Metabolite Involvement in Bromocriptine-Induced Prolactin Inhibition in Rats

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ABSTRACT

Bromocriptine (BCT) is a dopamine D2 receptor agonist used for the treatment of Parkinson’s disease and hyperprolactinemic disorders. After oral administration, BCT is metabolized into mono- or dihydroxylated metabolites. To study how these metabolites influence parent drug pharmacodynamics, we administered BCT to rats intravenously (1 mg/kg i.v.) and orally (10 mg/kg p.o.) and measured the inhibition of prolactin secretion. Despite similar areas under the curve for BCT, the duration of the effect was 36 h after oral and only 18 h after intravenous administration. Pharmacokinetic/pharmacodynamic models were used to correlate the concentration of BCT in the effect compartment with the lowering of prolactin. One of these models (effect compartment model) showed that the effective concentration (EC_{50}) at the site of action was much lower after oral (0.56 nM) than after intravenous administration (3.68 nM). In contrast, the EC_{50} values based on BCT metabolite data were in the same range for both administrations. These observations suggested the activity of one or more BCT metabolites. To confirm this hypothesis, hydroxylated metabolites of BCT (produced in vitro by rat liver microsomes) were administered i.v. (100 µg/kg) in rats. We found that monohydroxylated BCT was able to lower prolactin secretion like BCT. Dihydroxylated metabolites, as well as monohydroxylated metabolites, were effective in reducing prolactin secretion. Because we demonstrated that the concentration of hydroxylated metabolites after oral administration is 55-fold that of BCT, it can be concluded that BCT activity in the pituitary after oral administration is mediated by its metabolites.

BCT (2-bromo-α-ergocryptine) is a semisynthetic derivative of the ergot alkaloid family possessing dopamine agonist properties (Kebabian and Calne, 1979). Because of its inhibitory effect on prolactin secretion this drug has been used successfully for the treatment of prolactin-producing tumors and other hyperprolactinemic disorders, including idiopathic hyperprolactinemia and postpartum lactation (Thorner et al., 1980). BCT has also been useful in the treatment of acromegaly (Wass et al., 1977) and at higher doses in the treatment of Parkinson’s disease (Kopin, 1993).

When given orally to animals or humans, BCT is extensively metabolized by a hepatic first-pass effect. The main metabolites observed in the circulation are hydroxylated BCT and 2-bromolysergic acid (Maurer et al., 1982, 1983). Hydroxylated BCT metabolites have been implicated as possible active agents in the BCT-induced circling behavior of rodents (Reavill et al., 1980), hypothermia in rats (Silberberg et al., 1977) and behavioral effects in cats (Gonzalez-Lima et al., 1982, 1983). However, other authors failed to confirm these findings (Keller and Da Prada, 1979; Markey et al., 1979; Schran et al., 1985) and demonstrated that the duration of the hypothermic effect was better correlated with the concentrations of parent drug rather than with concentration of BCT plus metabolites. The presence and action of pharmacologically active metabolites in the central nervous system is clearly controversial, and this issue has not yet been resolved.

Several observations indicate that the effect of BCT in human pituitary is not correlated with the concentration of parent drug. In fact, it is known that prolactin inhibition persists when no BCT remains in the circulation (Katz et al., 1991). This was attributed to a prolonged action of the drug...
at the pituitary level rather than to a possible action of metabolites (Woolf, 1981).

To study the possible involvement of BCT metabolites at the pituitary level, we used a pharmacokinetic-pharmacodynamic model to correlate the concentration of BCT with its effect on in vivo prolactin secretion inhibition. We then produced hydroxylated metabolites by in vitro incubation of BCT with rat liver microsomes and demonstrated either in vitro or in vivo their dopaminergic activity on pituitary cells.

Materials and Methods

Animals. Male Sprague-Dawley rats were obtained from Iffa Credo (St-Germain sur l’Aberesle, France). They were maintained on a 12-h light/dark cycle with light from 7:00 a.m. to 7:00 p.m., in a temperature (21–22°C) and humidity (50 ± 10%)-controlled room. Rats were fed commercial rat chow (UAR, Villemoisson sur Orge, France), and tap water was available ad libitum. The rats were used after a 1-week acclimation period, at which time their body weights ranged from 250 to 300 g. All studies on animals comply with the French law on rules for animal experimentation, Decree 87–848, 19 October 1987.

