Caffeine metabolism during prolonged treatment of rats with antidepressant drugs

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Abstract:
Our previous studies showed that some of the tested antidepressants (tricyclics, SSRIs, mirtazapine, nefazodone) directly inhibited the metabolism of caffeine when added \textit{in vitro} to liver microsomes. The aim of the present study was to investigate a possible indirect effect of prolonged \textit{in vivo} administration of these antidepressants on the rate of caffeine oxidative metabolism: 1-N-, 3-N- and 7-N-demethylation and 8-hydroxylation in rat liver. The reactions were studied in liver microsomes of rats treated intraperitoneally (ip) for one day or two weeks with pharmacological doses of the drugs (imipramine, amitriptyline, clomipramine, nefazodone at 10 mg/kg; desipramine, fluoxetine, sertraline at 5 mg/kg; mirtazapine at 3 mg/kg), in the absence of the antidepressants \textit{in vitro}. One-day treatment with imipramine and amitriptyline decreased, while fluoxetine accelerated the metabolism of caffeine. Nefazodone stimulated 1-N-demethylation only. Fluoxetine given chronically increased exclusively 7-N-demethylation, while imipramine showed only such tendency. Sertraline and mirtazapine enhanced the rates of all caffeine oxidation pathways. We conclude that the tested antidepressant drugs may affect the metabolism of caffeine not only in a direct way (binding to the enzyme), but also indirectly \textit{via} inducing CYP1A2 (sertraline and mirtazapine) and CYP2C isoforms (fluoxetine, sertraline, mirtazapine) after prolonged administration. In addition, the presented data provide further experimental evidence for the importance of the subfamily CYP2C for the 7-N-demethylation of caffeine in the rat.

Key words:
caffeine metabolism, rat, cytochrome P450, antidepressants

Introduction

Caffeine (1,3,7-trimethylxanthine), one of the most widely and frequently ingested compounds throughout the world, is a useful enzymatic probe for its rapid and complete gastrointestinal absorption, its distribution throughout body water, and its low plasma protein binding, as well as for its short half-life, negligible first-pass metabolism, minimal renal elimination and biotransformation, the latter being virtually confined to the liver [12, 21, 23]. The compound undergoes 1-N-demethylation to theobromine, 3-N-demethylation to paraxanthine, 7-N-demethylation to theophylline and 8-hydroxylation to 1,3,7-trimethyluric acid. The literature data and our comparative study on caffeine metabolism at a broad substrate concentration range showed species- and concentration-dependent metabolism of caffeine differing in degree of oxidation in particular positions, as well as qualitative and quantitative contribution of CYP isoforms to particular oxidation pathways. The 3-N-demethylation of caffeine is a specific reaction for testing the activity of CYP1A2 in humans [1, 2,
21], while 8-hydroxylation is specifically mediated by CYP1A2 in the rat at a therapeutic concentration of the drug [15]. That is why caffeine is often used for phenotyping of human CYP1A2 [3, 23] and may be applied in the future for estimation of the CYP1A2 activity in pharmacological experiments in rats. However, our previous studies, carried out using rat cDNA-expressed CYP isoforms, liver microsomes, specific CYP inhibitors and inducers showed that caffeine may be used as a more universal “pharmacological tool” for simultaneous estimation of not only CYP1A2, but also of a few other CYP isoenzymes in pharmacological experiments in rats [15, 16]. The above-mentioned study indicated that theophylline formation was mainly catalyzed by the isoforms of the subfamily CYP2C. Thus, 7-N-demethylation of caffeine may be applied to the simultaneous, preliminary estimation of the CYP2C activity in the rat.

Caffeine has multiple pharmacological effects [10, 11]. As an adenosine receptor antagonist, caffeine increases the release of various neurotransmitters, and at higher concentrations inhibits phosphodiesterase. Due to its ability to interact with neurotransmission in different regions of the brain, caffeine displays psychomotor stimulant properties, promoting behavioral functions, such as vigilance, attention, mood and arousal [11]. Hence, mutual drug interactions between caffeine and other psychoactive drugs at a pharmacodynamic and a pharmacokinetic level are feasible [10], because they may lead to caffeine-related or medication-related side effects complicating psychiatric treatment. Therefore, caffeine is a highly interesting marker substance for the estimation of antidepressant effect on the activity of cytochrome P450 and prediction of interactions between drugs and environmental xenobiotics.

