ated with malarial prophylaxis with maloprim. *Br Med J* **286**: 1244–1245, 1983.
11. Cucinell SA, Israeli ZH, Dayton PG, Microsomal N-oxidation of dapsone as a cause of methaemoglobin formation in human red cells. *Am J Trop Med Hyg* **21**: 322–331, 1972.
12. DeGowin RL, Enues RB, Powell RD and Carson PE, The haemolytic effects of diphenylsulphone (DDS) in normal subjects and in those with glucose-6-phosphate dehydrogenase deficiency. *Bull World Health Org* **35**: 165–179, 1966.
13. Zuidema J, Hilbers-Moddermann ESM and Merkus FWHM, Clinical pharmacokinetics of Dapsone. *Clin Pharmacokinet* **11**: 299–315, 1986.
14. Weetman RM, Boxer LA, Brown MP, Mautich NM and Buchner RL, *In vitro* inhibition of granulopoiesis by 4 amino 4′ hydroxylamino phenyl sulphone. *Br J Hematol* **45**: 361–370, 1980.
15. Glader BE and Conrad ME, Haemolysis by diphenylsulphones: comparative effects of DDS and hydroxylamine-DDS. *J Lab Clin Med* **80**: 267–272, 1973.
16. Kramer PA, Glader BE and Li TK, Mechanism of methemoglobin formation by diphenylsulphones. *Biochem Pharmacol* **21**: 1265–1274, 1972.
17. Modderman ESM, Merkus FWHM, Zuidema J, Huikshoven H, Leiken DL, Controlled release of dapsone by intramuscular injection. In: *Controlled Release Delivery Systems* (Ed. Roseman Mansdorf), pp. 274–289. Marcel Dekker, New York, 1983.
18. Ganer A, Knobel B, Fryd CH, Rachmilewitz EA, Dapsone induced methaemoglobinaemia and haemolysis in the presence of familial haemoglobinopathy hasharon and familial methamoglobin reduction deficiency. *Israel J Med Sci* **17**: 703–704, 1981.
19. Bowden D and Tute MS, Structure activity relationships of antimycobacterial sulfones: a study using physicochemical constants. *Eur J Med Chem* **16**: 299–300, 1981.
20. Harrison JH and Jollow DJ, Role of aniline metabolites in aniline-induced hemolytic anemia. *J Pharmacol Exp Ther* **238**: 1045–1054, 1986.
21. Gibaldi M and Perrier D, *Pharmacokinetics* 2nd Edn., p. 445. Marcel Dekker, New York, 1982.
22. Grossman SJ and Jollow DJ, Role of DDS NOH in DDS-induced haemolytic anaemia. *J Pharmacol Exp Ther* **244**: 118–125, 1988.
23. Anders MW, Inhibition of microsomal drug metabolism by methylene-dioxybenzenes. *Biochem Pharmacol* **17**: 2367–2371, 1968.
24. Wright JT, Goodman RP, Bethel AMM and Lambert CM, Cimetidine and dapsone acetylation. *Drug Metab Dispos* **12**: 782–783, 1984.
25. Pelkonen O and Puurunea J, The effect of cimetidine on *in vitro* and *in vivo* microsomal drug metabolism in the rat. *Biochem Pharmacol* **29**: 3075–3080, 1980.
26. Rendic S, Ruff HH, Webea P and Kajfez F, Cimetidine and ranitidine: their interaction with human and pig liver microsomes and with purified cytochrome P-450. *Eur J Drug Metab Pharmacokinet* **9**: 195–200, 1984.
27. Meredith CG, Maldonado AL and Speeg KV Jr, The effect of ketoconazole on hepatic oxidative drug metabolism in the rat *in vivo* and *in vitro*. *Drug Metab Dispos* **13**: 156–162, 1985.
28. Santen RJ, Van der Bossche M, Symoens J, Brugmans J, DeCoster R, Site of action of low dose ketoconazole on androgen biosynthesis in man. *J Clin Endocrinol Metab* **57**: 732–736, 1983.
29. Speeg KV, Patwardhan RV, Avant GR, Mitchell MC and Schenker S, Inhibition of microsomal drug metabolism by histamine H_2-receptor antagonists studied *in vivo* and *in vitro* in rodents. *Gastroenterology* **82**: 89–96, 1982.
30. Daneshmend TK, Ketoconazole-cyclosporin interaction. *Lancet* **2**: 1342–1343, 1972.
31. Kedderis GL and Rickert DE, Inhibition of the microsomal N-hydroxylation of 2-amino-6-nitrotoluene by a metabolite of methimazole. *Biochem Biophys Res Commun* **113**: 433–438, 1983.
32. Weisburger JH and Weisburger EK, Biochemical formation and pharmacological toxicological and pathological properties of hydroxylamines and hydroxamic acids. *Pharmacol Rev* **25**: 1–66, 1973.
33. Uetrecht JP, Reactivity and possible significance of hydroxylamine and nitrous metabolites of procainamide. *J Pharmacol Exp Ther* **232**, 420–425, 1984.
34. Shear NH and Spielberg SP, *In vitro* evaluation of a toxic metabolite of sulfadiazine. *Can J Physiol Pharmacol* **63**: 1370–1372, 1985.
35. Woosley RL, Drayer DE, Reidenberg MM, Nies AS, Carr K and Oates JA, Effect of acetylator status phenotype on the rate at which procainamide induces antinuclear antibodies and the lupus syndrome. *New Engl J Med* **298**: 1157–1159, 1978.
36. Venkatesan K, Bharadwaj VP, Ramin G and Desikan KV, Study on drug interactions. *Leprosy in India* **52**: 229–236, 1980.
37. Peters JH, Murray Jr JF, Gordon GR, Gelber RM, Laing ABG, Effect of rifampicin on the disposition of dapsone in Malaysian leprosy patients. *Fed Proc* **36**: 996, 1977.
38. Peters JH, Murray JF, Gordon GR, Jacobsen RR, Metabolic–bacteriologic relationship in the chemotherapy of lepromatous patients with dapsone or dapsone-rifampicin. *Int J Leprosy* **46**: 115–116, 1978.

