J. steroid Biochem. Vol. 17, pp. 113 to 118, 1982
Printed in Great Britain.

0022-4731/82/010113-06$03.00/0

THE PHYSIOLOGICAL SIGNIFICANCE OF 11β-HYDROXYSTEROID DEHYDROGENASE IN THE RAT LUNG

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(*Received* 21 *September* 1981)

SUMMARY

We have examined the interconversion of cortisone (E) and cortisol (F) in rat lung homogenate and microsomal fraction and in the isolated rat lung perfused with Krebs bicarbonate solution containing 4.5% albumin. In the perfused lung the apparent K_m was 5.1 μM E and the V_{max} was 9 nmol·g^{-1}·min^{-1}. The ability of the lung to reduce E to F was enhanced both by 7 days prior exposure of the rat to an ambient temperature of 2°C and by starvation of the rat for 3 days. The activity was inhibited by adrenalectomy and castration of 7 days duration. Whereas little steroid oxidation occurred in the perfused lung, preparations of lung homogenates and microsomal fraction readily reduced or oxidised the 11-position of the corticoid molecule depending on the preponderance of either NADPH or NADP, respectively. We conclude, that the predominance of the reductive reaction in the whole rat lung under physiological conditions reflects the very active pentose-phosphate shunt in the lung, which produces NADPH. We suggest that this ability of the lung to activate E to F may exert a fine control over the arterial concentrtion of unbound, physiologically active, 11-hydroxylated steroid.

INTRODUCTION

11β-hydroxysteroid dehydrogenase (11β-HSD), the enzyme which inter-converts cortisone (E) and cortisol (F), is present in the lungs of the adult rat [1, 2], adult rabbit [3], fetal rabbit [3, 4] and the human fetus [5, 6]. In the isolated perfused rat lung (IPL) this enzyme readily reduces E to F and appears to dictate the F:E ratio in the lung tissue [2], moreover, because of the prominent position of the lung in the vascular system, the activity of 11β-HSD in lung may significantly influence the F:E ratio in the arterial blood. Therefore, as part of our investigation of the physiological role of 11β-HSD in the lung, we have examined whether the activity of the enzyme is altered in situations which are known to influence the plasma levels of corticosteroids. Specifically, we have examined the response to cold stress and starvation. In addition, we have measured activity in lungs following castration and adrenalectomy, when the levels of substrates should be zero.

As pointed out by Bush[7], most studies have been carried out in the presence of added cofactors, and depending on whether the oxidised or reduced form of the cofactor is used, the reaction can be forced in either the reductive or oxidative direction. The 11β-HSD enzyme has not been isolated in pure form, and we do not know whether there are one or two enzymes involved. In the present investigation we have partially characterised pulmonary 11β-HSD, and examined the role of the cofactors.

METHODS

Perfusion methods were similar to those previously described [8]. Briefly, male Porton rats (180–250 g) were anaesthetised with intraperitoneal sodium methohexitone [Eli Lilly (Australia) and Co.] 50 mg·kg^{-1}, tracheostomised and ventilated with 5% CO_2 in oxygen (tidal volume of 2.5 ml, 60 breaths·min^{-1} and end expired pressure of 2 cm H_2O). The thorax was then opened and cannulae were placed in the pulmonary artery via the right ventricle and in the left atrium. Without interrupting the circulation, the lungs were perfused at 10 ml·min^{-1} with Krebs bicarbonate solution containing 4.5% albumin via a Holter pump (model RL175-110, Extracorporeal Medical Specialities, Inc. PA). Finally, the lungs were removed from the thorax and placed in a closed chamber saturated with water vapour at 37°C. Perfusion and ventilation pressures were continuously monitored with two Statham transducers (model P23Dc) connected to a Grass Model 7C Polygraph (Grass Instruments, Quincy MA). In all cases the lungs were perfused for a 10 min period with a medium containing no steroid; the medium containing steroid was then introduced from a separate reservoir and the perfusion continued for the predetermined time. All perfusions involved

