Effects of Bromocriptine on Hepatic Cytochrome P-450 Monooxygenase System

Shabbir M. MOOCHHALA, Edmund J.D. LEE, Gwendolene T.M. HU, O.S. KOH¹ and Gordon BECKET¹

Department of Pharmacology, Faculty of Medicine and ¹Department of Pharmacy, Faculty of Science, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511

Accepted November 4, 1988

Abstract—We have evaluated the in vitro effects of bromocriptine (Br), on the hepatic cytochrome P-450 monooxygenase system of rats pretreated with saline phenobarbitone (PB) and β-naphthoflavone (BNF). Br inhibited ethoxyresorufin O-dealkylase (EROD) activity in liver microsomes of rats pretreated with saline and PB but not in BNF pretreated animals. Maximum inhibition of EROD activity by Br in the microsomes of saline and PB pretreated rats were 50%–60% of the control. In contrast, a dual effect was observed on aminopyrine N-demethylase activity (APD) by Br in microsomes of saline, PB and BNF pretreated rats. At a low concentration (25 μM), Br inhibited the activity of APD to a similar extent in all pretreatment groups; however, with higher concentrations of Br (50 μM to 300 μM), enhancement of APD activity was observed. Br (300 μM) increased the APD activity to 2–3 times the control level in microsomes of rats pretreated with saline, PB or BNF. Spectral studies revealed a Type I binding of Br to cytochrome P-450 from microsomes of saline and PB pretreated rats. A reverse type I binding was observed for BNF induced microsomes. In addition, Br also enhanced NADPH cytochrome c (P-450) reductase activity to a similar extent in all pretreatment groups. These results suggest that the inhibition of EROD activity may be due to direct binding by Br to certain isozymes of cytochrome P-450 and that the enhancing effect of Br on APD activity may be in part due to the activation of the NADPH cytochrome c reductase component of the cytochrome P-450 monooxygenase system.

Br (2-bromo-alpha-ergocriptine, Parlodel®), a semisynthetic ergot alkaloid, is a directly acting dopamine receptor agonist which is used primarily in the treatment of hyperprolactinemia. It is also effective in the management of prolactinomas, parkinsonism, acromegaly and for suppressing lactation (1).

Br is a lipophilic compound which contains a number of chemical groups that have the potential to be oxidized by cytochrome P-450-dependent monooxygenases in the liver. Indeed, it has been demonstrated that Br is extensively metabolized in the liver of both animals and man and that hydrolysis, isomerism and oxidation accounted for most of its metabolism (2). The many potential sites for oxidation by cytochrome P-450 led us to speculate that Br could interfere with the elimination of other drugs which are metabolized by the monooxygenase system in the liver. Such interference in the clearance of concurrently used drugs can result in either increased toxicity or reduced efficacy of these drugs. To date, only very few studies have reported drug interactions involving Br (3). None of these have indicated that Br interferes with the metabolism of other drugs.

Materials and Methods

Materials: Br methanesulfonate was a generous gift from Sandoz Ltd, Basel. Aminopyrine, ethoxyresorufin, β-naphthoflavone (BNF), equine heart cytochrome c, glucose-6-phosphate, glucose-6-phosphate...
dehydrogenase, NADP and NADPH were obtained from Sigma Chemical Company, U.S.A. Methanesulfonic acid was obtained from Aldrich Chemical Company, U.S.A. Sodium phenobarbital (PB) was obtained from Thornton and Ross, Ltd., U.K. All other chemicals used were commercial products of the highest available grade of purity.

**Animals:** Adult male Sprague-Dawley rats (200–250 g) obtained from the National University of Singapore, Animal Centre (Sembawang, Singapore) were used throughout the studies. Phenobarbital and β-naphthoflavone treated animals received three daily injections of the respective inducers (80 mg/kg, i.p.). Control animals received a similar volume of 0.9% sodium chloride solution. All animals were killed 24 hr after the last injection.

**Preparation of microsomes:** Rat liver microsomes were prepared as described by El Defrawy El Masry et al. (4). The microsomes were finally suspended in 50 mM potassium phosphate/potassium chloride buffer, pH 7.4, containing 20% glycerol (v/v) and stored at −80°C before use. Microsomal protein levels were determined by the method of Lowry et al. (5), using bovine serum albumin as the standard. Cytochrome P-450 levels in microsomes were determined by the method of Omura and Sato (6). An incubation mixture containing 6 mM glycerol was used throughout this study. Glycerol at this concentration had no effect on enzyme activity.