Biochemical Pharmacology, Vol. 39, No. 4, pp. 805–809, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Sex-dependent sensitivity to dapsone-induced methaemoglobinaemia in the rat

(*Received* 31 *July* 1989; *accepted* 23 *October* 1989)

Dapsone is a major component of the multidrug regimen for the treatment of leprosy [1]. It is also used in malarial prophylaxis [2], inflammatory disease [3, 4] and more recently in the therapy of *Pneumocystis carinii* in AIDS patients [5]. Dapsone is extensively metabolized in man and experimental animals to hydroxylamine derivatives [6–8]. Haematological side effects which occur during dapsone therapy such as methaemoglobinaemia [9], have been attributed to the hydroxylamine metabolite [10]. Reduced red cell lifespan due to haemolysis is especially marked in individuals with glucose-6-phosphate dehydrogenase deficiency or diminished glutathione reductase activity [11].

The rat is a useful model with which to investigate dapsone-induced methaemoglobinaemia [7]. However, it was noted during earlier studies conducted in our laboratory, that female rats were much less susceptible to dapsone-mediated toxicity compared with males. Sex differences in drug metabolism have been reported to have marked pharmacodynamic and toxicological consequences [12–14]. Hence, we wished to determine whether a sex-dependent sensitivity to dapsone-induced methaemoglobinaemia existed in the rat and to investigate the biochemical basis for this observation.

Materials and methods

Chemicals. 4,4′ Diaminodiphenyl sulphone was supplied by the Sigma Chemical Co. (Poole, U.K.). Dapsone hydroxylamine was synthesized as previously described [15]. All other chemicals were of reagent grade and were obtained from BDH Chemicals Ltd, (Poole, U.K.).

Protocol. Four groups (N = 5 per group) of male (groups 1 and 2) and female (groups 3 and 4) Wistar rats (200–250 g) were dosed intraperitoneally with 33 mg/kg of dapsone dissolved in dimethyl sulphoxide (200 μL). Blood samples (250 μL) were withdrawn from the tail veins of the rats while they were under light diethyl ether anaesthesia. Samples were removed pre-dose, then at 1, 2, 3, 5 and 24 hr.