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The following trivial steroid names and abbreviations are used: cortisone (E), 17,21-dihydroxy-4-pregnene-3,11,20-trione; cortisol (F), 11β,17,21-trihydroxy-4-pregnene-3,20-dione; 11-ketoprogesterone, 4-pregnene-3,11,20-trione.

the recirculation of 25 ml of medium through a reservoir, which was stirred vigorously. In the perfusions designed to characterise 11β-HSD, the medium contained various concentrations of E ranging from 0.11 to 98.6 μM; with concentrations above 0.11 μM, the labeled steroid was supplemented with unlabeled steroid. Each perfusion was run for a period of 10 min at which time the inflow tube was removed from the reservoir and the circuit allowed to run dry. The lung and medium were immediately placed on ice and the wet weight of the tissue determined. The tissue and medium were then extracted as described below. The amount of [^{14}C]-F produced was determined by removing the F spot from the TLC plate and eluting as described; the amount was corrected for recovery and the total amount of F in both lung tissue and medium was calculated. In perfusions in which we examined the effect of stress on the activity of 11β-HSD, the reservoir contained 0.1 μM [4-^{14}C]-cortisone and was recirculated through the lungs for 570 seconds; 500 μl aliquots were taken at the completion of 1, 2 and 4 cycles. At the completion of the perfusion, the circuit was again allowed to run dry and the wet weight of the lung lobes was determined. In these experiments only the amount of F appearing in the medium was measured. Duplicate 50 μl samples of the 500 μl aliquots of medium were counted: the remainder was extracted for steroids and these in turn were separated by TLC. The percentage of radioactivity in the F spot was determined and the number of moles calculated. The dry weight of the lung lobes was determined after freeze-drying. The absence of edema was monitored by the wet weight-as-to-dry weight ratio and the lungs with a ratio of greater than 5.5 were discarded (control lungs: 4.81 ± 0.55, mean ± SD of 121 lungs).

Lung homogenates

These were prepared as follows. The rat was killed by a sharp blow on the head and the lungs were rapidly removed and placed in 0.1 M phosphate buffer at pH 7.4 and 2°C. The major bronchi and vessels were dissected away and the remaining tissue was blotted dry and weighed. The lung was then homogenised in the buffer (1 g per 10 ml) using the TP10 shaft on an Ultra Turrax homogeniser (model TP18-10).

Microsomal fraction

This was prepared by standard methods [1]. The actual incubations were carried out in 15 ml glass tubes under aerobic conditions in a shaking water bath at 37°C. The substrate plus cofactor were placed in the tube in 0.75 ml of 0.1 M phosphate buffer at pH 7.4, then 0.25 ml of either the homogenate or microsomal fraction was added and the reaction started by placing the tube in the water bath. The reaction was stopped by placing the tube in ice and 27,000 d.p.m. of the relevant triated-steroid was added as a tracer for recovery.

Extraction procedures

The tissue was homogenised with the Ultra Turrax and then extracted three times with 10 ml of ethyl acetate. The pooled organic phase was then evaporated to dryness in a Buchler Vortex Evaporator (Searle Analytic Inc., NJ), reconstituted in 200 μl of ethanol and 10 μl were spotted on a silica gel thin layer plate (Macherey-Nagel-SILG-25/UV 254) and developed as previously described [2]. The spot corresponding to cortisol was then scraped into a mini-counting vial, together with 5 ml of scintillant (7 g of 2,5 diphenyloxazole, 300 ml of ethoxyethanol up to a liter with xylene) for counting in a Searle Mark III Beta Scintillation Counter (model 6880). The recovery from 250 extractions was 92.1 ± 7.1% mean ± SD. The identity of cortisol was checked as follows: the steroids in the sample were converted to their respective acetates [2], rechromatographed, and the ^{3}H:^{14}C ratio determined in the cortisol acetate spot. In addition, an aliquot of the original sample was chromatographed using the following solvent system: dichloromethane–methanol–water–glycerol (150:10:1:0.4, by vol.) and again the ^{3}H:^{14}C ratio determined in the cortisol spot. A constant ratio throughout these procedures indicated that the ^{14}C-metabolite was cortisol. Finally, the identity of E and F were verified by mass spectrometry [9]. Rat lungs were perfused for 72 min with a medium containing 50 μM unlabeled E, and the total vol. of medium was then extracted as above. The spots corresponding to E and F were scraped from the plate, eluted with dichloromethane–methanol (9:1, v/v), evaporated to dryness under nitrogen and reconstituted in ether for mass spectrometry. The mass spectra for the E and F extracted from the medium were identical to those obtained by directly spotting the E and F obtained from Sigma Chemical Company (St. Louis, MO), running the plate and scraping as for the test compounds (spectra not shown). The protein content of the microsomal fraction and homogenate was assayed by the method of Lowry[10].

