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EFFECTS OF THE GLUCOCORTICOID AGONIST, RU28362, AND THE ANTAGONIST RU486 ON LUNG PHOSPHATIDYLCHOLINE AND ANTIOXIDANT ENZYME DEVELOPMENT IN THE GENETICALLY OBESE ZUCKER RAT

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Abstract—The biochemical maturation of the lung in late gestation and in the young animal is regulated by glucocorticoids. The present study was aimed at dissociating the different glucocorticoid receptor sites involved in these regulatory functions. The obese Zucker rat was selected as a model for this study as it exhibits hypersensitivity to glucocorticoid hormone action by virtue of its elevated receptor numbers and activity. Two synthetic steroid analogues were administered to obese animals; RU28362, a specific type II receptor agonist, and the type II antagonist RU486. RU28362 promoted a strong catabolic effect, which was associated with reduced food intake and the abolition of growth in the rats. The agonist, RU28362, attenuated developmental increases in antioxidant enzyme activities, and altered the growth of the tissue. At the age studied, development of the lung phosphatidylcholine (PC) system was almost complete, but RU28362 increased disaturated PC 16:0/16:0 concentrations by almost 2-fold, and altered the molecular composition of total pulmonary PC. RU486 attenuated the growth of the rats and reduced their food intake. Treatment with the type II antagonist attenuated lung growth and increased the activities of pulmonary copper zinc (Cu/Zn) and manganese (Mn) superoxide dismutases. RU486 had no effect on lung PC concentrations and molecular composition. The data suggest a role for type I glucocorticoid receptors in the regulation of the antioxidant enzyme system in the lung, as type II antagonism will channel endogenous glucocorticoid binding to the type I site. Type II receptor binding would appear to play a role in regulating the lung PC content.

Late gestational maturation of pulmonary surfactant has been shown to be linked to changes in glucocorticoid status in the foetus [1–6]. Similarly maturation of antioxidant enzymes has been shown to be accelerated by treating the foetus with dexamethasone, a synthetic glucocorticoid, *in utero* [7]. Treatment of cultured foetal lung cells with synthetic glucocorticoids also increases antioxidant enzyme activities [8]. The overall effect of glucocorticoids is, therefore, the advance maturation of the lung.

Glucocorticoid action is mediated by two distinct receptor subtypes, best characterized in the brain [9], but also present in peripheral tissues [10]. Type I sites (MR) bind both mineralocorticoid and glucocorticoids and have only weak affinity for synthetic steroids. Type II (GR) sites bind glucocorticoids only, and are of high affinity for synthetic steroids [11, 12]. Corticosterone, in the rat, has dual metabolic actions differentially mediated by its specific binding to these sites [13]. Type II binding classically promotes catabolic functions, whilst type I binding promotes anabolic functions.

Specific steroid analogues have been developed which distinguish between type I and type II receptor binding. RU28362 is a highly potent type II agonist, with little or no affinity for type I sites [14]. RU486 is a potent antagonist of the same site, with no partial agonist activity [15]. RU486 is also an antagonist of the progesterone and androgen receptors [16].

We and others have previously reported that treatment of obese Zucker (*fa/fa*) rats with the antiglucocorticoid RU486 effectively abolishes their obesity [17, 18]. The central hypersensitivity of Zucker rats to glucocorticoid agonists [19–22] and antagonists [18], mediated by increased type I and type II receptor numbers [23], makes these animals a useful model for the study of glucocorticoid control of a number of physiological, behavioural and biochemical processes. In the current paper we use this model to examine the specific receptor sites involved in the regulation of pulmonary phospholipid synthesis and antioxidant enzyme activities. Although lung development was well beyond that of the foetal rat, the animals selected were of an age where the

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lung was, in biochemical terms, still undergoing maturational changes.

MATERIALS AND METHODS

Chemicals. All chemicals and reagents were purchased from the Sigma Chemical Co. (Poole, U.K.) or BDH (Poole, U.K.), with the exception of RU486 and RU28362, which were the kind gift of Dr D. Philibert (Roussell UCLAF, France).

Animals. Four-week-old obese (*fa/fa*) male rats were bred from heterozygote matings in the Southampton University animal house. They were fed PRD rat chow (Labsure, Minea, Cambs, U.K.) *ad lib.*, housed individually in wire-mesh cages in a room maintained at 22 ± 1° with a 14:10 light/dark cycle.

RU486 and RU28362 were dissolved in 70% ethanol diluted in 0.9% saline. RU486 (30 mg/kg body weight) was injected i.p. over an 11 day period. RU28362 was administered at a dose of 30 mg/kg body weight i.p. for 10 days. Control rats received injections of an equal volume of vehicle. Injections were administered at 9.00 a.m. each day. Six 4-week-old rats were killed on the first day of the experiment, without injection, to constitute a zero time control group.