In addition, three female and three male rats (both groups untreated) were anaesthetized with diethyl ether, and exsanguinated by cardiac puncture. Pooled blood from males and pooled female blood (1 mL/incubation, N = 4 per concentration) were separately exposed to dapsone hydroxylamine at 1, 3, 10 and 30 μM for 1 hr. To each incubation, the metabolite was added as a concentrated solution in 10 μL of HPLC grade methanol. A sample of blood (100 μL) was removed at 1 hr and assayed for methaemoglobin formation.

Preparation of hepatic microsomes. Microsomes were isolated from the pooled livers of four untreated female and four untreated male rats by the method of Purba *et al.* [16]. Their mean cytochrome P-450 contents were measured according to the technique of Omura and Sato [17] and found to be 0.65 nmol/mg microsomal protein (male) and 0.71 nmol/mg protein (females). Concentrated suspensions of the microsomes (10–15 mg microsomal protein/mL) were stored in 0.1 M phosphate buffer (pH 7.4). Microsomal protein determination was by the method of Lowry *et al.* [18]. Aliquots of suspensions were stored at −70°.

Metabolism of DDS by rat liver microsomes. [^{14}C]DDS (100 μM, 0.5 μCi) was incubated with male or female rat liver microsomes (0.5 mg protein) in phosphate buffer (pH 7.4, final volume 1 mL). Reactions were performed in quadruplicate and started by the addition of NADPH (1 mM), then incubated in a shaking water bath at 37° for 1 hr. NADPH was omitted from control incubations. Prior to the termination of the reactions by the addition of methanol (1 mL) 10 mM ascorbate was added to each incubation to preserve any dapsone hydroxylamine formed as previous studies have underlined the instability of this compound [6, 8]. The tubes were left at −20° overnight to precipitate all protein.

Analytical procedures. Samples from groups 1 and 3 plus the samples obtained from the *in vitro* incubation of rat blood with dapsone hydroxylamine were assayed for methaemoglobin levels relative to haemoglobin levels using the spectrophotometric technique of Harrison and Jollow [19].

Samples from groups 2 and 4 were assayed for dapsone concentrations using the HPLC method of Grossman and Jollow [7] for detection of dapsone in whole rat blood. Chromatography was performed on a Spectra-Physics 8700 chromatograph. Separation was achieved using a μBondapak C_{18} column (30 cm × 0.39 cm i.d. 10 μm, Waters Associates, Hartford, Cheshire, U.K.). The solvent mobile phase consisted of 0.1 M ammonium acetate, acetonitrile and methanol (66:12:22) flowing at 1.5 mL/min. The analyte was detected at 254 nm with a Pye Unicam UV detector. The retention times of dapsone and its internal standard were 4 and 7 min, respectively. Analysis of dapsone hydroxylamine was accomplished by radiometric HPLC using the method of Uetrecht *et al.* [15]. Dapsone and its hydroxylamine metabolite were identified chromatographically by comparison of their retention times with those of co-injected authentic unlabelled compounds (dapsone hydroxylamine, 8.5 min; dapsone, 10.5 min). After further precipitation of protein by centrifugation

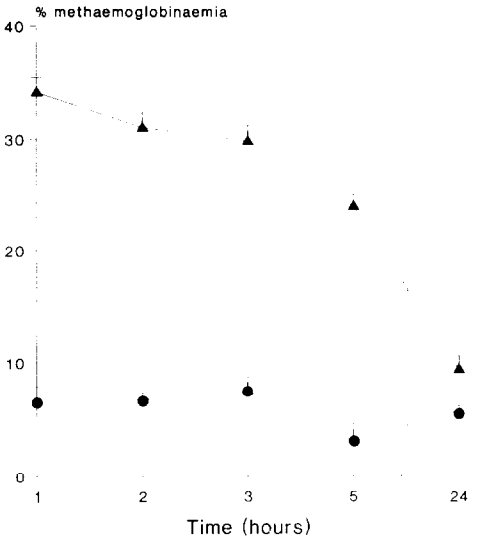

Fig. 1. Percentage methaemoglobinaemia against time after the administration of dapsone (33 mg/kg) to male rats (▲) and female rats (●) (N = 5 per group, values are means ± SE).