Our previous studies showed that some of the tested antidepressants (tricyclics, SSRIs, mirtazapine, nefazodone) directly inhibited the metabolism of caffeine when added in vitro to liver microsomes. Thus desipramine, sertraline, clomipramine and imipramine were most effective in inhibiting 1-N-demethylation (K<sub>i</sub> = 23.3–47.0 μM); imipramine, clomipramine and desipramine in repressing 3-N-demethylation (K<sub>i</sub> = 33.0–36.6 μM); desipramine and nefazodone in reducing 7-N-demethylation (K<sub>i</sub> = 23.3 and 66.7 μM, respectively); fluoxetine, imipramine and clomipramine in inhibiting 8-hydroxylation of caffeine (K<sub>i</sub> = 40.0–45.6 μM) [7, 9]. The aim of the present study was to investigate a possible indirect effect of prolonged in vivo administration of those antidepressants on the rate of caffeine metabolism in rat liver.

### Materials and Methods

#### Drugs and chemicals

Caffeine and its metabolites – theobromine, paraxanthine, theophylline and 1,3,7-trimethyluric acid, as well as NADP, DL-isocitric acid (trisodium salt), isocitric dehydrogenase were purchased from Sigma (St. Louis, USA). Imipramine hydrochloride was provided by Polfa (Jelenia Góra, Poland), amitriptyline by H. Lundbeck A/S (Copenhagen, Denmark), while clomipramine was from RBI (Natick, MA, USA) and desipramine from Ciba-Geigy (Wehr, Germany). Fluoxetine hydrochloride was purchased from Eli Lilly (Indianapolis, USA) and sertraline hydrochloride from Pfizer Corp. (Brussels, Belgium). Mirtazapine hydrochloride was donated by Organon (The Netherlands) and nefazodone by Bristol-Myers Squibb International, Ltd. (Uxbridge, UK). All organic solvents with HPLC purity were supplied by Merck (Darmstadt, Germany).

#### Animal procedures

All the experiments with animals were performed in accordance with the Polish governmental regulations (Animals Protection Act, DZ.U. 97.111.724, 1997). The experiments were carried out on male Wistar rats (230–260 g) kept under standard laboratory conditions. The investigated antidepressant drugs were administered intraperitoneally, twice a day for one day or two weeks at the following pharmacological doses: imipramine, amitriptyline, clomipramine and nefazodone 10 mg/kg, desipramine, fluoxetine and sertraline 5 mg/kg, mirtazapine 3 mg/kg. The control animals were injected with saline. The rats were sacrificed at 12 h (one-day treatment) or 24 h (two-week treatment) after the drug withdrawal, and liver microsomes were prepared by differential centrifugation in 20 mM Tris/KCl buffer (pH = 7.4), including washing with 0.15 M KCl according to a conventional method. The mentioned procedure deprives microsomes of the presence of parent drugs administered in vivo, which...
was confirmed in our experiment by using the HPLC method [8, 9].

**Caffeine metabolism in liver microsomes**

Studies into caffeine metabolism in liver microsomes were carried out at the linear dependence of product formation on time, protein and substrate concentration. Incubations were carried out in a system containing the liver microsomes (ca. 1 mg of protein/ml), a phosphate buffer (0.15 M, pH = 7.4), MgCl$_2$ × 6H$_2$O (6 mM), NADP (1.2 mM), DL-isocitric acid (6 mM) and isocitric dehydrogenase (1.2 U/ml). Caffeine metabolism was studied in the liver microsomes of control and antidepressant-treated rats at the substrate concentration of 800 μM. The final incubation volume was 1 ml. After a 50-min incubation, the reaction was terminated by adding 700 μl of a 2% ZnSO$_4$ and 50 μl of 2 M HCl.