Adrenalectomy and Castration

These were carried out by standard surgical techniques in rats anaesthetised with intraperitoneal sodium methohexitone. The rats were left for 7 days and maintained on 1% NaCl solution before the lungs were isolated.

Cold-stress rats

These were maintained for 7 days at 2°C, each rat in a separate cage. Rats were starved by removing all pellets for a 3 day period and allowing water *ad libitum*.

Solutions and chemicals

[4-^{14}C]-Cortisone (SA 58mCi mmol^{-1}) and [1.2.6.7(n)^{3}H]-cortisol (SA 81 Ci mmol^{-1}) were obtained from the Radiochemical Centre (Amersham,

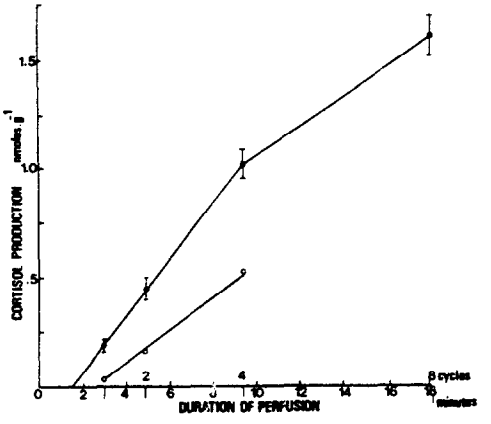

Fig. 1. Isolated rat lungs were recirculated at 10 ml·min^{-1} with Krebs bicarbonate solution containing 4.5% albumin and 0.1 μM [^{14}C]-cortisone. Following 1, 2, 4 and 8 cycles of the 25 ml reservoir through the pulmonary vascular bed, the reservoir was sampled and the amount of cortisol determined. The cortisol produced is expressed as nmol·g of wet lung^{-1}. The solid circles represent the mean ± SEM of 7 lung perfusions. The open circles represent the means of 2 lungs perfused under the same conditions, but in the presence 5 μM of the inhibitor 11-keto-progesterone. In both groups the production of cortisol was linear up until at least 4 cycles. The curves do not pass through the origin because of the deadspace of the circuit.

England). All other chemicals were purchased from the Sigma Chemical Company.

RESULTS

Recirculating a 25 ml reservoir containing 0.1 μM [^{14}C]-E through the isolated lungs resulted in progressively greater amounts of [^{14}C]-F in the reservoir (Fig. 1). This increase was linear with time up to 570 s after the introduction of the reservoir containing the radio-labeled steroid. After this, the rate of production of F decreased with the substrate depletion. 570 seconds is equivalent to 4 complete cycles of the reservoir through the lungs, at which stage 39.7 ± 3.4% (mean ± SD of 7 perfusions) of the [^{14}C]-E was converted to [^{14}C]-F. In the presence of 5 μM 11-keto-progesterone, the rate of reduction of E to F was greatly depressed. Although only two perfusions were performed in the presence of this inhibitor, the results were identical, and are also illustrated in Fig. 1.