Surgical procedures. The rats were anesthetized with flunthax (Pitman Moore, Meaux, France). An incision was made at the left and right groin and, respectively, the saphenous vein and femoral artery were exposed. A polyethylene catheter (PE 10, I.D., 0.28 mm; O.D., 0.68 mm; Clay Adams supplied by Becton Dickinson, Sparks, MD) was inserted into each vessel and held in position with a silk suture. The catheters were flushed with physiological serum containing 10% heparin to avoid coagulation, and their distal ends were sutured. The free end of the two catheters, emerging from the animals, were sutured. The second animal study was intended to compare directly the pharmacodynamic response, i.e., the lowering of plasma prolactin, with its main metabolites by two enzyme immunoassays specific for unchanged BCT or for the pool of hydroxylated metabolites (Valente et al., 1996). The hydroxylated metabolites were detected with antibodies directed against the bromoseryl part of the molecule. Unchanged BCT was detected by antibodies directed against the cyclopeptide structure of BCT. Enzymatic tracers were obtained by covalent coupling of BCT analogs to acetycholinesterase from the electric eel Electrophorus electricus. The specificity of antibodies was checked by cross-reactivity studies. Both assays have a limit of quantification of 50 pg/ml. The concentration of metabolites in each sample was calculated by subtracting the concentration determined by the assay measuring only untransformed BCT from the concentration determined by the assay measuring both BCT and its metabolites. Prolactin was determined by an enzyme immunoassay previously developed and validated in our laboratory (Duhau et al., 1991). The specificity of antibodies was checked by cross-reactivity studies. Both assays have a limit of quantification of 50 pg/ml. The concentration of metabolites in each sample was calculated by subtracting the concentration determined by the assay measuring only untransformed BCT from the concentration determined by the assay measuring both BCT and its metabolites. Prolactin was determined by an enzyme immunoassay previously developed and validated in our laboratory (Duhau et al., 1991).

Analytical methods. Plasma samples were analyzed for BCT and its main metabolites by two enzyme immunoassays specific for unchanged BCT or for the pool of hydroxylated metabolites (Valente et al., 1996). The hydroxylated metabolites were detected with antibodies directed against the bromoseryl part of the molecule. Unchanged BCT was detected by antibodies directed against the cyclopeptide structure of BCT. Enzymatic tracers were obtained by covalent coupling of BCT analogs to acetylcholinesterase from the electric eel Electrophorus electricus. The specificity of antibodies was checked by cross-reactivity studies. Both assays have a limit of quantification of 50 pg/ml. The concentration of metabolites in each sample was calculated by subtracting the concentration determined by the assay measuring only untransformed BCT from the concentration determined by the assay measuring both BCT and its metabolites. Prolactin was determined by an enzyme immunoassay previously developed and validated in our laboratory (Duhau et al., 1991).

Pharmacokinetic-pharmacodynamic modeling. The model proposed by Sheiner et al. (1979) was initially applied to the fitting of the pharmacokinetics and pharmacodynamics of BCT or BCT metabolites. To correlate the intensity of prolactin inhibition and the plasma concentration of either BCT or BCT metabolites, the pharmacokinetic-pharmacodynamic model was built up in three steps: (1) a pharmacokinetic model for characterization of drug absorption, distribution and elimination; (2) a link model between the plasma concentration and the concentration at the effect compartment (Cэфф), i.e., the pituitary; and (3) a pharmacodynamic model which relates the intensity of the drug effect to the Cэфф. Pharmacokinetic calculations and pharmacokinetic-pharmacodynamic modeling were performed using Sisphar software (Simed, Créteil, France). To obtain pharmacokinetic parameters for the modeling, two- and three-compartment models were fitted to the individual plasma concentrations of BCT or metabolites. Maximal concentrations of the drug in plasma (Cmax) and times of attaining these concentrations (Tmax) were evaluated from experimental data. Areas under the plasma concentration curves (AUC) were calculated according to the trapezoidal rule.

