had no remarkable effect on the increases. As might be expected from the dosage and short experimental interval, the liver sections displayed no unusual light microscopic changes compared with the control groups. Further studies might explore wider CsA dosage ranges in several species of either sex, isolation of the relevant molecular form of cytochrome P-450, and the nature of the drug binding sites of hepatocytes from the organs of operated and intact rats, possibly, by such approaches as photoaffinity labeling [28]. Although the liver, following partial removal, is unique in that regenerative changes take precedence over those involved in drug detoxification [29–32], our findings with CsA in the operated animal were similar to those found with the intact rat.

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Influence of the sulphation inhibitor, 2,6-dichloro-4-nitrophenol, on the production, and conjugation, of 4-hydroxybiphenyl generated from 4-methoxybiphenyl by rat isolated hepatocytes

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A number of aromatic xenobiotics are metabolized by the cytochrome P450-dependent microsomal monooxygenase (MMO)* system leading to the production or unmasking of a phenolic functional group which is followed by conjugation with glucuronic acid or sulphate at the newly generated phenolic group. It is recognised that the balance of these two phases of metabolism may play an important role in determining the overall biological effect of the parent xenobiotic, and for this reason it is important to identify the factors that might influence the balance of these two phases of metabolism.

* Abbreviations used: DCNP, 2,6-dichloro-4-nitrophenol; 4-MBP, 4-methoxybiphenyl; MMO, microsomal monooxygenase; 4-OHBP, 4-hydroxybiphenyl.

2,6-Dichloro-4-nitrophenol (DCNP) is a competitive inhibitor of aryl sulphotransferase [1] and has been used in a number of studies to decrease the sulphation of phenolic xenobiotics [2–4]. In this study we have investigated the influence of DCNP treatment on the production, and conjugation, of 4-hydroxybiphenyl (4-OHBP) generated by MMO-mediated metabolism from the model substrate 4-methoxybiphenyl (4-MBP) by rat isolated hepatocytes.

Materials and methods

The source and maintenance of the male Wistar rats used in these studies have been described previously [5], as have the techniques for the isolation of hepatocytes [5].

DCNP was obtained from Fluka AG (Buchs, Switzerland), whereas the other chemicals were obtained from sources previously documented [5, 6].

Incubation of hepatocytes with 4-MBP or 4-OHBP (both at 100 μM final concentration) and measurement of unconjugated 4-OHBP and its sulphate and glucuronic acid conjugates were carried out as described previously [5, 7], using an incubation medium of minimal essential medium + 10% calf serum. The concentration of 4-OHBP used in these studies was saturating for both pathways of conjugation. The amount of total 4-OHBP produced from 4-MBP was calculated by addition of the values obtained for the three metabolites. Sulphate-free medium was prepared by replacement of $MgSO_4$ in the complete medium [8] with an equimolar amount of $MgCl_2$. DCNP was added in a minimal volume of isotonic salt solution (max. of 80 μl in 4 ml cell suspension).

Statistical analysis was performed using a paired-sample Student's *t*-test.

Results and discussion

The sulphation of 4-OHBP added directly to the cells was inhibited in a concentration-dependent manner by DCNP, almost complete (97%) inhibition occurring at 50 μM DCNP. The glucuronidation of 4-OHBP was not inhibited by 5–50 μM DCNP (data not shown).

A similar magnitude of inhibition of sulphation by 50 μM DCNP was observed when 4-OHBP was generated by O-demethylation of 4-MBP (Table 1). This inhibition of sulphation was accompanied by significant ($P < 0.001$) increases in the amounts of 4-OHBP glucuronide, unconjugated 4-OHBP and the total amount of 4-OHBP. These changes in pattern of metabolism of 4-MBP were dependent on the concentration of DCNP, with maximal effect occurring at 50 μM (Fig. 1). Incubation of cells in sulphate-free medium produced similar alterations in the metabolism of 4-MBP (Table 2).

It is recognized that sulphotransferase acts as a high-affinity, low-capacity enzyme for the conjugation of phenolic aglycones, whereas UDP-glucuronosyltransferase acts as a low-affinity, high-capacity enzyme in competition with sulphotransferase [9, 10]. The findings of an increased glucuronidation of 4-OHBP and an increase in the amount of unconjugated 4-OHBP following inhibition of sulphation are consistent with this theory, and the increases in amounts of 4-OHBP glucuronide and unconjugated 4-OHBP noted in the DCNP study were linearly related to the decrease in amount of 4-OHBP sulphate ($r > 0.97$ and $P < 0.01$ in each case).

An increase in the extent of 4-MBP O-demethylation was consistently observed at high (>60%) levels of inhibition, the cause of which was not apparent from these studies.

Table 1. Influence of DCNP (50 μM) on the metabolism of 4-MBP in rat isolated hepatocytes

	Metabolite produced (nmol/2 × 10^6 cells/20 min)			
Incubation medium	4-OHBP Sulphate	4-OHBP Glucuronide	Unconjugated 4-OHBP	Total 4-OHBP
Control (−DCNP)	8.34 ± 0.62	5.00 ± 0.96	2.06 ± 0.41	15.40 ± 1.45
+DCNP (50 μM)	0.03 ± 0.02 (0.04)	13.69 ± 0.96 (274)	5.75 ± 0.73 (279)	19.47 ± 1.29 (126)

Values are mean ± SEM of 5 animals. Figures in brackets indicate the value as a percentage of the control value.

Table 2. Metabolism of 4-MBP in hepatocytes incubated in complete medium and sulphate-free medium

	Metabolite produced (nmol/2 × 10^6 cells/20 min)			
Incubation medium	4-OHBP Sulphate	4-OHBP Glucuronide	Unconjugated 4-OHBP	Total 4-OHBP
Complete*	7.50 ± 0.27	7.21 ± 0.23	2.29 ± 0.37	17.00 ± 0.62
Sulphate-free	2.45 ± 0.37 (33)	13.34 ± 0.58 (185)	4.22 ± 0.37 (184)	20.01 ± 0.99 (118)

Values are mean ± SEM of 4 animals. Figures in brackets indicate the value as a percentage of the control value.

*Contained inorganic sulphate at a final concentration of 1 mM.

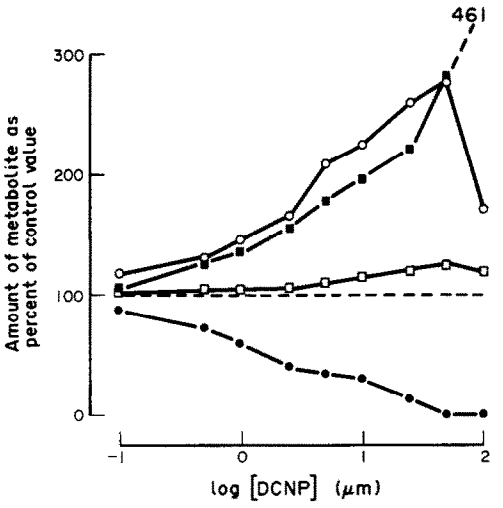

Fig. 1. Influence of different concentrations of DCNP on the pattern of metabolism of 4-MBP. Rat isolated hepatocytes were incubated in the presence of 4-MBP (100 μM) and DCNP (0.1–100 μM) for 20 min, after which time the amounts of metabolites produced were measured. Values are presented as the percent of control values (no DCNP) and are the mean of 5 experiments. Control values were those presented in Table 1: ●, 4-OHBP sulphate; ○, 4-OHBP glucuronide; ■, unconjugated 4-OHBP; □, total 4-OHBP.

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