Effect of stress on 11β-HSD activity in lung

In these experiments we exposed the rats to the stress, then the lungs were isolated and recirculated with a medium containing 0.1 μM [^{14}C]-E for a period of 570 s. Following 1, 2 and 4 cycles of the reservoir through the lungs, a 500 μl sample was taken from the reservoir and the amount of [^{14}C]-F determined. We took the mean of the rate of production of F after 2 and 4 cycles and expressed the result as nmol·g dry lung^{-1}·min^{-1}. Following 3 days starvation there was a significant increase in the rate of

Table 1. The effect of stress on the ability of the lung to reduce cortisone to cortisol

	Production of [^{14}C]-cortisol	N
Control	0.52 ± 0.067	8
Starvation	0.66 ± 0.056**	5
Ambient temperature 2 C	0.65 ± 0.077*	5
Adrenalectomy + castration	0.39 ± 0.064**	5

Results expressed as mean ± SD in nmol·min^{-1}·g dry lung^{-1}.

*$P < 0.05$, **$P < 0.025$. N is the number of lungs.

Rats were stressed by one of the following: starvation for 3 days, exposure to an ambient temperature of 2°C for 7 days, or castraton and adrenalectomy for 7 days. The lungs were then isolated, perfused at 10 l·min^{-1} with Krebs bicarbonate solution containing 4.5% albumin and 0.1 μM [^{14}C]-cortisone for 10 min. Samples of the recirculating perfusate were taken after 1, 2, and 4 cycles of the total initial reservoir through the pulmonary circulation, and the amount of [^{14}C]-cortisol was determined.

reduction of E to F (Table 1). Likewise, following 7 days at 2°C there was a significant increase in activity of 11β-HSD. Finally, lungs isolated from rats that had been adrenalectomised and castrated 7 days previously demonstrated a significantly lower 11β-HSD activity than lungs from control rats. As there was no difference between 11β-HSD activity in unoperated control rats and those that had been sham operated for adrenalectomy and castration, we have combined these results.

Characterisation of 11β-HSD in the isolated perfused rat lung

We perfused lungs for 10 min with concentrations of [^{14}C]-E varying from 0.11 to 98.6 μM and determined the actual amount of [^{14}C]-F produced by extracting both the medium and the lung tissue. Figure 2 is a Lineweaver–Burk plot of the results

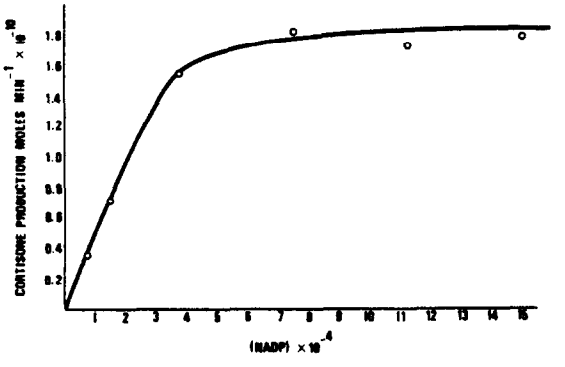

Fig. 2. A Lineweaver–Burk plot of the production of cortisol by the isolated perfused rat lung during 10 min perfusions with concentrations of [^{14}C]-cortisone varying from 0.11 to 98.6 μM. The lungs were perfused as in Fig. 1. The total amount of cortisol produced in both the lung tissue and the medium was then determined and expressed as nmol·g wet lung^{-1} min^{-1}. Each point represents the mean of 4 lungs.