To establish a relation between the drug concentration at the effect site (Cэфф) and the effect (E) a link model was defined as follows:

\[ \frac{dC_{эфф}}{dt} = K_аC_{эфф} - K_{ес}C_{эфф} \]

where \( C_{эфф} \) was the drug concentration in the effect compartment and \( C_{эфф} \) was the drug concentration in the plasma compartment. The

In vitro experiments. Anterior pituitary cell dispersion was obtained according to the methodology described by Hopkins and Farquhar (1973), in which 2 × 10⁶ cells/well were seeded into 12-well plates in Dulbecco’s Modified Eagle’s Medium (Gibco supplied by Life Technologies, Cergy-Pontoise, France) containing 1.5 g/l NaHCO₃, 2 mM glutamine, 50 µM penicillin, 50 µg/ml streptomycin and 10% fetal calf serum for 2 days under a 95% air and 5% CO₂ atmosphere. The monolayers were rinsed with 1 ml of phosphate buffer saline (PBS) and preincubated in the same medium for 1 h. Incubation was then performed with or without dopamine, BCT, monohydroxylated (M1/M2) and dihydroxylated (M3 and M4) metabolites at 10⁻³ M. After 1, 2 and 4 h of incubation, 100 µl of medium were collected and frozen at −20°C until prolactin measurement.

Binding studies. Receptor binding studies were performed with rat striatum as described previously (Terai et al., 1989) by CEREPR (Celle L’Evescault, France). The binding assay used 0.1 nM [³H]YTM 09151–2 (DuPont NEN, Les Ulis, France) as radioactive ligand, and nonspecific binding was determined with 10 µM (+)-butaclamol. After incubation with BCT or hydroxylated metabolites, the membrane preparations were filtered in a vacuum filtration pump using Whatman GF/B filters. The filters were then rinsed three times with 4 ml of cold saline and placed in scintillation vials with Formula 989 (DuPont NEN, Les Ulis, France). The radioactivity trapped on the filters was counted in a liquid scintillation counter (LS6000 Beckman, Gagny, France). Competitive inhibition studies allowed the calculation of the binding affinity constants of BCT and its metabolites.

"The first animal study was designed to assess the effect of the plasma pharmacokinetics of BCT and its metabolites on the pharmacodynamic response, i.e., the lowering of plasma prolactin by rat liver microsomes and demonstrated either in vitro or in vivo their dopaminergic activity on pituitary cells.
The concentration-response relation in the effect compartment was denoted recently by Dayneka et al. (1994): the concentration of the drug in the effect compartment producing 50% of the maximum effect; and $n$ is the Hill coefficient governing the slope and the shape of the effect concentration curve.

Additionally, the indirect pharmacodynamic response model proposed recently by Dayneka et al. (1993) and Jusko and Ko (1994) was applied to our data. The pharmacodynamics of the prolactin response for the average data from the six rats treated intravenously or orally were characterized by use of inhibition model I (Jusko and Ko, 1994):

$$dPRL/\text{dt} = k_{\text{in}}[1 - (C_{\text{PRL}}/EC_{50} + C_e^n)] - k_{\text{out}}PRL$$

where $PRL$ is the prolactin concentration in plasma; $EC_{50}$, the drug concentration which produces 50% of maximum inhibition; $n$, the Hill factor for sigmoidicity; $C_{\text{PRL}}$, the drug concentration in the plasma compartment; and $k_{\text{in}}$ and $k_{\text{out}}$, the rate of prolactin input and output from the central compartment, respectively. Pharmacokinetic and pharmacodynamic were fitted simultaneously.

For both pharmacodynamic analyses and at each time, the placebo effect was subtracted from the observed effect. Goodness of the fit of the curves was determined by visual inspection, residual analysis and Akaike criteria.