**Enzymatic assays:** The N-demethylation of aminopyrine in microsomes was determined by measuring the amount of formaldehyde produced (7), using the colorimetric procedure based on the Hantzsch reaction (8). Ethoxyresorufin O-dealkylase (EROD) activity was measured according to the method of Burke et al. (9). The activity of NADPH cytochrome c reductase was determined by the method of Williams and Kamin (10). The desired amounts of Br was dissolved in ethanol and added to the reaction mixture at a final ethanol concentration not exceeding 0.5%.

**Binding spectra:** The binding of Br with cytochrome P-450 was measured in a suspension of microsomes containing 2 mg/ml of microsomal protein in 0.1 M potassium phosphate buffer, pH 7.4 (11). Various amounts of the drug (ranging from 15 µM to 1.3 mM) dissolved in ethanol were added to the sample cuvette and an equal volume of ethanol was added to the reference cuvette. The ethanol concentration did not exceed 0.5% (v/v). Binding spectra were recorded from 350 nm to 500 nm in a Shimadzu UV-3000 spectrophotometer. The possible formation of metabolite intermediate complexes were examined by incubating Br (1 mM) with microsomes (2 mg protein/ml) and NADPH (0.5 mM) for 20 min at 37°C as described by Franklin (12). The difference spectrum between 400 nm and 500 nm was obtained by comparing this incubation mixture to an identical mixture incubated for only 15 sec.

**Results**

**Effects of bromocriptine on liver microsomal ethoxyresorufin O-deethylase (EROD) and aminopyrine N-demethylase (APD):** To determine the effect of Br on drug metabolism, we used liver microsomes prepared from rats pretreated with saline, PB and BNF. PB and BNF pretreatment were chosen since these compounds are well known inducers of different cytochrome P-450 isozymes (13). Ethoxyresorufin and aminopyrine were chosen as model substrates since liver microsomes from BNF and PB pretreated animals are known to metabolize these compounds at much higher rate than liver microsomes from control animals (13).

Figures 1 and 2 show the effects of Br on cytochrome P-450 dependent ethoxyresorufin O-deethylase (EROD) and aminopyrine N-demethylase (APD) activities. Br inhibited EROD activity (Fig. 1) to about 50%–60% (Br concentration ranged between 50 µM–100 µM) of the control activities in liver microsomes from saline and PB pretreated rats. In contrast, Br exhibited little inhibitory activity (<10% of control activity) in BNF-induced microsomes. It is of interest to note that in the control and PB induced microsomes, Br inhibited EROD to a lesser degree at higher concentrations (150 µM–300 µM) than at lower concentrations.

On the other hand, Br exhibited atypical effects on APD activity (Fig. 2). Br at 25 µM inhibited APD activity by 30% of the control...
Fig. 1. Inhibition of ethoxyresorufin O-deethylase activity by bromocriptine in liver microsomes from saline (□), phenobarbitone (PB) (+) and ß-naphthoflavone (BNF) (◇) pretreated rats. Control activities equaled 19.4±0.4, 50.4±19.8 and 2810±81 pmol resorufin formed/min/mg protein for control, PB and BNF induced microsomes, respectively (S.E.M. of 4 different experiments).

Fig. 2. Enhancement of aminopyrine N-demethylase activity by bromocriptine in liver microsomes from saline (□), phenobarbitone (PB) (+) and ß-naphthoflavone (BNF) (◇) pretreated rats. Control activities equaled 69±6, 185±19 and 83±15 nmoles HCHO formed/hr/mg protein for control, PB and BNF induced microsomes, respectively (S.E.M. of 4 different experiments).
in saline, PB and BNF-induced microsomes; but as the concentration of Br was increased (above 50 µM), APD activity was enhanced in a dose-dependent manner by approximately 200–300%.

**Bromocriptine induced difference spectra of microsomal cytochrome P-450**: To determine whether Br interacted directly with the cytochrome P-450 enzymes in liver microsomes prepared from rats pretreated with various inducers, spectral binding studies were performed (Fig. 3). The addition of Br to microsomes from saline and PB pretreated rats caused a type II difference spectrum (characterized by an absorbance maximum at 425 nm and an absorbance minimum around 390 nm–406 nm), whereas a reverse type I spectral change (characterized by an absorbance maximum at 420 nm and an absorbance minimum at 380 nm) was observed with microsomes from BNF pretreated rats. In all studies, the absorbance changes of Br followed Michaelis-Menten kinetics and increased with increasing concentration. The spectral dissociation constant (K_s) calculated from double reciprocal plots, were 27 µM and 15 µM for the control and PB induced microsomes, respectively. The spectral dissociation constant obtained from BNF-induced microsomes was 100 times higher than those of the other pretreatment groups (K_s=2 mM).