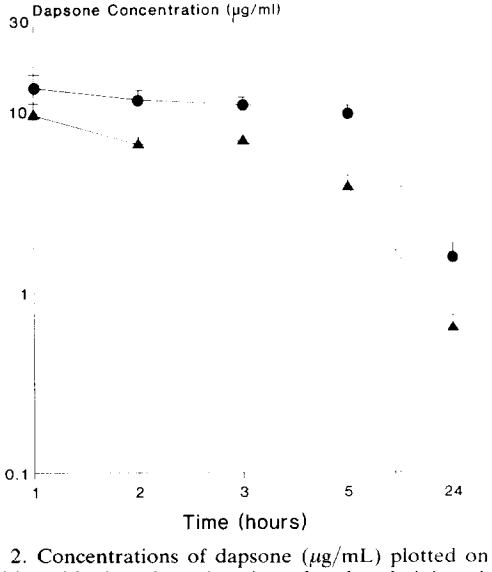

Fig. 2. Concentrations of dapsone (μg/mL) plotted on a semi-logarithmic scale against time after the administration of dapsone (33 mg/kg) to male rats (▲) and female rats (●) (N = 5 per group, values are means ± SE).

(650 *g* for 20 min), aliquots (100 μL) of the supernatant containing approximately 130,000 dpm were injected onto the column for measurement of 8–10,000 dpm associated with dapsone hydroxylamine. Separation was achieved using the C_{18} μBondapak column. The solvent mobile phase consisted of water, acetonitrile, acetic acid and triethylamine (80:20:1:0.05 v/v) flowing at 1.2 mL/min. Eluate was again monitored at 254 nm and collected in 30 sec fractions which were then mixed with 4 mL of scintillant fluid for measurement of radioactivity.

Calculations. Both the curve for percentage methaemoglobin $AUC_{Met\ Hb\ 0\text{–}24\ hr}$ and the $AUC_{(0.24\ hr)}$ for dapsone blood concentrations were estimated by the trapezoidal rule [20]. The AUC from 24 hr to infinity for blood dapsone concentrations was calculated by the ratio $C_{24/\beta}$ where C_{24} was the blood level of dapsone at 24 hr. The AUC to infinity (AUC) for dapsone was obtained from the sum of the $AUC_{(0\text{–}24)}$ and $AUC_{(24\ hr\text{–}\infty)}$. The terminal phase elimination rate constant (β) was determined by least squares regression analysis of the post distributive blood dapsone concentration-time data for all five time points and the half-life ($T_{\frac{1}{2}}$) from the ratio $0.693/\beta$. Clearance was calculated by the formula

$$Cl = \frac{DOSE}{AUC}.$$

Statistical analysis was accomplished by the Wilcoxon signed rank test accepting $P < 0.05$ as statistically significant.

Results and discussion

Within 1 hr of dapsone administration to the male rats, a rapid and acute methaemoglobinaemia resulted (33.1 ± 2.8%), compared with background values of 4.5 ± 1.1%. This is in agreement with previous studies in the male rat [7, 8]. Twenty-four hours post dosing in the male rats, levels of methaemoglobin had fallen to less than 10% (9.4 ± 3.7%). In sharp contrast (Fig. 1) administration of dapsone to female rats resulted in low initial levels of methaemoglobin (6.4 ± 3.8% at 1 hr) which were not significantly different from background levels (3.9 ± 1.0%). At 24 hr, the females still exhibited low levels of methaemoglobinaemia (5.7 ± 1.8%). Over the period of the study, the $AUC_{(met\ Hb\ 0\text{–}24\ hr)}$ for female rats (120.4 ± 35.6) was less than one third of that of the males (455.4 ± 6.2).

However, dapsone plasma levels in the females were significantly higher than those of the males (Fig. 2). The AUC for dapsone measured in female rats (167.5 ± 34.6 μg hr/mL) was more than double that of the males (78.3 ± 11.7 μg hr/mL, $P < 0.01$). There were no significant differences between the half-lives of dapsone in the respective groups (6.5 ± 2.3 hr vs 7.02 ± 1.4 hr). However, the clearance of dapsone in the male rats (7.15 ± 1.06 mL/min) was more than twice that of the females (3.4 ± 0.72 mL/min) and this was reflected in the rapid formation of methaemoglobin in the males. However, the reduced ability of the female rats to clear dapsone from blood was associated with a lack of haemoglobin oxidation in these animals.