**Preparation of metabolites.** Metabolites were obtained by rat liver microsomal incubations as described previously with minor modifications (Peyronneau et al., 1994). Male Sprague-Dawley rats were treated intraperitoneally with dexamethasone, a cytochrome P450 3A specific inducer. Rat liver microsomes were prepared as reported previously (Kremers et al., 1981) and stored at -80°C before use. BCT (10.5 mg in 1 ml of methanol) was incubated with rat liver microsomal suspension containing 200 nmoI of cytochrome P450 and an NADPH-generating system (0.5 mM NADP+, 5 mM glucose 6-phosphate and 1 U/ml glucose-6-phosphate dehydrogenase) in 100 ml of a 0.1 M phosphate buffer, pH 7.4, for 30 min at 37°C. Incubation was stopped by addition of 150 ml dichloromethane and stirred on ice for 10 min. The mixture was centrifuged and the organic phase was reduced by evaporation. Metabolites were purified by HPLC at room temperature on a C18 Ultrabase 5 μm column (250 × 7.5 mm; SFC, Neuilly Pliance, France). The mobile phase included 10% acetonitrile and 1 g/l ammonium carbonate in water (solvent A) and acetonitrile (solvent B). Elution was performed at a flow rate of 2 ml/min. A gradient of solvent B increased linearly from 0% to 100% in 45 min followed by 10 min of solvent A. BCT and metabolites were detected at 305 nm. HPLC/UV profiles of BCT metabolites were identical with those obtained previously (Peyronneau et al., 1994). Peak fractions corresponding to mono- and dihydroxylated metabolites (structures shown fig. 1) were collected and dried under vacuum. The quantity of metabolites was determined by UV spectrophotometry at 305 nm. Starting with an incubation of 10.5 mg of BCT, approximately 0.9 and 1.3 mg of mono- and dihydroxylated metabolites were recovered, respectively.

**Results**

To analyze the respective effects of BCT and its metabolites, we first chose to generate, through two different routes of administration, a pharmacokinetic situation for which the ratio of BCT metabolites/untransformed BCT was different and to observe the consequences on the pharmacological response. Because no metabolites of BCT were commercially available, such an approach was necessary before undertaking in vitro production of metabolites. Because of the 10% bioavailability of BCT (Maurer et al., 1982), we decided to administer BCT orally at a dose 10-fold greater than the dose used for i.v. administration.

The plasma concentration-time profiles shown in figure 2 indicate that the choice of these routes and doses allowed production of similar BCT concentrations after either oral or intravenous administration. In contrast, concentrations of BCT metabolites were much higher after oral administration because of the marked first-pass effect. Individual profiles were modeled according to a two-compartment model for metabolites profile after intravenous administration of BCT or a three-compartment model for other profiles. The pharmacokinetic parameters are given in table 1. The equations allowing the description of the mean drug profiles shown in figure 2 were: $C_p = 1.52e^{-0.065t} + 80.05e^{-0.65t} + 335e^{-2.94t}$ (BCT, i.v.), $C_p = 0.42e^{-0.018t} + 94.34e^{-0.02t} - 74.1e^{-0.41t}$ (BCT, p.o.), $C_p = 10.1e^{-0.022} + 1190e^{-0.32t}$ (BCT metabolites, i.v.) and $C_p = 31.6e^{-0.011} + 2976e^{-0.159t} - 2892e^{-1.21t}$ (BCT metabolites, p.o.). The areas under the curves for untransformed BCT after i.v. or oral administration were in the same range (299 vs. 329 ng/ml/h), whereas the AUC for immunoreactive metabolites were approximately 5-fold greater (3,893 vs. 18,050 ng/ml/h) after oral administration. The ratio of immunoreactive metabolites to untransformed BCT was 55 after oral administration.

Plasma prolactin profiles are depicted in figure 3. After administration by both routes, plasma prolactin levels decreased strikingly. However, the reduction was significantly longer for oral BCT. Although the BCT plasma concentrations at 24 h were the same after both routes of administration, prolactin had returned to the basal level after i.v. administration, and was still significantly reduced after oral administration. This observation led to the suggestion that the apparent change in prolactin inhibition after the oral dose, as compared with the intravenous dose, may be attributed to one or more immunoreactive BCT metabolites present at higher concentrations. To confirm this hypothesis, the relation between the drug concentration and the effect was evaluated by means of pharmacokinetic-pharmacodynamic models. With use of the effect-compartment model,
routes of administrations. No assay. Rat samples were taken 18 h after administration, fractionated by HPLC and separately detected by enzyme immunoassay. To identify each of the immunoreactive metabolites, they were fractionated by HPLC and separately detected by enzyme immunoassay. Rat samples were taken 18 h after administration, a time at which BCT activity could be attributed to its metabolites, were pooled and chromatographed. The immunoreactive profile was compared with that obtained after chromatography of BCT metabolized by rat microsomes. As shown in figure 4, the immunoreactive peaks for the rat samples were representative of metabolites obtained after BCT incubation with rat microsomes. For the rat sample, untransformed BCT represented less than 5% of the total immunoreactivity. The identity of the metabolites has been confirmed by mass spectrometry (Peyronneau et al., 1994). The main immunoreactive (M1/M2) peak corresponded to a mixture of stereoisomers of 8'-hydroxybromocriptine. The two other peaks (M3 and M4) corresponded to stereoisomers of 8',9'-hydroxybromocriptine. It should be pointed out that M1/M2 metabolites were not been fully separated under the conditions used for chromatography, because each isomer undergoes partial transformation into the other isomer upon storage or during purification.

