Table 1. Drug concentration producing 50 per cent inhibition of PEA and 5-HT deamination*

Inhibitor	PEA (M)	5-HT (M)
Iprindole	$2{\cdot}5 \times 10^{-5}$	9×10^{-5}
Imipramine[11]	$2{\cdot}5 \times 10^{-5}$	10×10^{-5}
Amitriptyline	$3{\cdot}0 \times 10^{-6}$	9×10^{-5}

* Reaction mixtures contained 3·6 nmoles PEA or 5-HT and varying amounts of drug and mitochondrial protein in 2 ml of 0·05 M potassium phosphate buffer, pH 7·4. The value for drug concentration producing 50 per cent inhibition was obtained from graphs for each inhibitor as presented in Fig. 1.

HT, a specific type A MAO substrate [13], is illustrated in Fig. 1. Under the conditions used, 50 per cent inhibition of PEA and 5-HT deamination is achieved at iprindole concentrations of approximately $2{\cdot}5 \times 10^{-5}$ M and 9×10^{-5} M respectively. These data indicate that, at concentrations less than 2×10^{-4} M of this tricyclic antidepressant drug, the type B form of the oxidase is more susceptible to inhibition than is the type A form of MAO.

A comparison of the concentrations of iprindole and two widely used antidepressant drugs, imipramine and amitriptyline, required to inhibit PEA and 5-HT deamination by 50 per cent is presented in Table 1. These results indicate that the ability of iprindole to inhibit the type A and B forms of rabbit MAO is similar to that of imipramine. Amitriptyline, however, is considerably more potent as an inhibitor of the type B form of the oxidase than either of the other two antidepressant agents, although the ability of amitriptyline to inhibit the A form of MAO is similar to that of the other tricyclic drugs.

It has been shown previously that imipramine reversibly binds to MAO and that inhibition of PEA and 5-HT deamination could only be detected at substrate concentrations near or less than that of the inhibitor [11]. This may partly explain why Gluckman and Baum [4] had previously failed to notice any decrease of tyramine deamination by iprindole. In their experiments they used a 50-fold excess of substrate (0·05 M tyramine) compared to that of iprindole (0·001 M). Results of experiments in this paper reveal that a 15- and 50-fold excess of the drug concentration is required to inhibit 1·8 μM PEA and 5-HT by 50 per cent respectively.

Mosnaim *et al.* [14] and Fischer *et al.* [15] have suggested that a depletion of endogenous PEA may be one of the biochemical lesions in depression. Mosnaim *et al.* [16] have shown that the mean concentration of PEA in rabbit brain increases after acute or chronic treatment with either imipramine or iprindole. Whether or not an increase in brain PEA is actually involved in alleviating the symptoms of depression, the data obtained by Mosnaim *et al.* [16] are consistent with the view that imipramine and iprindole can also inhibit the type B form of MAO *in vivo*.

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Competitive inhibition of glucuronidation by *p*-hydroxyphenyl hydantoin

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5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH), the major diphenylhydantoin metabolite, as well as diphenylhydantoin itself, is present in hepatic tissues [1] and homogenates [2] of animals treated with diphenylhydantoin. It is

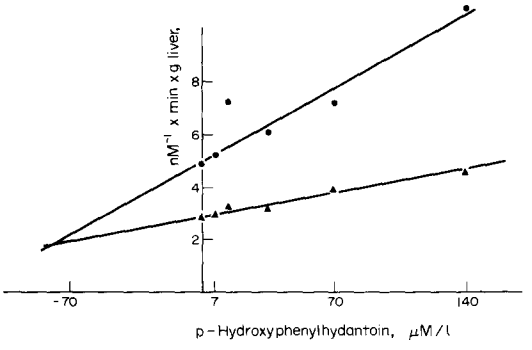

Fig. 1. Inhibition of the conjugation of *p*-nitrophenol by different concentrations (μM) of 5 (*p*-hydroxyphenyl)-5-phenylhydantoin. Activity was determined simultaneously in duplicate and the averages of 4 homogenates are plotted according to Dixon [15]; ▲ 0·14 mM, ● 0·07 mM *p*-nitrophenol, respectively.