Dapsone and its monoacetyl derivative are known to be extensively and rapidly N-hydroxylated to hydroxylamines in the male rat, and these metabolites induce the formation of methaemoglobin *in vivo* and *in vitro* [6, 7]. *In vitro*, in the present study, radiometric analysis of incubations containing dapsone, NADPH and male rat microsomes revealed a peak corresponding to the retention time of dapsone hydroxylamine (Fig. 3). Metabolic conversion of radiolabelled dapsone to the hydroxylamine in male rats was 0.3 ± 0.3% without NADPH, and 8.6 ± 0.61% with NADPH. In contrast, metabolic conversion by female rat microsomes was 0.2 ± 0.04% without NADPH and 1.77 ± 0.8% with NADPH. Hence, male rat liver microsomes converted more than four times the amount of dapsone to the hydroxylamine compared with female rat microsomes. From Fig. 4, it is clear that the absence of methaemoglobin in the female rats was not due to any pharmacodynamic differences in the sensitivity of whole blood to the direct toxicity of dapsone hydroxylamine *in vitro*. Therefore it is likely that the lack of metabolism of dapsone to *N*-hydroxy derivatives by female rats protects them from the known haematological side effects of those metabolites. Thus, our findings are in agreement with pre-

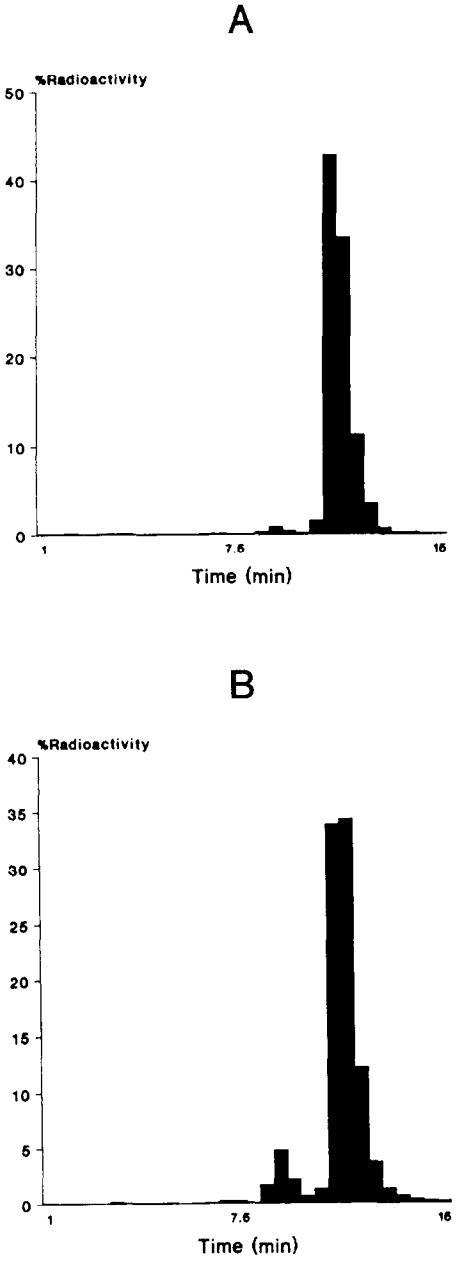

Fig. 3. High performance liquid radiochromatogram of extracts of incubations containing either female (A) or male (B) rat liver microsomes (0.5 mg protein, NADPH and dapsone 100 μM, 0.5 μCi) performed at 37° for 1 hr. The peaks eluted at 8.5 and 10.5 min were dapsone hydroxylamine and dapsone, respectively.

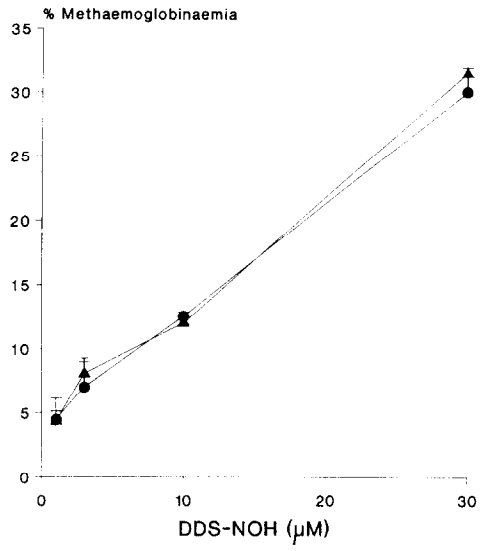

Fig. 4. Incubation of whole blood (1 mL per incubation) obtained from male (▲) and female (●) rats with dapsone hydroxylamine (1–30 μM) (N = 4 per incubation, values are means ± SE).

vious studies which indicated that N-hydroxylation is a prerequisite for dapsone toxicity *in vivo* [7, 21].

