Table 2. Effects of *N*-acetyldopa and γ-glutamyldopa on the life span of mice inoculated with B16 melanoma

Treatment (concn)	Survival time (days) Median	Range	%ILS*
Experiment 1			
Control	46	37–53	
N-Acetyldopa (500 mg/kg)	46	40–61	0
N-Acetyldopa (1000 mg/kg)	54	44–69	17 ($P < 0.01$)
γ-Glutamyldopa (500 mg/kg)	45	40–54	−2
γ-Glutamyldopa (1000 mg/kg)	47	35–54	2
Experiment 2			
Control	36	31–38	
N-Acetyldopa (1000 mg/kg)	40	32–45	11 ($P < 0.05$)

B16 melanoma cells were inoculated i.p. in young male C57BL/6 × DBA/2 F_1 mice, 10 animals per group, following the National Cancer Institute protocol [17]. Treatment of mice was started on day 1 and continued daily for 12 days. Drugs, dissolved in 1 ml of 0.9% NaCl, were adjusted to pH 7 with $NaHCO_3$ powder and were given i.p. once a day. Control animals received i.p. injection of 1 ml of 0.9% NaCl.

* Percentage of increase in the life span of treated versus control animals.

than in HeLa cells. The results obtained indicate that in pigmented melanoma cells the cytotoxicity is mediated by both hydrogen peroxide and tyrosinase, while in non-pigmented melanoma cells it is mediated mostly by hydrogen peroxide produced by autoxidation of the drugs.

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Drug effects on output of prostacyclin from isolated lungs

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Exogenous arachidonic acid (AA) is metabolized by lung tissue to a variety of biologically active cyclo-oxygenase products (COP) including prostaglandins (PG) and thromboxane (Tx). Synthesis of COP from endogenous AA esterified in lung lipids is stimulated by a variety of stimuli–chemical, physical or immunological [1]. In perfused isolated lungs, the active COP derived from either source of substrate which appear in effluent from lung are accompanied by inactive metabolites of these products, usually via the action of 15-hydroxy PG dehydrogenase (PGDH) [2–4]. Inactivation of PGs can also be demonstrated directly by perfusing PGE_2 or $PGF_{2\alpha}$ through the

pulmonary circulation [5]. The overall effect of drugs affecting both PG inactivation and COP synthesis in lung were found to be more complex than we had predicted [6]. We report here in further detail the effects in guinea-pig and rat isolated lungs of four drugs—bromcresol green, dipyridamole, nafazatrom and sulphinpyrazone—on COP synthesis from exogenous and endogenous AA correlated with their effects on PG inactivation.

Materials and methods

Male Wistar rats (200–250 g) and Dunkin–Hartley guinea pigs (180–200 g) were used. Rats were anaesthetized with pentobarbitone (60 mg/kg; i.p.) and guinea pigs were killed by cervical dislocation. Lungs were removed and perfused via the pulmonary artery with Krebs solution (gassed with 95% O_2; 5% CO_2 and warmed to 37°) at 8 ml/min [7]. After a 10-min period of perfusion, the assays were started.

Inactivation of PGE_2 was measured with rat or hamster stomach strips superfused with lung effluent [7]. The responses to bolus injections of PGE_2 (300–1000 ng) into the pulmonary circulation were compared, in a bracket assay, with those to injections of PGE_2 (10–100 ng) given directly to the assay tissues. Production of PGs from lung was assayed on rat stomach strip superfused with lung effluent, following exogenous AA (1–10 μg) given by bolus injection into the perfusate flow entering the pulmonary circulation. The biological activity thus produced in lung effluent was assayed using PGE_2 as a standard and expressed as ng PGE_2 equivalents.

Output of PGI_2 was studied by radioimmunoassay of 6-oxo-$PGF_{1\alpha}$. A solution of AA (final concentration, 40 μM in rat and 4 μM in guinea-pig lungs) was infused (0.1 ml/min) into the lung perfusate for 3 min. Lung effluent was collected during the infusion and for a further 7 min. To study PG synthesis from AA in lung lipids (endogenous AA), the calcium ionophore A23187 was injected (0.1–1.0 μg) into the perfusate entering the lung. The lung effluent was collected for 5 min immediately following the ionophore.

A sample of lung effluent (100 μl) from either procedure was assayed directly or after five-fold dilution with Tris-buffered saline, for 6-oxo-$PGF_{1\alpha}$. The assay procedures [8] and the specificity of the antibody [9] have been described previously. The drugs in the concentrations used did not affect the RIA of 6-oxo-$PGF_{1\alpha}$.