**Formation of metabolite-intermediate complexes with cytochrome P-450**: This procedure was carried out to show that Br does not require metabolic activation by liver microsomes to achieve its inhibitory potency. Br (1 mM) was incubated with microsomes and 0.5 mM NADPH for periods up to 20 min. No difference spectra was apparent when the spectra of these microsomes were compared to similar mixtures that were incubated for 15 sec; this suggests the absence of metabolite intermediate complexes (data not shown).

**Effects of bromocriptine on NADPH cytochrome c reductase**: The apparent enhancement of APD activity by Br raised the possibility that Br may not only be interacting with cytochrome P-450 itself, but also on some other necessary component of the monooxygenase system. In an experiment conducted using the alternative electron acceptor, ferricytochrome c, we found that Br caused a dose-dependent enhancement (approximately 2–4-fold) in reductase activity in microsomes of all three pretreatment groups (Fig. 4). This effect on reductase activity may account for the 2–3-fold enhancement of APD activity.

**Discussion**

To date, studies on the effect of Br on the metabolism of other drugs have not been reported. This study was an initial step taken to explore the possibility that Br may not only be interacting with cytochrome P-450 itself, but also on some other necessary component of the monooxygenase system. In this study, we report that Br can both inhibit and enhance cytochrome P-450 dependent oxidative metabolism of other drugs in the liver. This effect depended on the type of substrate and concentration of Br used, and it also depended on the pretreatment of rats with selective inducers of hepatic cytochrome P-450.

The inhibitory effect of Br on EROD activity appeared to arise from its ability to bind directly to the cytochrome P-450 component of the monooxygenase system. The different potency of Br to inhibit EROD activites in different pretreatment groups may be the result of different binding affinities of Br to different isozymes catalyzing O-
dealkylation. The binding of Br to microsomal P-450 seen spectrometrically (Fig. 3) is confirmatory of an interaction of Br with cytochrome P-450 of affinity similar to that observed for the inhibition of EROD activity. Microsomes from saline and PB pretreated rats showed binding affinities similar to their inhibitory potency. On the other hand, a much larger concentration of Br (Ks=2 mM) is required to elicit spectral change in microsomes from BNF pretreated rats. Br at the concentration range between 10 μM to 300 μM had little inhibitory effect on EROD activity. In addition, Br elicited type II spectral perturbation with microsomes of saline and PB pretreated rats, which is strongly suggestive of the formation of a nitrogenous ligand to the haem iron of cytochrome P-450 (14). The reverse type I binding spectrum observed when Br was added to microsomes from BNF pretreated rats is characteristic of the interaction at the hydrophilic site of the iron (14). Although EROD activity can be induced by either PB or BNF pretreatment, the EROD activity in PB-induced microsomes was shown to be inhibited to a greater degree by Br. These results strongly suggest that Br selectively inhibits EROD activity catalyzed by certain isozyme(s) of cytochrome P-450, especially those that are found constitutively or induced by PB. Studies of the interaction of Br with purified cytochrome P-450 isozymes will help resolve these possibilities.

The atypical effect of Br on APD activities in control, PB and BNF induced microsomes suggest that Br modulates the monooxygenase system by more than one mechanism. Such an effect is not uncommon as other studies in vitro have revealed that several compounds have dual effects on the cytochrome P-450 monooxygenase system. These compounds inhibit the activity of some forms of cytochrome P-450 while enhancing the activities of others (15). Aminopyrine N-demethylation is at present thought to be catalyzed by at least four different isozymes of cytochrome P-450 (16). As the oxidative metabolism (N-demethylation) of aminopyrine requires a flow of electrons from NADPH cytochrome c reductase (also called cytochrome P-450 reductase), stimulation of this enzyme may enhance cytochrome P-450-
dependent N-demethylase activity. Our results show that Br caused a dose-dependent increase in NADPH cytochrome c reductase activity in control, PB and BNF induced microsomes. Br enhanced NADPH cytochrome c reductase activity to an extent similar to its enhancement of APD activity. This suggests that Br's enhancing effect on APD activity is probably due in part to the activation of NADPH cytochrome c reductase. Further studies involving purified NADPH cytochrome c reductase may help resolve the site(s) on the reductase on which Br might be acting. These include 1) interaction between NADPH and NADPH cytochrome c reductase and 2) interaction between NADPH cytochrome c reductase and cytochrome P-450.