**Determination of caffeine and its metabolites**

Caffeine and its four primary metabolites were assessed using the HPLC method based on Rasmussen et al. [20] as previously described [7]. Briefly, after incubation, samples were centrifuged and the water phase containing caffeine and its metabolites was extracted with 6 ml of an organic mixture consisting of ethyl acetate and 2-propanol (8:1, v/v). The residue obtained after evaporation of the microsomal extract was dissolved in 100 μl of the mobile phase described below. An aliquot of 20 μl was injected into the HPLC system. The Merck-Hitachi chromatograph, “LaChrom” (Darmstadt, Germany), equipped with a L-7100 pump, an UV detector and a D-7000 System Manager was used. The analytical column (Supelcosil LC-18, 15 cm × 4.6 mm, 5 μm) was from Supelco (Bellefonte, USA). The mobile phase consisted of 0.01 M acetate buffer (pH = 3.5) and methanol (91:9, v/v). The flow rate was 1 ml/min (0–26.5 min), followed by 3 ml/min (26.6–35 min). The column temperature was maintained at 30°C. The absorbance of caffeine and its metabolites was measured at a wavelength of 254 nm. The compounds were eluted in the following order: theobromine (9.7 min), paraxanthine (15.8 min), theophylline (16.9 min), 1,3,7-trimethyluric acid (23.4 min), caffeine (30.5 min). The sensitivity of the method allowed for quantification of theobromine as low as 0.001 nmol, paraxanthine as low as 0.004 nmol, theophylline as low as 0.005 nmol, 1,3,7-trimethyluric acid as low as 0.01 nmol and caffeine as low as 0.005 nmol in one sample. The accuracy of the method amounted to 2.1% (theobromine), 1.2% (paraxanthine), 1.3% (theophylline), 2.3% (1,3,7-trimethyluric acid) and 2.9% (caffeine). The intra- and inter-assay coefficients of variance were below 4% and 6%, respectively.

**Results**

Of the antidepressants studied, only imipramine and amitriptyline decreased the rate of 1-N-demethylation, 3-N-demethylation and 8-hydroxylation of caffeine after one-day (i.e. 24 h) exposure to the drugs (to about 50–75% of the control). Such an effect was not observed after chronic treatment with the two antidepressants (Fig. 1, 2, 4). One-day treatment with fluoxetine accelerated the metabolism of caffeine (Fig. 1–4), especially 1-N-demethylation (up to 160% of the control) (Fig. 1). Nefazodone stimulated only caffeine 1-N-demethylation after 24 h exposure to the antidepressant (to 140% of the control) (Fig. 1). Fluoxetine given chronically increased exclusively 7-N-demethylation (to 165% of the control), while imipramine showed such a tendency (Fig. 3). Sertraline and mirtazapine enhanced the rates of all caffeine oxidation pathways: 1-N-demethylation increased to about 145% of the control (Fig. 1) and 3-N-demethylation, 7-N-demethylation and 8-hydroxylation rose to about 180% of the control (Fig. 2–4).

**Discussion**

Our previous studies showed that some of the tested antidepressants (tricyclics, SSRIs, mirtazapine, nefazodone) directly inhibited the metabolism of caffeine when added in *vitro* to liver microsomes and suggested that the four oxidation pathways of caffeine metabolism might be catalyzed to different degree by individual rat CYP isoforms [7, 9]. The later investigation carried out using selective CYP inducers suggested that CYP2C11 might be engaged in the 7-N-demethylation of caffeine in the rat [16]. Recent results obtained using cDNA-expressed CYPs indicated
Fig. 1. The influence of one-day and two-week treatment with antidepressant drugs on 1-N-demethylation of caffeine in rat liver microsomes. Microsomes were incubated with 600 μM caffeine. All values are the means ± SEM of 7–8 animals; ** p < 0.05, *** p < 0.01, **** p < 0.001 (Duncan test), compared with control. Absolute control value was 0.0206 ± 0.001 nmol of theobromine/mg of protein/min. IMI = imipramine, DMI = desipramine, AMI = amitriptyline, CLP = clomipramine, FLX = fluoxetine, SRT = sertraline, MIRT = mirtazapine, NEF = nefazodone.

Fig. 2. The influence of one-day and two-week treatment with antidepressant drugs on 3-N-demethylation of caffeine in rat liver microsomes. Microsomes were incubated with 600 μM caffeine. All values are the means ± SEM of 7–8 animals; ** p < 0.05 (Duncan test), compared with control. Absolute control value was 0.0200 ± 0.001 nmol of paraxanthine/mg of protein/min. For further explanation, see Figure 1.
Fig. 3. The influence of one-day and two-week treatment with antidepressant drugs on 7-N-demethylation of caffeine in rat liver microsomes. Microsomes were incubated with 800 μM caffeine. All values are the means ± SEM of 7–8 animals; * p < 0.05, ** p < 0.01 (Duncan test), compared with control. Absolute control value was 0.0388 ± 0.002 nmol of theophylline/mg of protein/min. For further explanation, see Figure 1.