Arachidonic acid (Sigma) was dissolved in ethanol and stored under nitrogen at −20°. After evaporating the ethanol with a stream of nitrogen, the residue was taken up in 0.9% w/v NaCl solution and converted to the sodium salt of AA with Na_2CO_3. This aqueous solution was freshly made for each day's experiments. Prostaglandin E_2 (Sigma) was stored in methanol solution at −20°. Methanol was evaporated and the PGE_2 redissolved in 0.9% w/v NaCl solution and diluted further to the desired concentration.

Radioactive 6-oxo-$PGF_{1\alpha}$ (5, 8, 9, 11, 12, 14, 15 (n) ^{3}H-6-oxo-$PGF_{1\alpha}$; 100 Ci/mmole) was obtained from New England Nuclear (Boston, MA). The calcuim ionophore was a gift from Dr W. Dawson (Lilly Research Centre, Windlesham, Surrey). We are also grateful for gifts of indomethacin (Merck Sharp & Dohme, Hoddesdon, Herts) sulphinpyrazone (Ciba-Geigy, Horsham), dipyridamole (Boehringer-Ingelheim, U.K.) and nafazatrom (Bayer, U.K.). Bromcresol green was obtained from BDH Chemicals Ltd. (Poole). The antiserum to 6-oxo-$PGF_{1\alpha}$ was a generous gift from Dr J. A. Salmon (Wellcome Foundation, Beckenham, Kent).

Results are given as mean values (± SEM) from N lungs unless otherwise stated. In most experiments, except those using the calcium ionophore, each lung provided its own control value before drug infusion was started and those results have been analysed with a paired *t*-test. With ionophore, only one injection was given to each lung and thus control and drug treated values were obtained from different lungs. Here the unpaired *t*-test was used to assess significance of the difference between means. A value of $P < 0.05$ was taken to denote a significant difference.

Results

Bioassays. The concentrations of the drugs used in these experiments were chosen by preliminary studies of their ability to inhibit pulmonary inactivation of exogenous PGE_2 in either rat or guinea-pig lung. The values selected (bromcresol green, 10 μM; dipyridamole, 10 μM; nafazatrom, 37 μM and sulphinpyrazone, 20 μM) all gave good inhibition in either lung preparation (Table 1). In guinea-pig lung the control inactivation of PGE_2 was 98 ± 1% (N = 20) and dipyridamole decreased this to 67% of the normal value, i.e. to about 65%. This means that survival of PGE_2 rose from 2% to 35%, a 17-fold increase in biological activity. Inactivation in control rat lungs was 95 ± 1% (N = 18) and all the drugs gave easily measurable increases in survival, in excess of 4-fold.

The COP synthesized from exogenous AA were bioassayed on the rat stomach strip. This tissue responds to most of the PGs and TxA_2 with a contraction and provides an overall estimate of the formation of biologically active

Table 1. Effects of drug treatment on COP metabolism in isolated lungs

	Inactivation of COP	Synthesis of COP		
		Exogenous AA		Endogenous AA
	Exogenous PGE_2 inactivation (bioassay)	COP output (bioassay)	6-oxo-$PGF_{1\alpha}$ output (RIA)	6-oxo-$PGF_{1\alpha}$ output (RIA)
Guinea-pig lung				
Bromcresol green (10 μM)	65 ± 6*	58 ± 6*	103 ± 4	194 ± 32*
Dipyridamole (10 μM)	67 ± 6*	116 ± 8	67 ± 20	413 ± 114*
Nafazatrom (37 μM)	66 ± 10*	36 ± 6*	31 ± 7*	302 ± 77*
Sulphinpyrazone (20 μM)	85 ± 3*	21 ± 10*	21 ± 4*	508 ± 184*
Rat lung				
Bromcresol green (10 μM)	79 ± 2*	115 ± 17	—	—
Dipyridamole (10 μM)	81 ± 7*	83 ± 8	136 ± 46	405 ± 91*
Nafazatrom (37 μM)	67 ± 3*	95 ± 9	157 ± 67	651 ± 150*
Sulphinpyrazone (20 μM)	82 ± 6*	65 ± 13*	—	—

* Significantly different from control; $P < 0.05$.

Results are expressed as a percentage of control, i.e. drug-free, experiments. The values in the Table are the mean results (± SEM) from 4 or 5 lungs for each drug.

COP. With guinea-pig lung, the output of bioassayable COP was decreased by all the drugs except dipyridamole (Table 1) and sulphinpyrazone was the most potent inhibitor of COP synthesis. The results with rat lung were different. Relatively less COP were formed than in guinea-pig lung before any drug treatment (1.8 ± 0.2 and 12 ± 1 ng PGE_2 equiv./μg AA respectively) and only one drug, sulphinpyrazone, showed a clear inhibition (Table 1).

