfusion.

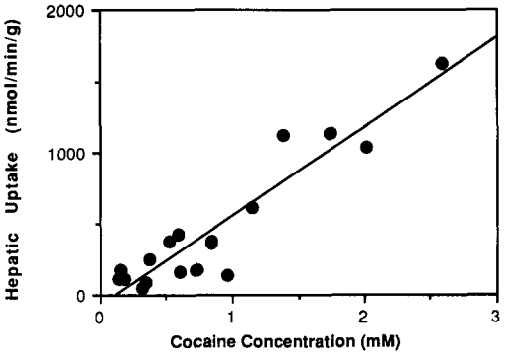

Fig. 9. Correlation between the concentration of cocaine infused and the hepatic uptake of cocaine. Conditions are described in the legend of Figs. 1 and 2. $r = 0.932$, $P < 0.001$. (N = 18.)

Only dibucaine produced a large increase in pressure, but, unlike the effect produced by cocaine, the increase was irreversible and the onset of the increase was delayed. Both tetracaine and dibucaine dramatically decreased bile flow (Fig. 7B) and increased LDH release into the perfusate (Fig. 8). Release of LDH was barely detectable during cocaine infusion. The dibucaine- and tetracaine-treated livers also became edematous and pale. These results indicate that local anesthetics do not produce the same effect as cocaine and that dibucaine and tetracaine in millimolar concentrations are acutely toxic to the perfused liver.

Tropacocaine, a structural analog of cocaine lacking the carboxymethyl group at position 2, does not produce liver damage in mice [5] and does not block re-uptake of norepinephrine. Infusion of tropacocaine was associated with increased pressure and decreased bile flow. These changes were quantitatively smaller and occurred at higher concentrations than those seen with cocaine. Tropacocaine (2.2 mM) produced a 78% increase in perfusion pressure in contrast to the 174% increase seen with 2.0 mM cocaine. Tropacocaine concentrations of 4.4 and 4.9 mM were required to produce a decrease in bile flow of 81 and 83%, similar to the 78% decrease seen with 2.59 mM cocaine.

Hepatic transport of cocaine. Hepatic uptake of cocaine was estimated from the difference in the amount infused and the amount recovered in the post-liver perfusate over the 15-min infusion period. A tracer amount of radiolabeled cocaine was added to the infusate for these studies, and the amount of cocaine was calculated from the specific radioactivity. This net uptake of cocaine increased linearly as the concentration of infused cocaine increased (Fig. 9). The appearance of radioactivity in the bile following infusion of radiolabeled cocaine indicated that cocaine and/or its metabolites (cocaine-equivalents) were excreted into bile. Unlike hepatic uptake, biliary excretion of cocaine-equivalents declined as the concentration of infused cocaine increased. The highest total excretion, 5.18 μmol (5.4% of infused dose), occurred with 0.15 mM cocaine, and the lowest, 1.44 μmol (0.11%), occurred with 2.59 mM cocaine. In all 18 experiments, the concentration of cocaine in the bile at peak excretion remained relatively constant at 4.3 ± 0.3 μg cocaine/μl bile (12.65 ± 0.88 mM). As the total bile volume decreased, the total excretion of cocaine decreased.

DISCUSSION

These studies have shown that cocaine at millimolar concentrations reversibly increases perfusion pressure and alters bile flow. These effects of cocaine are not shared by other local anesthetics. Procaine and lidocaine at equimolar concentrations did not produce cocaine-like effects. The pressure increase produced by dibucaine was not reversible, and the effects of dibucaine and tetracaine on bile flow were also qualitatively different from that of cocaine. Norcocaine and tropacocaine, both structurally related to cocaine, also produced an increase in pressure. Thus, the observed effects of cocaine are related to its structure, not to its local anesthetic properties.

Since the perfusion flow was controlled by a pump and remained constant, the increases in perfusion pressure produced by cocaine were attributed to vasoconstriction. Other possible causes of increased resistance such as edema, air embolism and clogging of sinusoids by debris are unlikely because the pressure increases were reversible and the physical appearance of the liver remained normal.

The mechanism by which cocaine induces vasoconstriction is speculative at this point. The present studies, however, indicate that the vasoconstriction is not mediated via release of known mediators. This conclusion is based on the lack of inhibition of cocaine-induced vasoconstriction both by alpha-adrenergic receptor blockers such as phentolamine, yohimbine, and prazosin, and by the cyclooxygenase inhibitor indomethacin. Compounds with alpha-adrenoceptor activity did produce vasoconstriction in our system, and this effect was inhibited by alpha-adrenergic blockers. Since serotonin failed to produce vasoconstriction by itself, it is unlikely that the effect of cocaine is mediated by the release of serotonin. Although norcocaine, a toxic metabolite of cocaine, produced vasoconstriction, the effect of

cocaine is not dependent on its metabolism. Chloramphenicol and SKF-525A inhibit microsomal drug metabolism [16, 17], yet neither compound altered the response to cocaine. These results suggest that cocaine-induced vasoconstriction may be due to a direct effect of cocaine on the hepatic vasculature. It is tempting to propose that the effect of cocaine may be mediated via as yet unknown mediators and/or receptors. Binding sites for cocaine have been reported in liver and brain [18, 19], but it is not clear whether they represent specific cocaine receptors or transporters for dopamine or norepinephrine.