Throughout the study the rats were weighed and food intake determined at 9.00 a.m. daily. The rats were killed by decapitation between 9.00 and 10.00 a.m. on the last day of the study. Tissues were rapidly dissected, rinsed in ice-cold saline and frozen in liquid nitrogen prior to storage at −80°.

Enzyme assays. Lung antioxidant enzyme activities [Cu/Zn and Mn superoxide dismutases (Cu/Zn and

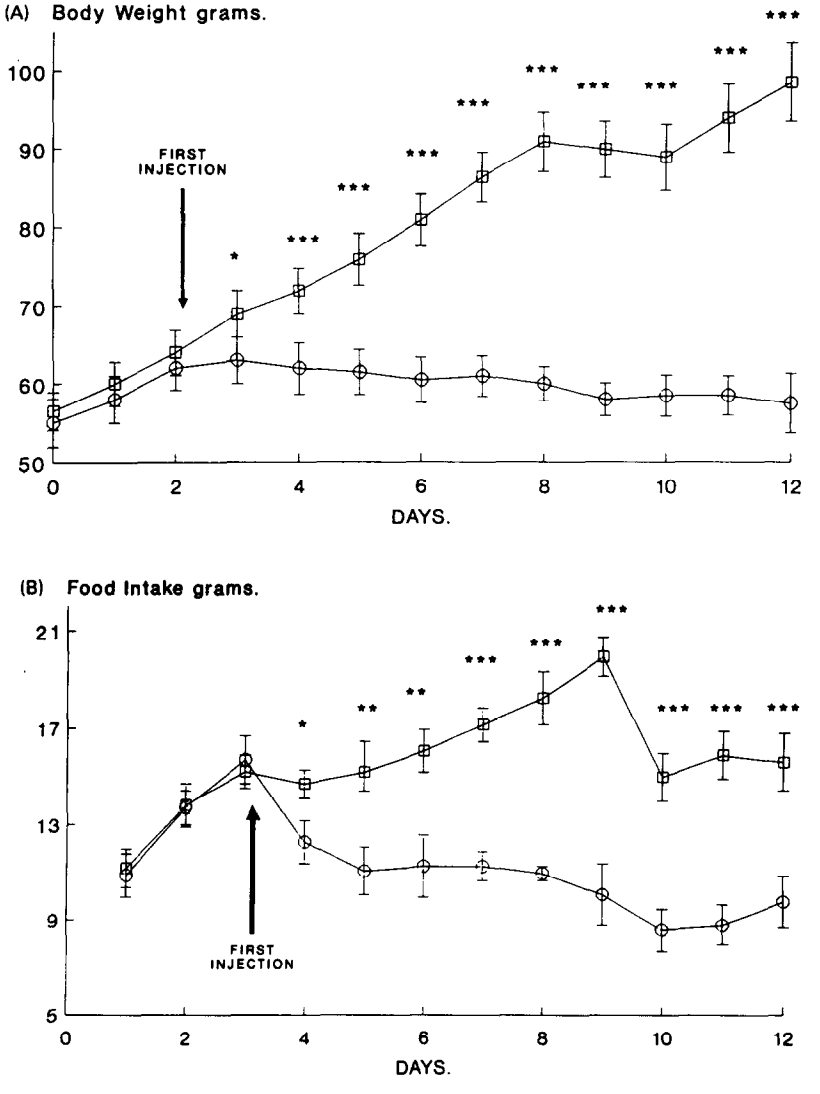

Fig. 1. The effect of RU28362 treatment on the body weight (A) and food intake (B) of obese male Zucker rats. RU28362 was injected s.c. at 9.00 a.m. each day for 10 days. Data shown are mean ± SEM for seven control and five treated animals. *, **, *** Indicate statistically significant differences ($P < 0.05$, 0.01, 0.001, respectively).