Fig. 4. The influence of one-day and two-week treatment with antidepressant drugs on 8-hydroxylation of caffeine in rat liver microsomes. Microsomes were incubated with 800 μM caffeine. All values are the means ± SEM of 7–8 animals; * p < 0.05, ** p < 0.01 (Duncan test), compared with control. Absolute control value was 0.1181 ± 0.011 nmol of 1,3,7-trimethyluric acid/mg of protein/min. For further explanation, see Figure 1.
that 8-hydroxylation, the main oxidation pathway of caffeine in the rat at its therapeutic concentration, is specifically catalyzed by CYP1A2 [15]. 1-N- and 3-N-demethylations were also preferentially catalyzed by CYP1A2, but to a lesser degree than 8-hydroxylation, while 7-N-demethylation was mainly mediated by the isoforms of the subfamily CYP2C.

The contribution of the CYP2C isoforms to the metabolism of caffeine was also confirmed by the results of the present work concerning the effect of chronic treatment with antidepressants on the metabolism of caffeine, when the data were compared with the effects of these drugs on the activities of CYP isoforms found in our previous studies [4, 5, 13]. Given chronically, fluoxetine (a CYP2C6 and CYP2C11 inducer) increased exclusively 7-N-demethylation, while imipramine (a CYP2C6 inducer and a CYP3A inhibitor) showed only such tendency. Sertraline (a CYP2C6 and CYP3A inducer) and mirtazapine (a CYP2C6 inducer) enhanced the rate of all caffeine oxidation pathways. The obtained results indicate that chronic treatment with fluoxetine (and imipramine) induces caffeine metabolism by enhancing the activity of CYP2C only, while sertraline and mirtazapine – mainly by elevating the activity of CYP2C and CYP1A2.

The results obtained in our experiment (two-week treatment) indicate that mirtazapine is an effective inducer of CYP2C (an increase in 7-N-demethylation) and CYP1A2 (an increase in 8-hydroxylation). This effect may be due to its possible influence on enzyme regulation. It is known that aryl hydrocarbon receptor (AhR) plays a very important role in the regulation of CYP1A genes [24]. On the other hand, constitutive androstane receptor (CAR) plays an important role in the regulation of CYP2C genes [14, 18]. Recent studies suggest that CYP1A2 may also be regulated by CAR, not only by AhR [17]. It is of interest that CAR is up-regulated in response to AhR activation [19]. However, the mechanism of CYP1A2 and CYP2C induction by mirtazapine needs further investigation.

Like in the case of mirtazapine, chronic treatment with sertraline significantly increased the rate of all caffeine oxidation pathways, which also suggests induction of CYP1A2 and CYP2C isoforms. On the other hand, our previous in vivo studies demonstrated that sertraline decreased the activity of rat CYP2D after a one-day and chronic treatment [7]. However, this isoform is not involved in the metabolism of caffeine [15]. Thus, the effect of sertraline on the metabolism of caffeine in the liver of rats may be due mainly to its possible influence on enzyme regulation. Probably, the above-mentioned receptors (CAR and/or AhR) may be also involved in this effect.

In contrast to mirtazapine and sertraline, chronic treatment with fluoxetine exclusively accelerated only 7-N-demethylation of caffeine. Since our recent study using rat cDNA-expressed CYPs indicated that 7-N-demethylation was governed by isoforms of the subfamily CYP2C (mainly CYP2C6 and CYP2C11) [15, 16] it means that chronic treatment with fluoxetine significantly enhanced the activity of CYP2C, measured as the rate of 7-N-demethylation of caffeine. The above data agree with our previous results showing increases in the activities of rat CYP2C6 and CYP2C11 after prolonged administration of fluoxetine [4, 5]. Thus the results concerning the effect of fluoxetine on the metabolism of caffeine and on the activity of CYPs provide practical evidence for a principal role of CYP2C in the 7-N-demethylation of caffeine [4, 5, 13, 16].

In conclusion, the obtained results indicate that the tested antidepressant drugs may affect the metabolism of caffeine not only in a direct way (binding to the enzyme), but also indirectly via inducing CYP1A2 (sertraline and mirtazapine) and CYP2C isoforms (fluoxetine, sertraline, mirtazapine) after prolonged administration. In addition, the presented data provide further experimental evidence for the importance of the subfamily CYP2C for the 7-N-demethylation of caffeine in the rat.

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