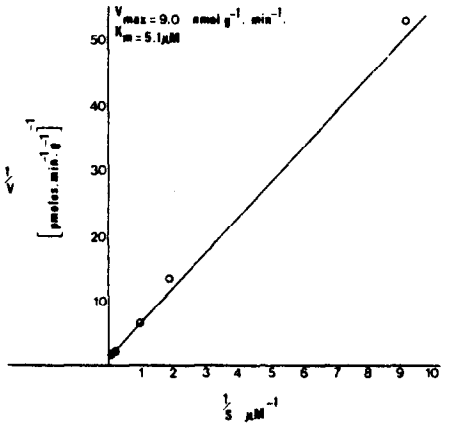

Fig. 3. The lung microsomal fraction was incubated for 30 min at 37°C under aerobic conditions with 0.138 mM [^{14}C]-cortisol and varying concentrations of NADP. The amount of cortisone produced was determined. Each point represents the mean of three different incubations. The apparent K_m determined from a Lineweaver–Burk plot was 0.56 mM of NADP. In all subsequent incubations we used 1 mM NADP.

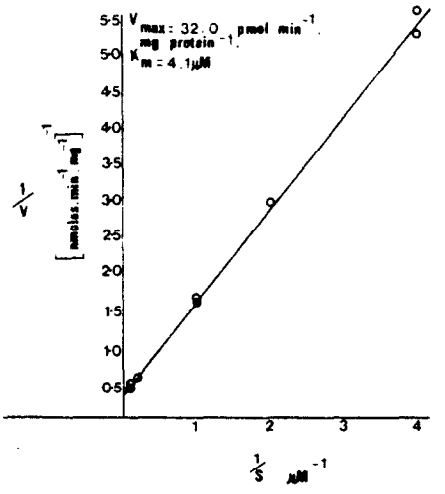

Fig. 5. Lung homogenates were incubated with cortisol. Other conditions as described for Fig. 4.

from these experiments. The V_{max} was 8.98 nmol·g wet lung^{-1}·min^{-1} and the K_m was 5.11 μM.

Sub-cellular location of 11β-HSD

Serial dilutions of the different cellular fractions were incubated for 20 min at 37°C with [^{3}H]-F made up to 10 μM with unlabeled F and NADP (final concentration 0.75 mM). 78% of the 11β-HSD activity was found in the microsomal fraction.

Characterisation of 11β-HSD

We determined the optimal concentration of cofactor by incubating various concentrations (0.075 to 1.5 mM) of either NADP or NADPH and 0.138 mM of either F or E for 30 min. Figure 3 shows the curve from the experiments using NADP and F which showed that the K_m was 0.56 mM. In all subsequent incubations we used 1 mM cofactor and established that when using substrate concentrations of between 0.2 and 10 μM the reaction remained zero order with respect to the substrate over the 15 min period of the incubation. Lineweaver–Burk plots for the homogenates are shown in Figs 4 to 5 for both E and F; the kinetic constants are included in the figures. The plots for the microsomal fraction are presented in Figs 6 and 7.

DISCUSSION

In the present experiments we have confirmed that the isolated perfused rat lung reduces the keto group in the 11-position of the E molecule and that the F formed appears rapidly in the vascular compartment.

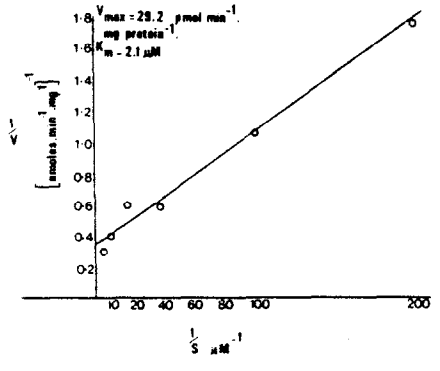

Fig. 4. Lung homogenates were incubated for 15 min under aerobic conditions at 37°C and pH 7.4 with various concentrations of cortisone and 1 mM of NADPH. Each point represents the mean of duplicate observations. The Lineweaver–Burk plot was constructed using the linear regression program of the TI 59 programmable calculator (Texas Instruments, Inc., Dallas, Texas).