Radioimmunoassay of 6-oxo-$PGF_{1\alpha}$. Two of the drugs used here, dipyridamole and nafazatrom, have been reported to increase PGI_2 production in other systems [10, 11]. We therefore assessed the effects of the drugs on PGI_2 production in lung, by RIA of 6-oxo-$PGF_{1\alpha}$ in lung effluent. The basal (i.e. in the absence of exogenous AA) concentrations of 6-oxo-$PGF_{1\alpha}$ were below the detection limit of our assay (0.2 ng/ml) and did not exceed that limit during treatment of the lungs with any drug.

In guinea pig lung, there was no change in 6-oxo-$PGF_{1\alpha}$ output from exogenous AA in the presence of dipyridamole and bromcresol green but, with nafazatrom and sulphinpyrazone, the output of 6-oxo-$PGF_{1\alpha}$ fell to about 25% of the untreated value (27 ± 7 ng/ml, N = 20). The output of 6-oxo-$PGF_{1\alpha}$ from endogenous AA stimulated by the calcium ionophore A23187 in control lungs (30.4 ± 6.9 ng/ml, N = 9) was markedly increased by all four drugs.

The results obtained in rat lung (Table 1) with dipyridamole and nafazatrom were slightly different. From exogenous AA, output of 6-oxo-$PGF_{1\alpha}$ (3.8 ± 0.6 ng/ml, N = 9) was unchanged but from endogenous AA under ionophore stimulation, output was greatly increased (4- to 6-fold) over the control levels (0.6 ± 0.1 ng/ml, N = 9).

Discussion

In these experiments we have shown that the effects of the drugs on PGI_2 synthesis in isolated lung depended on the source of AA used as substrate.

In guinea-pig lung, all the drugs increased PGI_2 output (measured as 6-oxo-$PGF_{1\alpha}$) from endogenous AA but they did not increase output from exogenous AA perfused through the lungs. Indeed, two drugs, sulphinpyrazone and nafazatrom, inhibited PGI_2 output from exogenous AA and in the bioassay bromcresol green also inhibited output of bioassayable PGs. The drugs used here have pharmacological activities other than inhibiting PG inactivation. For instance, dipyridamole decreases adenosine uptake by lung [12], sulphinpyrazone inhibits cyclooxygenase activity [13] and nafazatrom blocks 5-lipoxygenase in neutrophils [14]. It is possible that, for any one drug at a single concentration, the combination of activities might produce the difference in effect we report here. However, the fact that all four drugs with such diverse activities identified the same difference between endogenous and exogenous AA and that this difference was observed in the lungs of two different species would indicate that the difference in action on synthesis from exogenous and endogenous AA is a real phenomenon.

The mechanism by which PGI_2 output was increased is not obvious. The other activities exhibited by these drugs, already discussed above, do not provide any common feature to explain our findings. Direct stimulation of PGI_2 synthesis has been demonstrated for nafazatrom [15] and dipyridamole [10, 11]. For nafazatrom, this stimulation has been attributed to an increased conversion of PGH_2 to PGI_2 and thus should be equally observed with exogenous or endogenous substrate. Inhibition of lung uptake of exogenous AA is another possible explanation of the difference between exogenous and endogenous substrate. However, bromcresol green increased lung uptake of ^{14}C-AA [16], whereas dipyridamole decreased uptake [17]. In the latter paper, dipyridamole also did not affect 6-oxo-$PGF_{1\alpha}$ output from exogenous AA but output from endogenous substrate was increased by about 50%.

Another possible explanation is the inhibition of PGE_2 inactivation that we have demonstrated here. Exogenous PGI_2 is not inactivated in the pulmonary circulation [18, 19] but PGI_2 formed in the lung from *endogenous* substrate is metabolized and 6,15-dioxo-$PGF_{1\alpha}$ derivatives are found in these conditions [4, 20, 21]. Thus inhibitors of PGDH (nafazatrom; [22]) or of PG uptake (bromcresol green, dipyridamole and sulphinpyrazone; [23, 24]) could decrease the *in situ* metabolism of PGI_2 and increase the amount of 6-oxo-$PGF_{1\alpha}$ in lung effluent. However, this explanation should hold for PGI_2 synthesis *in situ* from either exogenous or endogenous substrate.

Thus we can offer no satisfactory explanation of the results reported here. Perhaps the use of more specific inhibitors of PGDH [25] might help to elucidate the mechanism underlying the divergences in the effects of drugs on the metabolism of exogenous and endogenous AA in lung.