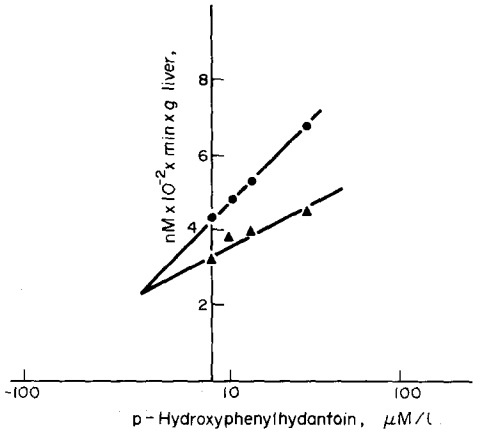

Fig. 2. Inhibition of the conjugation of 4-methyl-umbelliferone by different concentrations (μM) of 5 (*p*-hydroxy phenyl)-5-phenylhydantoin. Results were obtained and plotted as in Fig. 1. ▲ 0·5 mM, ● 0·25 mM 4-methyl-umbelliferone, respectively.

still present 6–9 hr after parent drug administration. HPPH inhibits diphenylhydantoin metabolism in hepatic microsomes [3], especially hydroxylation of this drug [4]. Finally, HPPH is eliminated as a glucuronide. The latter fact led us to investigate its ability to modify *in vitro* conjugation of the artificial substrates, *p*-nitrophenol and 4-methyl-umbelliferone. Because of the nonspecificity of UDP-glucuronyltransferase (EC 2.4.1.17, acceptor unspecific) (UDPGT) most substrates are themselves potent inhibitors of the *in vitro* conjugation, like steroids [5], bilirubin [6], hydroxychinolin [7].

METHODS

The UDP-glucuronyltransferase preparations were obtained from livers of male Sprague–Dawley rats weighing 200–220 g, (Centre des Oncins, Lyon, France), fed *ad lib.* Animals were killed by decapitation. The livers were quickly removed at 4°. Further handling was done at 4° in a cooled room. The tissues were cut with scissors and then homogenized (5% w/v) with a glass–Teflon homogenizer in ice for 1 min at 2800 rev/min. The medium contained 0·25 M sucrose, 1 mM EDTA, 1 mM Tris and HCl to give a final pH of 7·4. Triton X-100 (0·1% w/v) was added to activate UDPGT [8, 9]. Preparations were stored in melting ice at least 20 min before use.

5-(*p*-hydroxyphenyl)-5-phenylhydantoin (a gift from Dr A. J. Glazko, Parke Davis, Ann Arbor, Michigan) was added to the reaction vials as a methanol solution in concentrations indicated on the figures. The methanol was evaporated off, as it has been found to compete with *p*-nitrophenol [10]. Enzyme activity was determined with *p*-nitrophenol (method 1) or with 4-methyl-umbelliferone (method 2) as proposed by Frei [11]. In method 1, the reaction mixture (100 μl) contained 0·14 mM *p*-nitrophenol, 1 mM UDP-glucuronic acid (UDPGA) (Boehringer, Mannheim, Germany) 0·5 mM $MgCl_2$, and 30 μl homogenate giving a final concentration of Triton X-100 of 0·03 per cent. Incubation (37°) was stopped after 10 min by adding 200 μl of 10% trichloracetic acid followed by centrifugation for 2 min at 20,000 rev/min. Potassium hydroxide (10 μl) was added to the supernatant. The amount of unconjugated *p*-nitrophenol was measured at 405 nm in an Eppendorf Photometer. In method 2, the activity was determined with 0·5 mM 4-methyl-umbelliferone, 2·1 mM UDPGA, 12 mM $MgCl_2$ and 10 μl homogenate in a final volume of 60 μl, Triton X-100 0·016 per cent. Incubation (37°) was stopped after 5, 10 and 15 min. Fluorescence of free 4-methyl-umbelliferone was measured in aminoethylpropanol buffer (pH 10·5) using an Eppendorf device for fluorimetric determinations; filters I 313·366 nm, II 340·3000 nm).

Each determination was performed in duplicate.