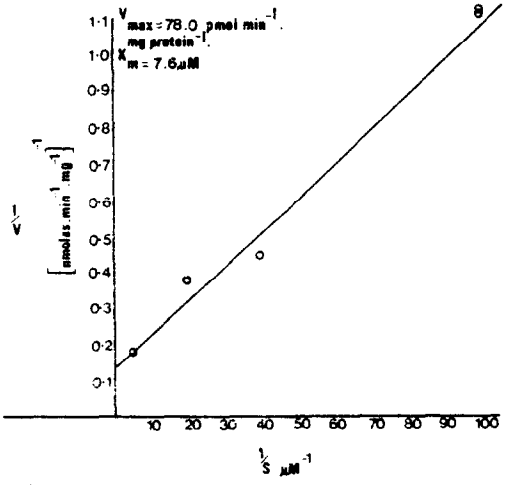

Fig. 6. Lung microsomal fraction incubated with cortisone. Other conditions as described for Fig. 4.

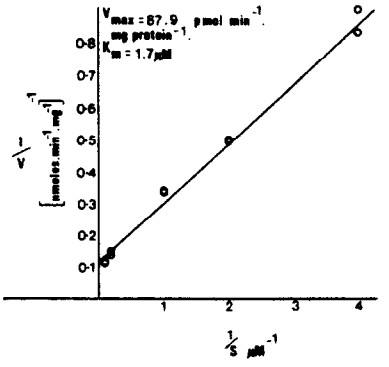

Fig. 7. Lung microsomal fraction incubated with cortisol. Other conditions as described for Fig. 4.

However, the influence of this conversion on the concentration of 11β-hydroxylated corticoids in arterial plasma is unclear. There are a number of problems in relating our results to the situation *in vivo*. First, the endogenous corticoids in the rat are not E and F, but 11-dehydrocorticosterone (A) and corticosterone (B). However, we have shown previously that the isolated perfused rat lung readily reduces A to B in a fashion comparable to that with E to F (unpublished results). Second, our perfusing medium did not contain corticosteroid binding globulin (CBG). Third, we perfused the lungs at 10 ml·min^{-1} which is about 20% of normal cardiac output in the rat.

We could find no data on the plasma concentration of A in the rat, although a number of reports suggest that the total plasma concentration of B is of the order of 20 μg·100 ml^{-1} [11, 12]. Burton and Jeynes[13] have reported that the A:B ratio in mouse plasma is unity. Furthermore, we previously found that the E:F ratio in the rabbit is 2.5 [14]; for the purpose of the following discussion, we will assume that the A:B ratio in rats is unity, that is, the concentration of A is also 20 μg·100 ml^{-1}, or 0.58 μM. However, because of the very high binding affinity of B for CBG, the concentration of unbound B will be very low compared to that of A, which has only a very low binding affinity [15, 16]. Hence, the concentration of unbound A must approximate the total plasma concentration of the steroid, whereas the concentration of unbound B will remain near zero until the binding sites on the CBG are saturated. From our present results we can calculate that introducing 0.58 μM of A into the pulmonary artery of the IPL results in the generation of 0.9 nmol of B per 100 ml of medium per min. At this stage we do not know whether this is perfusion limited, but if so, the generation of B from A would be some five times higher at the more physiological flow rate of 50 ml·min^{-1}. If B was normally 95% bound to CBG, then there would be 2.9 nmol of unbound B per 100 ml of plasma. In this case the lung would be making a very significant contribution to the arterial concentration of unbound, physiologically active, B. The actual significance would depend on the degree of saturation of CBG binding sites at the time.