Metabolites were then produced from rat liver microsomes in sufficient quantity for in vitro and in vivo pharmacological studies. With membrane preparations of rat striatum, we showed that BCT and its metabolites were able to specifically displace the binding of [3H]YM-09151–2 with binding affinity constants in the same range, i.e., 70, 20, 13 and 21 nM for BCT, metabolites M1/M2, metabolite M3 and metabolite M4, respectively. In rat primary pituitary cultures, each metabolite was able to inhibit prolactin secretion with a potency similar to that of BCT or dopamine (fig. 5). They were then administered intravenously to rats at the dose of 100 µg/kg and their effects on prolactin secretion inhibition were compared with those of BCT or vehicle. As shown in figure 6, only the mixture of metabolites M1/M2 inhibited prolactin secretion. Compared with monohydroxylated, the absence of in vivo effect for metabolites M3 and M4 could be attributed to their faster total clearance as shown by their plasma kinetics after intravenous administration (fig. 7). The plasma profiles were described by first-order biexponential disposition with $C_p = 2.09e^{-0.15t} + 57.8e^{-0.77t}$ (metabolites M1/M2), $C_p = 0.46e^{-0.08t} + 7.59e^{-4.41t}$ (metabolites M3) and $C_p = 0.74e^{-0.14t} + 31.50e^{-3.99t}$ (metabolites M4). The clearances were 0.37, 10.85 and 3.19 l/h/kg for metabolites M1/M2, M3 and M4, respectively.

**Discussion**

Several observations indicate that the effect of BCT on human prolactin secretion is not correlated with the concentration of parent drug. Indeed, there is evidence that prolactin inhibition persists when BCT is undetectable in the circulation (Thorner et al., 1980; Katz et al., 1991). This effect has been attributed to a prolonged action of the drug at the pituitary level rather than to a possible action of BCT metabolites. It has been demonstrated that in vitro, inhibition of prolactin release from isolated rat pituitary cells is maintained after withdrawal of BCT. This prolonged effect may be caused by a slow dissociation rate from the receptors or by continuous release of BCT from nonspecific sites such as membrane lipids (Woolf, 1981; Hanna and Shin, 1992).

To confirm or deny this hypothesis, we first proposed an integrated pharmacokinetic-pharmacodynamic model to determine the possible involvement of BCT metabolites in the time course of prolactin inhibition after i.v. or oral administr
The sustained inhibition of prolactin secretion observed for the oral route and the differences in BCT pharmacodynamic parameters between the two routes provided evidence for action of the metabolites at the pituitary level. We then demonstrated that hydroxylated metabolites bind to the dopamine D2 receptor and were able to lower prolactin excretion of primary pituitary cells to an extent similar to BCT. Finally, we have shown that monohydroxylated BCT metabolites have the same potency in vivo as BCT in lowering circulating prolactin levels. Under our experimental conditions, dihydroxylated metabolites were ineffective because their plasma concentrations were insufficient because of rapid clearance from the circulation. However, these metabolites are active in vitro and are observed at high concentrations after oral administration of BCT (fig. 4). They therefore should also contribute to the in vivo inhibition of prolactin secretion. Because the concentrations of metabolites are well above that of BCT after oral administration, it may be concluded that they contribute mainly to the hypoprolactinemic effect of BCT.

