The effect of dexamethasone on the activity of 11β-hydroxysteroid dehydrogenase in the foetal rabbit lung during the final stage of gestation

MOIRA A. LUGG, TERENCE E. NICHOLAS*, *Department of Human Physiology, School of Medicine, The Flinders University of South Australia, Bedford Park, South Australia*, 5042

The observation of Liggins (1969) that glucocorticoid administration accelerates lung maturation in the sheep foetus has since been repeated in other species including the rabbit (Kotas & Avery, 1971), monkey (Delemos & McLaughlin, 1973) and man (Liggins & Howie, 1972). The glucocorticoids act to increase the amount of pulmonary surfactant on the alveolar surface and hence the stability of the alveoli (Kotas, Fletcher & others, 1971). Farrell & Zachman (1973) concluded that this action of glucocorticoids was via induction of choline phosphotransferase, a key enzyme in the principal pathway of synthesis of surfactant-type phosphatidylcholine in lung. Oldenberg & Van Golde (1977) recently confirmed this work and found in addition that these drugs also enhanced the activity of lysophosphatidylcholine acetyltransferase. Research emphasis has been on the ability of glucocorticoids to induce enzymes involved in pulmonary phospholipid synthesis, to the neglect of possible effects on other enzymes. One such enzyme is 11β-hydroxysteroid dehydrogenase (11β-HSD), which interconverts cortisol and cortisone. Previously we have reported that the isolated perfused lung of the adult rat rapidly reduces the inactive steroids cortisone and 11-dehydrocorticosterone to the active glucocorticoids cortisol and corticosterone respectively, while little activity is observed in the reverse direction (Kim & Nicholas, 1975; Nicholas & Kim, 1975). This ability is also present in the foetal rabbit lung and increases rapidly during the last five days of gestation (Nicholas, Johnson & others, 1976; Torday, Olson & First, 1976). The possibility that glucocorticoids induce 11β-HSD was suggested by Smith, Torday & Giroud, (1973), who found that cortisol induced the enzyme in human foetal lung cells taken at mid-gestation and maintained in culture.

In the present communication we have tested the hypothesis that glucocorticoids induce 11β-HSD in the foetal rabbit lung *in vivo*. In these experiments we have used dexamethasone (9α-fluoro-16α-methylprednisolone), a very potent glucocorticoid, and one which is used to accelerate foetal lung maturation in the human (Ballard & Ballard, 1976).

The following experiments were performed on English shorthair rabbits. Initial mating was taken as zero time of gestation. On the day of injection the doe was anaesthetized (sodium pentobarbitone: 30 mg kg^{-1}, i.v.), the uterus was exposed by laparotomy and the foetuses in one horn were injected intramuscularly, through the wall of the uterus, with dexamethasone. The foetuses in the other horn were injected with an equivalent volume of saline (25 μl). Two days later the doe was again anaesthetized and the foetuses delivered by caesarian section. Breathing was prevented by leaving the membranes around the head of the foetus. The foetal lungs were rapidly excised and kept at 4°. Eighty to 150 mg portions were accurately weighed and then minced at 4° in 25 ml Erlenmeyer flasks.

Incubation with [^{14}C]*cortisone.* The tissue was incubated for 30 min in Krebs bicarbonate solution (pH 7·4) containing 4·5% albumin and [^{14}C]cortisone (100 mg of tissue per 5 ml medium) and equilibrated with 5% CO_2 in oxygen at 37°. The flasks were shaken at 100 times min^{-1}. The incubation was terminated by placing the flasks in ice and approximately 3×10^5 d min^{-1} of [^{3}H]cortisol was added as a tracer to determine recovery. The medium and tissue were then homogenized with an Ultra Turrax homogenizer and extracted three times with 10 ml of ethyl acetate. The pooled organic phase was then evaporated to dryness in a Buchler vortex evaporator, reconstituted in 200 μl of ethanol and 10 μl were spotted onto a silica gel thin layer chromatography plate (Macherey Nagel-SILG-25/UV 254) and developed as previously described (Nicholas & Kim, 1975). The spot corresponding to cortisol was scraped into a mini-counting vial, scintillation fluid added and the vial counted in a Searle Mark III Beta Scintillation Counter (No. 6880). The identity of cortisol was checked as follows: the steroids in the sample were converted to their respective acetates (Nicholas & Kim, 1975), rechromatographed and the ^3H : ^{14}C ratio determined in the cortisol acetate spot. In addition, an aliquot of original sample was chromatographed using the following solvent system: dichloromethane–methanol–water–glycerol (150 : 10 : 1 : 0·4), and again the ^3H : ^{14}C ratio determined in the cortisol spot. A constant ratio throughout these procedures indicated that the ^{14}C-metabolite was cortisol.