Although sex differences in drug metabolism are frequent and widely investigated [22–24], sex differences in methaemoglobin formation are rarely reported [25]. Studies with 4-aminopropiophenone showed female beagles to be more sensitive to methaemoglobin formation than male beagles, due to a sex difference in the N-hydroxylation of the compound [25]. Indeed, a number of factors have been identified in man which influence susceptibility to dapsone-induced methaemoglobinaemia, such as erythrocytic enzyme deficiencies [26, 27] or variation in the ability to metabolize the drug to the hydroxylamine [8]. The present study underlines the toxicological consequences of variation in the metabolic activation of dapsone.

In summary, we have shown in the rat a marked sex difference, not only in the plasma disappearance of dapsone, but also in the tendency to develop dapsone mediated methaemoglobinaemia. Females may be protected from dapsone mediated oxidation of haemoglobin as a consequence of a lack of production of the toxic N-hydroxylated metabolites of the drug.

Acknowledgements—M.D. Coleman is a Wellcome Fellow in Toxicology. B. K. Park is a Wellcome Senior Lecturer.

Department of Experimental Therapeutics
University of Liverpool
Liverpool L69 3BX
U.K.

M. D. COLEMAN*
M. J. WINN†
A. M. BRECKENRIDGE
B. K. PARK

REFERENCES

1. Shepard CC, Leprosy today. *New Engl J Med* **307**: 1640–1641, 1982.
2. Bruce-Chwatt LJ, *Essential Malariology*, pp. 181–182. Heinemann, 1982.
3. Lang PG, Sulfones and sulfonamides in dermatology today. *J Am Acad Dermatol* **1**: 479–492, 1979.
4. Grindulis KA and McConkey B, Rheumatoid arthritis, the effects of treatment with dapsone on heamoglobin. *J Rheumatol* **11**: 776–778, 1984.
5. Green ST, Goldberg DJ, Leach J, Christie PR and Kennedy DH, AIDS-related *Pneumocystis carinii* pneumonia successfully treated with dapsone and trimethoprim. *Br J Clin Pharmacol* **26**: 487–488, 1988.
6. Israili ZH, Cucinell SA, Vaught J, Davis E, Zesser JM and Dayton PG, Studies of the metabolism of DDS in man and experimental animals. Formation of *N*-hydroxy metabolites. *J Pharmacol Exp Ther* **187**: 138–151, 1973.
7. Grossman SJ and Jollow DJ, Role of DDS NOH in DDS-induced haemolytic anaemia. *J Pharmacol Exp Ther* **244**: 118–125, 1988.
8. Coleman MD, Breckenridge AM and Park BK, Bioactivation of dapsone to a cytotoxic metabolite by human hepatic microsomal enzymes. *Br J Clin Pharmacol* **28**: 389–395, 1989.
9. Cucinell SA, Israeli ZH, Dayton PG, Microsomal N-oxidation of dapsone as a cause of methaemoglobin formation in human red cells. *Am J Trop Med Hyg* **21**: 322–333, 1972.
10. Modderman ESM, Merkus FWHM, Zuidema J, Huikshoven H and Leiken DL, Controlled release of dapsone by intramuscular injection. In: *Controlled Release Delivery Systems* (Ed. Roseman Mansdorf), pp. 274–289. Marcel Dekker, New York, 1983.
11. Zuidema J, Hilbers-Moddermann ESM and Merkus FWHM, Clinical pharmacokinetics of Dapsone. *Clin Pharmacokinet* **11**: 299–315, 1986.
12. Winn MJ and Park BK, The effects of 17β-oestradiol or testosterone on the response to S-warfarin in castrated male rats. *J Pharm Pharmacol* **39**: 958–960, 1987.
13. Winn MJ, Clegg JAD and Park BK, (1987) An investigation of sex-linked differences to the toxic and to the pharmacological actions of difenacoum: studies in mice and rats. *J Pharm Pharmacol* **39**: 219–222, 1987.
14. Munoz FG and Fearon Z, Sex related differences in acetominophen toxicity in the mouse. *Clin Toxicol* **22**: 149–156, 1984.
15. Uetrecht J, Zahid N, Shear NH and Biggar WD, Metabolism of DDS to a hydroxylamine by human neutrophils and mononuclear cells. *J Pharmacol Exp Ther* **245**: 247–279, 1988.
16. Purba HS, Maggs JL, Orme MLE, Back DJ and Park BK, The metabolism of 17α ethinyloestradiol by human liver microsomes: formation of catechol and chemically reactive metabolites. *Br J Clin Pharmacol* **23**: 447–453, 1987.
17. Omura T and Sato R, The carbon monoxide binding pigment of liver microsomes. *J Biol Chem* **239**: 2370–2378, 1964.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
19. Harrison JH and Jollow DJ, Role of aniline metabolites in aniline-induced hemolytic anemia. *J Pharmacol Exp Ther* **238**: 1045–1054, 1986.
20. Gibaldi M and Perrier D, In: *Pharmacokinetics* 2nd Edn, p. 445. Marcel Dekker, New York, 1982.
21. Manfredi G, de Panfilis G, Zampetti M and Allegra F, Studies on dapsone induced haemolytic anaemia. *Br J Dermatol* **100**: 427–432, 1979.
22. Kato R, Takashashi A, Ohshima T and Hosoya E, Effect of morphine administration on the hydroxylamine of steroid hormones by rat liver microsomes. *J Pharmacol Exp Ther* **174**: 211–220, 1970.
23. Kato R and Gillette JR, Effect of starvation on