It remains to be determined whether the action of metabolites on the pituitary is also found in the central nervous system, in particular in the corpus striatum. Disagreement is apparent in published reports. By use of specific inhibitors of BCT hydroxylation, it has been demonstrated that some central nervous actions of BCT, such as hypothermia in rats or

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pharmacokinetic and pharmacodynamic parameters of BCT and its metabolites after i.v. (1 mg/kg) or p.o. (10 mg/kg) dose of BCT</th>
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<tr>
<td></td>
<td>Bromocriptine</td>
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<td></td>
<td>i.v.</td>
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<td>Pharmacokinetic parameters</td>
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<tr>
<td>AUC (ng/ml · hr)</td>
<td>299 ± 71</td>
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<tr>
<td>C_{max} (ng/ml)</td>
<td>403 ± 105</td>
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<tr>
<td>T_{max} (hr)</td>
<td>0.08</td>
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<tr>
<td>Rate constants</td>
<td>5.46 ± 0.67</td>
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<tr>
<td>n (Hill)</td>
<td>0.85 ± 0.13</td>
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<td>Pharmacodynamic parameters</td>
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<td>Effect-compartment model</td>
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<td>CE_{50} (nM)</td>
<td>3.7 ± 1.0</td>
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<tr>
<td>K_e0 (hr^{-1})</td>
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<td>E_{max} (%)</td>
<td>90.1 ± 1.1</td>
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<tr>
<td>n (Hill)</td>
<td>1.94 ± 0.81</td>
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<td>Indirect pharmacodynamic model</td>
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<tr>
<td>CE_{50} (nM)</td>
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<tr>
<td>K_{out} (hr^{-1})</td>
<td>0.18</td>
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<td>n (Hill)</td>
<td>3.88</td>
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a Mean ± S.E.M.
b First sampling time.
c Elimination rates except for values noted by d.
d Absorption rate.
e Based on mean profiles.

Fig. 3. Inhibition of prolactin profiles after intravenous administration of 1 mg/kg (■) or oral administration of 10 mg/kg (□) of BCT and placebo (- - - - , without symbols). Values are the means ± S.E.M. (n = 6). For clearer presentation S.E.M. (mean value of 19%) for placebo are not indicated. The basal level (100%) for prolactin were (means ± S.E.M): 10.8 ± 0.9 (BCT, i. v.), 14.8 ± 0.9 (BCT, p. o.) and 11.3 ± 2.1 (placebo) ng/ml.
hallucinatory-like behavior in cats, are at least partly dependent on BCT metabolism (Silbergeld et al., 1977, Gonzalez-Lima et al., 1987). Contradictory reports, however, have shown that hypothermia and BCT-induced cerebral dopamine turnover were caused by BCT itself and did not require previous biotransformation into active metabolites (Keller and Da Prada, 1979; Schran et al., 1985). This points to the passage of BCT metabolites through the blood-brain barrier or to the metabolization of BCT by monoxygenases present in the brain. We recently used the microdialysis technique to show that the metabolites were undetectable in striatum after BCT administration to rats (Renouf-Granveau et al., in press, 1997). The metabolites should therefore not contribute significantly to the effect of BCT in the central nervous system.

The evidence that hydroxylated metabolites are active at the pituitary level in rats suggests that these metabolites may be active in humans. Studies on in vitro BCT hepatic biotransformations have shown that metabolite patterns are identical in rats and humans (Maurer et al., 1983; Peyronneau et al., 1994). So far, the respective concentrations of BCT metabolites in human plasma after clinical therapeutic doses have been only partially studied with carbon 14-labeled BCT (Schran et al., 1980). A very low BCT-to-metabolites ratio was observed. With the same immunoassays as presented here, we have shown in women given oral BCT that the ratio of immunoreactive metabolites to untransformed BCT is in the same range as that noted in our rat study (Valente, D., unpublished observation). Recently, the intravaginal route has been proposed as a substitute to avoid the
secondary effects of BCT noted with oral administration, such as nausea or vomiting (Ginsburg et al., 1988). Compared with the same oral dose, intravaginal administration results in higher BCT concentrations because of the absence of a first-pass hepatic effect. However, inhibition of prolactin secretion is much lower than that recorded after the same oral dose (Vermesh et al., 1988; Katz et al., 1991). These observations suggest that BCT metabolites contribute to the action of BCT at the pituitary level in humans.

In conclusion, previous work suggested that the duration and maintenance of the pharmacological effect of BCT at the pituitary level resulted mainly from slow dissociation from specific receptor sites and/or from continuous release from nonspecific binding sites. In contrast, the present results show that it is BCT metabolites that mainly contribute to the prolonged action of BCT in rats. Although confirmation in humans is required, this finding may have clinical implications because BCT has a large spectrum of established and potential therapeutic applications and is subject to continuous biopharmaceutical and pharmacological developments.

References


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