Solutions and chemicals. [4-^{14}C]Cortisone (specific activity 58 mCi $mmol^{-1}$) and [1,2,6,7(n) ^{3}H]cortisol (specific activity 38 Ci $mmol^{-1}$) were obtained from the Radiochemical Centre, Amersham. Dexamethasone sodium phosphate was obtained from Charles E. Frosst (Australia). All other chemicals and solutions used were obtained or prepared as previously reported (Nicholas & Kim, 1975).

In the first series of experiments, foetuses were injected on day 25 of gestation and on day 27 the lungs were incubated with 0·1 mM [^{14}C]cortisone. This concentration of cortisone is four times the Km for 11β-HSD in rat liver (Bush, 1969). Initially we injected a dose of 10 μg of dexamethasone, as Ballard & Ballard (1976) have suggested that most previous studies used doses of

* Correspondence.

glucocorticoid greatly in excess of that required for acceleration of lung maturation. Table 1 shows that no difference was found in the rate of reduction of cortisone to cortisol in the lungs of foetuses injected with dexamethasone and those injected with saline. We then increased the dose of dexamethasone to 50 μg, similar to that used by Farrell & Zachman (1973), and again found no difference compared to the controls in the rate of reduction of cortisone to cortisol.

In a second series of experiments we investigated whether dexamethasone might have an effect only at a critical time of gestation. We injected foetuses of 22, 23, 25 and 28 days of gestation with either 50 μg of dexamethasone or an equivalent volume of saline, and two days later removed the lungs and incubated them in a medium containing 0·2 μM [^{14}C]cortisone. The use of this lower concentration of cortisone avoided any possibility of substrate inhibition. Previously we had established that at 0·2 μM cortisone the substrate does not become a limiting factor during a 30 min incubation with lung tissue from either a day 30 rabbit foetus or an adult rabbit. Table 2 shows that the ability of the foetal lung to reduce cortisone to cortisol increased from day 24 to day 30, however, at no stage did dexamethasone enhance this ability.

There is abundant evidence that exogenous glucocorticoids stimulate pulmonary surfactant production and so increase the rate of maturation of the foetal lung. Furthermore, evidence from Murphy (1974) suggests that endogenous glucocorticoids may also be important in lung maturation. Therefore the presence in the foetal lung of an enzyme which converts cortisone to cortisol is of great potential importance in lung maturation, particularly in light of the fact that foetal plasma contains high concentrations of cortisone throughout gestation (Murphy & Diez d'Aux, 1972). Our present results confirm the previous finding that the rabbit foetal lung has the ability to reduce cortisone to cortisol which increases during the final third of gestation (Nicholas & others, 1976). Our results do not confirm those of Smith & others (1973) that corticoids induce 11β-HSD in foetal lung, however, the following differences in design must be considered. First, we used a different species. Second, we attempted to induce an enzyme *in vivo* rather than in a cultured cell preparation. The latter must be viewed with some reservation following the report of Mason, Dobbs & others (1977) that alveolar type II cells after one day in culture show differences in structure, in total amount of DNA and in the phospholipid composition when compared with freshly isolated type II cells. Third, although we chose dexamethasone rather than cortisol because the former is more potent, the ability of a corticoid to induce

Table 1. *Foetuses were injected on day 25 of gestation with dexamethasone (10 or 50 μg) or saline.* Two days later the lungs were incubated in a medium containing 0·1 mM [^{14}C]cortisone, and the cortisol produced was measured.