At first glance, the lack of inhibition by alpha-adrenoceptor antagonists seen in our studies appears to contradict earlier literature on cocaine. There are numerous reports on potentiation of the effects of norepinephrine by cocaine in arteries and veins [20–22]. More recently, Pitts *et al.* [23] reported a pressor response in rats after intravenous administration of cocaine. This response was inhibited by phentolamine but not by reserpine. In contrast, Moore *et al.* [24] found that the increase in arterial pressure and the decrease in uterine blood flow in pregnant ewes after cocaine were not inhibited by phentolamine. The fact that cocaine enhances the effect of norepinephrine does not rule out the possibility that it can also have an effect on the vasculature not mediated by catecholamines. The concentration of cocaine, the species, and the vascular bed seem to be important factors in determining the effects of cocaine.

In the liver, cocaine-induced vasoconstriction may be generalized or localized primarily to pre-hepatic (portal vein and its major divisions), intrahepatic, or post-hepatic (hepatic vein) regions. The location of the vasoconstriction may influence liver function. If the vasoconstriction were primarily pre-hepatic, the hepatocytes would not be subjected to high pressure, and their function should not be impaired since perfusate flow rate is unchanged. Intrahepatic constriction may lead to shunting of perfusate resulting in inadequate oxygenation in portions of the liver. Post-hepatic vasoconstriction would subject the liver parenchyma to high interstitial pressure leading to swelling of the liver and impairment of liver function. Since the cocaine-induced increase in perfusion pressure was associated with an impairment of liver function (decreased bile flow) and was not associated with swelling, it is likely that the intrahepatic microvasculature is the site of cocaine action.

Bile formation is one of the essential functions of the liver. The effect of cocaine on bile formation is related to its concentration. Bile flow increased in response to lower concentrations (≤ 0.5 mM) of cocaine and decreased at higher concentrations (≥ 0.8 mM). The reversible cholestatic effect (decrease in bile flow) at higher concentrations indicates that liver function is reversibly impaired by cocaine. This impairment was not due to major damage to plasma membranes because cocaine did not increase the release of LDH. Taken together, these results show for the first time that rat liver function is altered by acute administration of cocaine.

The mechanism by which cocaine decreases bile formation is not clear. Changes in bile flow were significantly correlated with changes in perfusion pressure. This observation may be simply a coincidence (if both pressure and bile flow changes are dependent on cocaine concentration, a certain degree of correlation is expected), and a direct effect of cocaine leading to cholestasis cannot be ruled out. It is also possible that the cholestatic effect is secondary to the increase in perfusion pressure. In some experiments pressure did not increase although cocaine concentration was moderate to high, and in these experiments bile flow did not decrease. Anoxia has been shown to decrease bile flow [25] and, if shunting occurs, anoxia during vasoconstriction may play a role in decreasing bile formation. The decrease in oxygen uptake following the pressure increase (Fig. 4) supports the hypothesis that cocaine is causing intrahepatic vasoconstriction and shunting.

The mechanism of the choleresis induced by lower concentrations of cocaine is not clear. The choleretic effect of a drug is usually calculated from the increase in bile flow divided by the amount of drug excreted in the bile [12]. The choleretic effect of cocaine varied from 2.44 to 46.01 μl bile/μmol cocaine excreted. It is likely that this wide variation was caused by the sum of two processes: (1) cocaine induces choleresis directly, and (2) cocaine indirectly decreases bile formation by producing vasoconstriction. As the concentration of cocaine increases, the indirect effect of cocaine may be masking the direct effect. Although many inhibitors have been studied, it has not yet been possible to prevent the vasoconstriction in order to study the effects of cocaine concentration alone on bile formation. The nonspecific vasorelaxant papaverine partially inhibited the vasoconstriction, but at the 100 μM concentration necessary for that effect, papaverine itself inhibited bile flow.

The present studies also show that cocaine was transported by the liver and excreted in bile. Since net hepatic uptake was linearly related to cocaine concentration, the uptake process was not saturable at the concentrations studied. Thus, hepatic uptake may involve diffusion rather than a specific transport mechanism. Although cocaine equivalents appeared in bile, biliary excretion represented a maximum of 5% of the infused dose. Thus, biliary excretion is not a major route of cocaine excretion.

In summary, cocaine induced vasoconstriction in a reversible, concentration-dependent manner in isolated perfused rat livers. This effect was not mediated via known mediators and was not shared by other local anesthetics. In addition, cocaine decreased bile formation at concentrations that produce vasoconstriction. Cocaine was transported by the liver and excreted to a limited extent in bile.

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