RESULTS AND DISCUSSION

Low concentrations of 5-(*p*-hydroxyphenyl)-5-phenylhydantoin were found to inhibit competitively both *p*-nitrophenol (Fig. 1) and 4-methyl-umbelliferone (Fig. 2) conjugation, with K_i 0·08 mM and 0·04 mM, respectively. Concentrations above 50 μM HPPH caused an uncompetitive inhibition of 4-methyl-umbelliferone conjugation.

This inhibition makes it difficult to interpret *in vitro* investigation of UDP-glucuronyltransferase activity after *in vivo* administration of diphenylhydantoin. Remaining HPPH, bound to proteins in crude enzymatic preparations, could inhibit the measurement of activity with synthetic substrates.

The competitive inhibition of *p*-nitrophenol and of 4-methyl-umbelliferone conjugation by HPPH *in vitro*, may indicate that these three substrates are conjugated by the same enzyme.

Mulder [12, 13] demonstrated that *o*-aminophenol, *p*-nitrophenol, phenolphthalein, 4-methyl-umbelliferone and bilirubin could be conjugated by the same enzyme, on the same site and that 8-hydroxychinolin [7], phenolphthalein, 4-methyl-umbelliferone and *p*-nitrophenol inhibited conjugation for each other.

Further measurements are now being performed with HPPH itself as substrate of UDP-glucuronyltransferase [14] to determine whether or not diphenylhydantoin is an inducer of conjugation.

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Adenosine uptake by erythrocytes of man, rat and guinea-pig and its inhibition by hexobendine and dipyridamole

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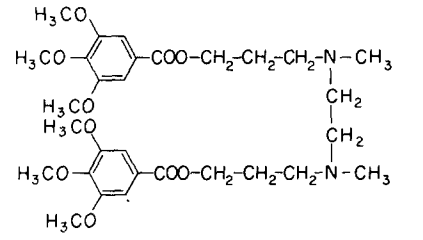
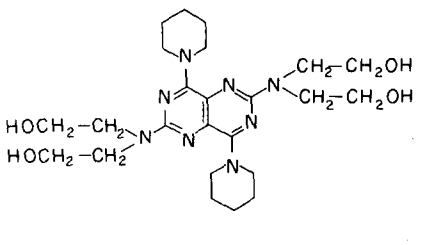
Adenosine is rapidly eliminated from the plasma by the blood cells [1], by the heart [2, 3], and by the lungs [4]. In the guinea-pig, this elimination is very sensitive to dipyridamole and hexobendine. Both drugs exert their inhibitory effect by decreasing the permeability of the cell membrane for adenosine. In the rat, however, the uptake of adenosine into the lungs is about 100 times less sensitive to dipyridamole and hexobendine than in the guinea-pig, while the uptake into the heart is not inhibited at all by these drugs [5, 6].

The uptake of adenosine into the erythrocytes of the guinea-pig, rat and man and the inhibition of this uptake by dipyridamole and hexobendine should provide some information about the sensitivity of adenosine elimination to both drugs in man in comparison with the cited animals.

Blood was collected from the right ventricle of guinea-pigs and rats and from the cubital vein of men. 0·05 mg/ml heparin was added. After centrifugation at 3500 rev/min the supernatant plasma was replaced by ice-cold saline. This procedure was repeated four times. Two ml of the erythrocyte





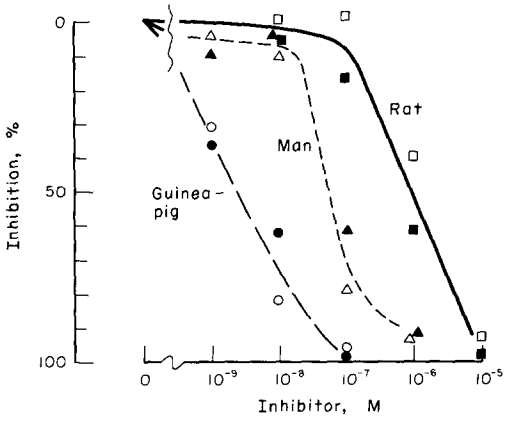


Fig. 1. Inhibition of ^{14}C-adenosine net uptake into erythrocytes of guinea-pigs (○●), men (△▲) and rats (□■) by different concentrations of hexobendine (open symbols) and dipyridamole (closed symbols).