Dexamethasone (μg)	Production of cortisol	
	Dexamethasone	Saline
10	73·6 ± 1·57 (16)	76·9 ± 2·25 (20)
50	76·0 ± 3·26 (14)	75·8 ± 2·18 (10)

Each value represents the mean ± s.e.m. expressed as nmol g^{-1} h^{-1}. The number of incubations is indicated in parentheses.

Table 2. *Foetuses at different stages of gestation were injected with dexamethasone (50 μg) or saline.* Two days later the lungs were incubated in a medium containing 0·2 μM [^{14}C]cortisone and the cortisol produced was measured.

Day of incubation	Production of cortisol	
	Dexamethasone	Saline
24	0·60 ± 0·05 (6)	0·55 ± 0·08 (6)
25	0·99 ± 0·05 (19)	0·94 ± 0·05 (13)
27	1·20 ± 0·04 (16)	1·22 ± 0·08 (12)
30	1·80 ± 0·08 (10)	1·68 ± 0·16 (6)

Each value represents the mean ± s.e.m. expressed as nmol g^{-1} h^{-1}. The number of incubations is indicated in parentheses.

11β-HSD may not be a function of its glucocorticoid activity. Finally, although we took care in the present experiments not to disturb the foetuses unduly during the injection, it is possible that the stress of the manoeuvre increased endogenous concentrations of corticoids following both saline and dexamethasone injection. This may have disguised any difference between test and control animals (Rooney, Gobran & others, 1976). We feel that this is unlikely for the values of cortisone to cortisol conversion in the present experiments are similar to those we found previously in foetuses which had not been injected (Nicholas & others, 1976).

In conclusion, dexamethasone administered directly to the rabbit foetus *in utero* does not alter the activity of 11β-HSD.

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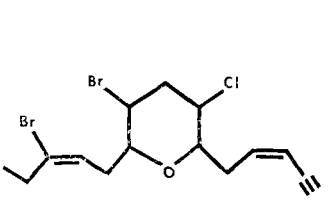
Novel substances of marine origin as drug metabolism inhibitors

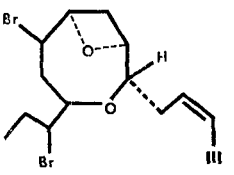
PUSHKAR N. KAUL*, SHRINIVAS K. KULKARNI, ETSURO KUROSAWA†, *The University of Oklahoma Health Sciences Center, College of Pharmacy, Oklahoma City, Oklahoma, U.S.A. and †Department of Chemistry, Hokkaido University, Sapporo, Japan*

Several compounds and fractions from the extracts of marine invertebrates have been found to possess cardiovascular, central nervous system and anticancer activities (Kaul, 1972; Kaul, Kulkarni & others, 1977; Schmitz, Campbell & others, 1977). The extracts of one of the marine organisms, *Aplysia dactylomela*, commonly known as sea hare, potentiated the pentobarbitone induced sleep-time in mice. The bioassay guided fractionation of this extract led to the isolation of dactylyne (I), a dibromochloro cyclic ether which was the active principle responsible for the potentiation (Kaul, Kulkarni & others, 1978). Several other halogenated cyclic ethers (II–V) have also been found in the algae of *Laurencia* genus (Irie, Suzuki, Masamune, 1968; Irie, Izawa & Kurosawa, 1970; Fukuzawa, Kurosawa & Irie, 1972). We now report that laureatin (II), isolaureatin (III) and laurefucin (IV) isolated from the red alga *Laurencia nipponica* and laurencin (V) from *L. glandulifera* also prolong the pentobarbitone

* Correspondence.



Dactylyne
(I)



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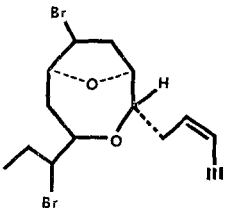
(II)



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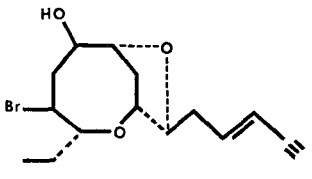
(III)



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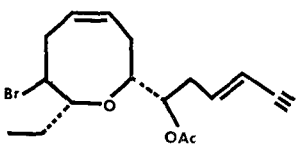
(IV)



Laurencin
(V)