* To whom correspondence should be addressed.
† Present address: Department of Pharmacology, University of Alabama at Birmingham, Alabama 35294, U.S.A.

NADPH-dependent enzymes in liver microsomes of male and female rats. *J Pharmacol Exp Ther* **150**: 279–284, 1965.
24. Skett P, Mocle A, Rafter J, Sahlin L and Gustafsson JA, The effects of gonadectomy and hypophysectomy on the metabolism of imipramine and lidocaine by the liver of male and female rats. *Biochem Pharmacol* **29**: 2759–2762, 1980.
25. Bright JE, Woodman AC, Harris TC and Wood SG, Sex differences in the production of methaemoglobinaemia by 4 amino-propiophenone. *Xenobiotica* **17**: 79–83, 1987.
26. Ganer A, Knobel B, Fryd CH and Rachmilewitz EA, Dapsone induced methaemoglobinaemia and haemolysis in the presence of familial haemaglobinopathy hasharon and familial methaemoglobin reduction deficiency. *Israel J Med Sci* **17**: 703–704, 1981.
27. DeGowin RL, Bennett Eppes R, Powell RD and Carson PE, The haemolytic effects of diaphenylsulfone (DDS) in normal subjects and in those with glucose 6-phosphate-dehydrogenase deficiency. *Bull World Health Org* **35**: 165–179, 1966.

Biochemical Pharmacology, Vol. 39, No. 4, pp. 809–811, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

The relative abilities of MPTP and MPP^+ to compete with [^{3}H]dopamine for the rat and marmoset striatal dopamine uptake site

(*Received* 19 *June* 1989; *accepted* 2 *November* 1989)

The principal neurotoxic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in man and non-human primates is a profound destruction of the nigrostriatal dopamine system, causing severe motor impairments similar to those observed in Parkinson's disease [1–4].

Pretreatment of monkeys with monoamine oxidase B inhibitors fully or partially protects against the biochemical and behavioural impairments [4, 5], indicating that oxidation of MPTP to 1-methyl-4-phenylpyridinium (MPP^+) may be required to cause neurotoxicity [6, 7]. This may occur in glial or neuronal cells containing monoamine oxidase B, with the subsequent uptake of MPP^+ via the dopamine uptake process into the dopamine neurone [8, 9]. The partial protection afforded by dopamine uptake inhibitors against MPTP induced toxicity in the primate would support this hypothesis [10].