The results of this study raise the possibility of a drug interaction occurring during the clinical use of Br. The range of concentrations of Br used in this study is well above the serum concentrations which are found during the clinical use of Br in man (1). However, tissue concentrations of Br have been shown to be considerably higher than serum concentrations in animal experiments. For example, the concentration of Br in the liver was approximately 13 times higher than the serum concentration 1 h following the administration of a single dose of Br to rats (3). It is therefore possible that the effects observed in this study could be extrapolatable to the clinical context. Additional studies are being carried out in our laboratory to determine whether Br can influence the metabolism of cytochrome P-450 in vivo. Preliminary studies in our laboratory have shown that a single dose of Br (2 mg/kg, i.p.) could prolong zoxazolamine paralysis time significantly by three-fold (vehicle pretreated rats = 140±14 min, Br pretreated rats = 333±34 min). Zoxazolamine is metabolized by isozymes of cytochrome P-450 induced by 3-methylcholantherene or β-naphthoflavone.

In summary, Br appears to be both an inhibitor and enhancer of cytochrome P-450-dependent drug metabolism in the liver and therefore has the potential to cause drug interactions clinically. The enhancing effect on APD activity could be due in part to its enhancing effect on NADPH cytochrome c reductase, whereas its inhibitory effect is probably due to its direct binding to a certain isozyme(s) of cytochrome P-450.

Acknowledgment: This work was supported by a grant from the National University of Singapore (RP 870372).

References

1 Vance, M.L., Evans W.S. and Thorner M.O.: Bromocriptine Ann. Intern. Med. 100, 78–91 (1984)
2 Maurer, G., Schreier, E., Delabarde, S., Nufer, R. and Shukla, A.P.: Fate and disposition of bromocriptine in animals and man. Eur. J. Drug Metab. Pharmacokinet. 8, 51–62 (1983)
3 Schran, H.F., Tse, F.L.S. and Bhuta, S.I.: Pharmacokinetics and pharmacodynamics of bromocriptine in the rat. Biopharm. Drug Dispos. 6, 301–311 (1985)
4 El Defrawry El Masry, S., Cohen, G.M. and Mannering, G.J.: Sex dependent differences in drug metabolism in the rat. Drug Metab. Dispos. 2, 267–284 (1974)
5 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)
6 Omura, T. and Sato, R.: The carbon monoxide binding pigment of liver microsomes. J. Biol. Chem. 239, 2370–2378 (1964)
7 Sladek, N.E. and Mannering, G.J.: Introduction of drug metabolism II. Qualitative differences in the microsomal N-demethylating systems stimulated by polycyclic hydrocarbons and by phenobarbital. Mol. Pharmacol. 5, 186–199 (1969)
8 Nash, T.: The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. J. Biol. Chem. 55, 412–416 (1953)
9 Burke, D.M., Prough, R.A. and Mayer, R.T.: Characteristics of a microsomal cytochrome P-448 mediated reaction. Ethoxyresorufin O-deethylation. Drug Metab. Dispos. 5, 1–8 (1977)
10 Williams, C.H. and Kamin, H.: Microsomal triphosphopyridine nucleotide—cytochrome c reductase of liver. J. Biol. Chem. 237, 587–595 (1962)
11 Schenkan, J.B., Remmer, H. and Estabrook, R.W.: Spectral studies of drug interaction with hepatic microsomal cytochrome P-450. Mol. Pharm. 3, 113–123 (1967)
12 Franklin, M.R.: The influence of cytochrome P-450 induction on the metabolic formation of 455 nm complexes from the amphetamines. Drug Metab. Dispos. 2, 321–326 (1974)
13 Lu, A.Y.H. and West, S.B.: Reconstituted mammalian mixed function oxidase, requirements,
specificities and other properties. *In Hepatic Cytochrome P-450 Monooxygenase System*, Edited by Schenkman, J.B. and Kupfer, D., p. 523–544, Pergamon Press, Oxford (1982)

14 Schenkman, J.B., Sligar, S.G. and Cinti, D.L.: Substrate interaction with cytochrome P-450. *Pharmacol. Ther.* 12, 43–71 (1981)

15 Cinti, D.L.: Agents activating the liver microsomal mixed function oxidase system. *In Hepatic Cytochrome P-450 Monooxygenase System*, Edited by Schenkman, J.B. and Kupfer, D., p. 717–739, Pergamon Press, Oxford (1982)

16 Imaoka, S., Inoue, K. and Funae, J.: Aminopyrine metabolism by multiple forms of cytochrome P-450 from rat liver microsome. *Arch. Biochem. Biophys.* 265, 159–170 (1988)