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Unresponsiveness of female rat hepatic monooxygenases to physiological levels of testosterone

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Biotransformation of a wide variety of xenobiotics and endogenous compounds is accomplished by a class of microsomal hemoproteins known as the cytochrome P-450s [1, 2]. A copious body of compelling evidence is now available demonstrating the existence of sex differences in the hepatic metabolism of drugs in rats [1, 3, 4]. In general, males exhibit higher rates of metabolic conversion of various substrates including hexobarbital, aminopyrine and ethylmorphine than do females [1, 3, 4]. This sex difference in metabolism has been attributed to the stimulatory effects of androgen, since orchidectomy increases the plasma half-life of these compounds with a concomitant decrease in the activities of hepatic monooxygenases, whereas testosterone treatment results in a reversal of this castration effect [1, 3, 4]. In a similar vein, past reports have shown that androgen exposure in the neonatal period is also important, imprinting a basic male pattern of metabolism and an ability to respond to androgens later in life [4, 5]. In contrast, neonatal or adult ovariectomy has little effect on the hepatic monooxygenases, indicating that estrogen is not necessary for the imprinting or maintenance of the female type of drug metabolism [3–5].

Unfortunately, most studies of androgenic regulation of drug metabolism have used suprapharmacological doses of androgens and, thus, any sex-related differences in the responsiveness of hepatic monooxygenases to physiological levels of these hormones are unknown. In the present study we have examined the effects of a physiological dose of testosterone on the hepatic monooxygenase system of gonadectomized male and female rats.

Materials and methods

Animals. Three-month-old male and female Sprague–Dawley rats [Crl:CD(SD)BR, Charles River Breeding Laboratories, Inc., Wilmington, MA] were maintained under standardized conditions of light (7:00 a.m.–7:00 p.m.) and temperature (20–23°) on a diet of laboratory chow and water. Animals were housed 2–3 to a cage on hardwood bedding chips.

Treatments. Male rats were castrated through scrotal incisions and female rats were ovariectomized by a dorsal paralumbar approach [6]. All control animals were sham operated. Two weeks after surgery, half of the gonadectomized animals were injected subcutaneously with testosterone propionate (TP, 0.20 mg/kg daily), while the remaining castrates and all of the sham-operated rats received an equivalent amount of the corn oil vehicle for the 45-day treatment period. Rats were rapidly killed by decapitation between 9:00 and 10:00 a.m. on the day following the last injection.

Livers were perfused with ice-cold 0.9% saline and then removed, trimmed, weighed, minced into small pieces, and homogenized in 2 vol. of cold 0.25 M sucrose containing 50 mM Tris–HCl (pH 7.4). The homogenate was treated as described previously [7].

Hexobarbital hydroxylase activity was determined by our modification [6] of the radioenzyme procedure of Kupfer and Rosenfeld [8]. The assay measures the rate of microsomal conversion of radioactive hexobarbital, 5-[2-^{14}C]cyclohexenyl-3,5-dimethylbarbituric acid (14.4 mCi/mmol; NEN Research Products, Boston, MA), to 3-hydroxyhexobarbital. Linear kinetic data about the enzyme were obtained using ten different hexobarbital concentrations (0.035 to 0.4 mM). Apparent Michaelis constants (K_m) and maximal velocities (V_{max}) were obtained from linear regression models of the data using the method of Hofstee [9]. The correlation coefficients for all Hofstee plots were positive, exceeded 0.95, and were statistically significant ($P < 0.05$).

Total cytochrome P-450 was quantified by measuring the carbon monoxide difference spectra after reduction with dithionite [10]. Cytochrome b_5 and total microsomal heme were measured according to the procedures of Omura and Sato [10] and Falk [11] respectively. Microsomal protein was determined by the method of Lowry *et al.* [12] with bovine serum albumin as the standard.

Quantitation of serum testosterone levels was carried out by our previously reported method [13] using a specific antiserum obtained from Radioassay Systems Laboratories, Inc. (Carson City, CA).

Experimental groups were compared by analysis of variance and Student's *t*-test.

Results and discussion

The prolonged period of orchidectomy produced expected [1, 3, 14] but profound decreases in ventral prostate and seminal vesicle weights and concentrations of serum testosterone (Table 1), as well as declines to female-like levels of hexobarbital hydroxylase, cytochromes P-450 and b_5, and heme (Table 2). Daily administration of 200 μg/kg of testosterone increased the serum concentration of the hormone in the orchidectomized rats to intact values. Concomitantly, the restoration of physiological levels of serum testosterone increased the weights of the ventral prostate and seminal vesicles and the concentrations of hepatic hexobarbital hydroxylase, cytochromes P-450 and b_5, and heme to the pre-castration levels. As expected [1, 3, 15], gonadectomy in the female rats produced no alterations in the activities of the hepatic monooxygenases and the concentrations of hepatic heme (Table 2). In contrast to the male, daily treatment with 200 μg/kg of testosterone had no effect on the levels of hepatic hexobarbital hydroxylase, cytochromes P-450 and b_5, and heme, in spite of the fact that the serum concentration of testosterone was increased to male-like values (Table 1).

Our data concerning sex differences in cytochrome P-450 and hexobarbital hydroxylase are in agreement with previously reported results [1, 3]. It is noteworthy, however, that the hepatic monooxygenase system in the gonadectomized female rats was unresponsive to the action