As expected from the work of Bush, Hunter and Meigs[20], 11-keto-progesterone markedly depressed the rate of reduction of E to F. The reductive activity of 11β-HSD was enhanced following stress involving either starvation or exposure to low temperature. Although we did not measure the concentration of plasma corticoids in these rats, others have reported an elevation under such conditions. The increase in activity may then reflect actual enzyme induction due to increased levels of substrate, however, it is also possible that it reflects an increase in the amount of available cofactor. In the fetal rabbit, Torday, Olson and First[4] found that the increased 11β-HSD activity found between days 25 and 30 of gestation was due to an increase in the level of NADPH, the preferred cofactor for this enzyme. We found that the activity of 11β-HSD was reduced 7 days after adrenalectomy and castration; this is consistent with the finding of Lax, Ghraf and Schriefers[17] that gonadectomy reduced the ability of microsomal fraction of rat liver to oxidise F to E. Our results suggest that the activity of pulmonary 11β-HSD is under some degree of hormonal control and that the activity responds to physiological stress. Hence, the possibility exists that the activity of this enzyme in lung may play a role in the overall homeostatic mechanism controlling the plasma concentrations of corticoids. Certainly the activity of 11β-HSD in lung would influence the concentration of corticosterone actually in the lung tissue.

Previously we had reported that the isolated perfused rat lung demonstrated only marginal ability to oxidise F to E and it was possible that only an 11-reductase enzyme was present in this tissue. Our present results clearly demonstrate that, in the presence of NADP, preparations of both lung homogenate and microsomes readily oxidise the hydroxyl group in the 11-position. Apparently the direction of the reaction is determined by the normal preponderance of NADPH in the lung. This is consistent with the very active pentose-phosphate shunt in this tissue [18, 19]. Our present results are consistent with those of Koerner[1] in that we also found that the majority of the 11β-HSD activity (78%) was in the microsomal fraction. In fact, the K_m value for the oxidation of F was only one fifth of that for the reduction of E; it was one sixth of the K_m for the oxidative reaction reported by Bush, Hunter and Meigs[20] in rat liver microsomes. Whereas this may reflect a difference between the lung and liver, these workers used an acetone-dried powder of the microsome fraction, so that the two sets of results are not directly comparable. It does seem, however, that the affinity of the enzyme–cofactor complex in the oxidative direction is greater than that in the reverse direction, again emphasising that under physiological conditions the direction of the reaction is dictated by the NADPH: NADP ratio. Our results with lung homogenates present a picture similar to that with the microsomal

fraction, although the apparent K_m values for the oxidative and reductive directions are closer in value. In the homogenates it is possible that the substrate is brought in contact with other enzymes, resulting in complicating reactions which are not apparent in either the isolated lung or in the microsomal fraction. Previously we had shown that perfusion of the isolated lung with E resulted in the formation of predominantly F, with only a very small fraction converted to more polar compounds, possibly conjugates. However, we have not checked whether this is also the case with the lung homogenate, and other products may result from the incubation. Such complicating reactions may account for the differences in apparent K_m values found between the microsomal fraction and homogenate. Certainly there is closer agreement in the values for K_m for the microsomal and the IPL.

There appear to be species differences in the ability of the lung to interconvert E and F. Whereas the rat and rabbit readily reduce E to F (1–4), the human [6] and guinea pig [2] lungs demonstrate little such ability. The difference between the human lung and the rabbit lung is also apparent in the fetus [3, 6]. More surprising is the apparent difference between different strains of rats. The lungs of the Porton rats used in the present experiments demonstrated a markedly greater ability to reduce E to F than those of the previously used Sprague–Dawley rats [2]. However, such a comparison is difficult because the experiments were performed seven years apart and in different countries.

In conclusion, we have shown that the activity of the 11-reductase enzyme in the rat lung changes in situations known to affect the level of 11-hydroxylated corticoids in plasma. Although the amount of 11-hydroxylated corticoid generated by this pulmonary enzyme is small compared with the total amount of these compounds present in plasma, it may play an important role in adjusting the concentration of the unbound, physiologically-active corticoid. We suggest that the predominance of the reduction reaction over the oxidation reaction in the lung is due to a high ratio of NADPH:NADP in this tissue; this ratio may also account for the apparent species differences.

Acknowledgements—We are grateful for the technical assistance of Heather Barr and Sharon Colquhoun and the helpful comments of Dr Robert Rush. Support was provided by the National Health and Medical Research Council of Australia.

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