In contrast to the pronounced effects observed in the primate, rodents appear considerably less sensitive to MPTP induced toxicity, although the effects that do occur either *in vivo* or in cell cultures are also attenuated by monoamine oxidase B inhibitors [11–13]. In the rodent, as in the primate, the effects of MPTP may again be mediated by MPP^+, which has potent neurotoxic effects in cell cultures [14], although the reason for differences in *in vivo* sensitivity to MPTP between the rodent and primate species remains uncertain.

It was hypothesized that such species differences may relate to the second stage of the neurotoxic process involving MPTP and/or MPP^+ uptake into the dopamine neurones. Here we investigate this possibility and compare the abilities of MPTP, MPP^+ and uptake inhibitors to inhibit [^{3}H]dopamine uptake in striatal synaptosomal preparations from the rat and marmoset.

Methods and materials

Female Hooded Lister rats (Bradford bred) weighing 250–300g and male or female common marmosets (*Callithrix jacchus*) weighing 280–320g were killed by cervical dislocation and decapitation, respectively. The brains were immediately removed. The striatum was dissected out and homogenized in a glass–Teflon homogenizer (clearance 0.11–0.15 mm) by six movements up and down in 10 mL ice-cold 0.32 M sucrose followed by centrifugation at 1000 *g* (4°) for 10 min (Beckman L8-70 ultracentrifuge). The supernatant was retained and centrifuged at 48,000 *g* for 15 min (4°). The resulting pellet was gently resuspended in ice-cold 0.27 M sucrose at a concentration of 0.5–0.8 and 0.4–0.5 mg protein/mL for the rat and marmoset, respectively.

Krebs buffer (900 μL) was gassed using 95% O_2/5% CO_2 containing 115.0 mM NaCl, 4.97 mM KCl, 1.0 mM $CaCl_2$, 1.22 mM $MgSO_4$, 1.20 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 μM nialamide, 0.8 mM ascorbic acid and 0.1 μM [^{3}H]dopamine (50 Ci/mmol) in the absence (total uptake) or presence of uptake-competing compounds (2.0×10^{-10}–2.0×10^{-5} M, six concentrations) which were added to reaction tubes in triplicate and preincubated at 37° for 3 min. [^{3}H]Dopamine uptake was initiated by the addition of 100 μL of the crude synaptosomal preparation (or 100 μL 0.27 M sucrose for filter blank). The reaction was allowed to proceed at 37° for 6 min before termination by rapid filtration through pre-wet Whatmann GF/B filter paper, followed immediately by washing with 9.0 mL ice-cold Krebs buffer for 6 sec. The filtration and washing procedure was performed using a semi-automatic membrane harvester (Brandel). The filter discs were placed in 10.0 mL 'Insta-Gel' scintillant (Packard) and counted for tritium by liquid scintillation spectroscopy (Tri-Carb 1900 CA, Canberra Packard) at approximately 47% efficiency. Protein estimation was performed by the method of Bradford [15] using bovine serum albumin as the standard.

[^{3}H]Dopamine (50 Ci/mmol, Amersham International, Amersham, U.K.), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (Research Biochemical Incorporated, RBI), 1-methyl-4-phenylpyridinium (RBI), GBR12909 1-[2-[bis-(4-fluorophenyl) methoxyl]-ethyl-4-[3-phenyl-propyl]-piperazine dihydrochloride (RBI), desipramine hydrochloride (Sigma Chemical Co., Poole, U.K.), hemicholinium-3 (Sigma), mazindol (Sandoz, Middlesex, U.K.), fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN), benztropine mesylate (Merck Sharp & Dohme, Herts, U.K.) and cocaine hydrochloride (May & Baker, Dagenham, U.K.).

All drugs were dissolved in distilled water except mazindol and GBR 12909 which were dissolved in a minimum quantity of dilute hydrochloric acid and made to volume with distilled water. All drugs were used as received.

Results and discussion

Preliminary experiments established that [^{3}H]dopamine uptake into the synaptosomes was linear with respect to time of incubation (up to 15 min). Furthermore, the uptake was comparable (1.8 ± 0.5 and 2.4 ± 0.2 pmol/min/mg protein, mean ± SE of 4–7 determinations) in rat and marmoset striatal preparations respectively.

The uptake of [^{3}H]dopamine into rat and marmoset striatal synaptosomes was inhibited by nanomolar concentrations of the selective dopamine uptake inhibitor