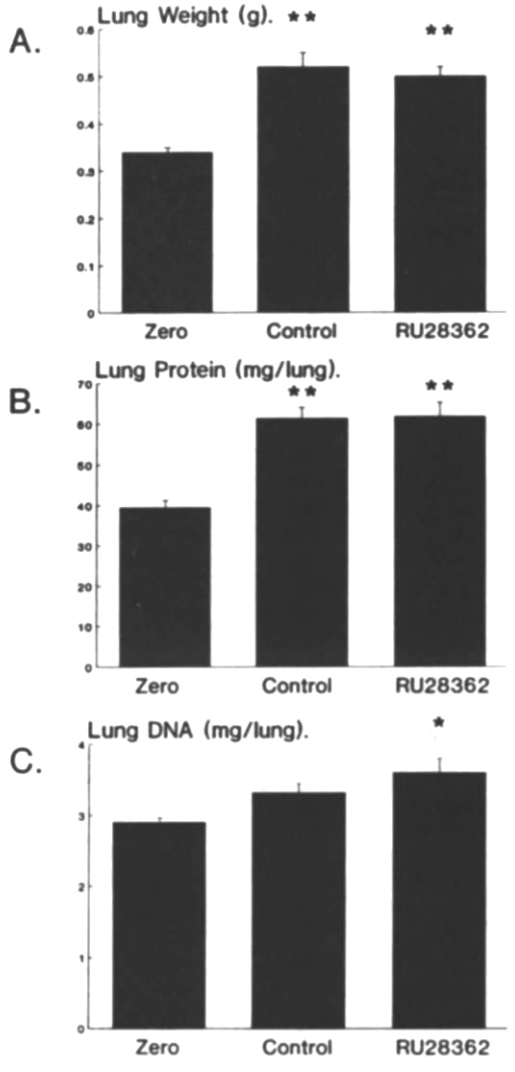

Fig. 2. The effects of RU28362 treatment on lung weight (A), lung protein content (B) and lung DNA content (C) of obese male Zucker rats. RU28362 was injected s.c. at 9.00 a.m. each day for 10 days. Data shown are mean ± SEM for five zero time, seven control and five treated animals. *, ** Indicate statistically significant differences between control and RU28362 groups relative to zero time controls ($P < 0.05$, 0.01, respectively).

Mn SOD*), catalase (CAT) and glutathione peroxidase (GPX)] were determined in a single Tris–HCl-buffered homogenate preparation, as reported by Rickett and Kelly [24]. All lung enzyme activities were expressed per milligram DNA, determined by the method of Richards [25]. Hepatic tyrosine aminotransferase (TAT) activity was assayed as previously reported [22] and expressed per milligram protein, determined by the method of Bradford [26]. All assays were performed in batches of 15–20 samples, and to avoid interassay variations, results were standardized against a quality control (guinea pig liver).

* Abbreviations: CAT, catalase; Cu/Zn SOD, copper zinc superoxide dismutase; GPx, glutathione peroxidase; Mn SOD, manganese superoxide dismutase; P/O, ratio of C16 to C18 fatty acids; TAT, tyrosine aminotransferase; PC, phosphatidylcholine.

Analysis of lung phospholipids. Total lipid was extracted from lung homogenates using the method of Bligh and Dyer [27]. Individual molecular species of phosphatidylcholine (PC) were identified and quantified by the fluorescence detection HPLC method of Postle [28].

Corticosterone determination. Corticosterone in rat plasma was determined by radioimmunoassay, as described previously [18].

Statistical analysis. Data were analysed by one-way or two-way analysis of variance (ANOVA), as appropriate, followed by a Tukey test for individual comparisons where differences were indicated.

RESULTS

The effects of the glucocorticoid agonist RU28362

The effect of RU28362 on the body weights of 4-week-old male obese rats is shown in Fig. 1A. The weight of control rats increased steadily throughout the experimental period. Rats receiving RU28362 displayed an immediate cessation of growth, with a slight loss of weight (5%) over the last 8 days of treatment. Food intake was significantly ($P < 0.001$) decreased (27%) by RU28362 treatment, from the first day following initial agonist administration (Fig. 1B). The gonadal white adipose depot weight was reduced by 70% in RU28362 treated rats (0.16 ± 0.05 vs 0.56 ± 0.08 g, $P < 0.001$). A comparison of the lung weights of 4-week-old zero time control obese rats with control animals at the end of the study, indicated that lung growth was occurring in animals of this age (Fig. 2A). Lung protein concentrations (Fig. 2B) increased over the study period, while lung DNA increased, but not significantly (Fig. 2C). In RU28362-treated rats lung weight, protein and DNA were all significantly increased relative to zero time controls. Increased DNA concentrations in the lungs of RU28362-treated rats may indicate that the lungs of the animals contained more cells than those of the controls, and unaltered protein content indicates these cells may be smaller than those in control lungs.

Hepatic TAT activity was significantly elevated in RU28362-treated animals (RU28362; 34.20 ± 2.70 vs control; 26.10 ± 1.80 μmol/min/mg protein, $P < 0.05$). Pulmonary Cu/Zn SOD, CAT and GPX increased significantly (35%, 45% and 130%, respectively) in control animals relative to the zero time controls (Fig. 3). The activity of Mn SOD did not change significantly over the period studied (Fig. 3A). In RU28362-treated rats Cu/Zn SOD activity was increased relative to zero time controls but the developmental increase was significantly attenuated by the drug treatment (Fig. 3B). GPx and CAT activities in RU28362-treated animals were not significantly different to those of zero time controls (Fig. 3C and D).

The effects of the glucocorticoid antagonist RU486

The effect of RU486 on the body weight of 4-week-old obese male rats is shown in Fig. 4A. Over the 11 days of treatment the normal growth of RU486-treated rats was attenuated (53%), beginning on the fifth day of treatment, and becoming significant by the ninth day. Food intake (Fig. 4B) was also reduced (19%) by RU486 treatment, but

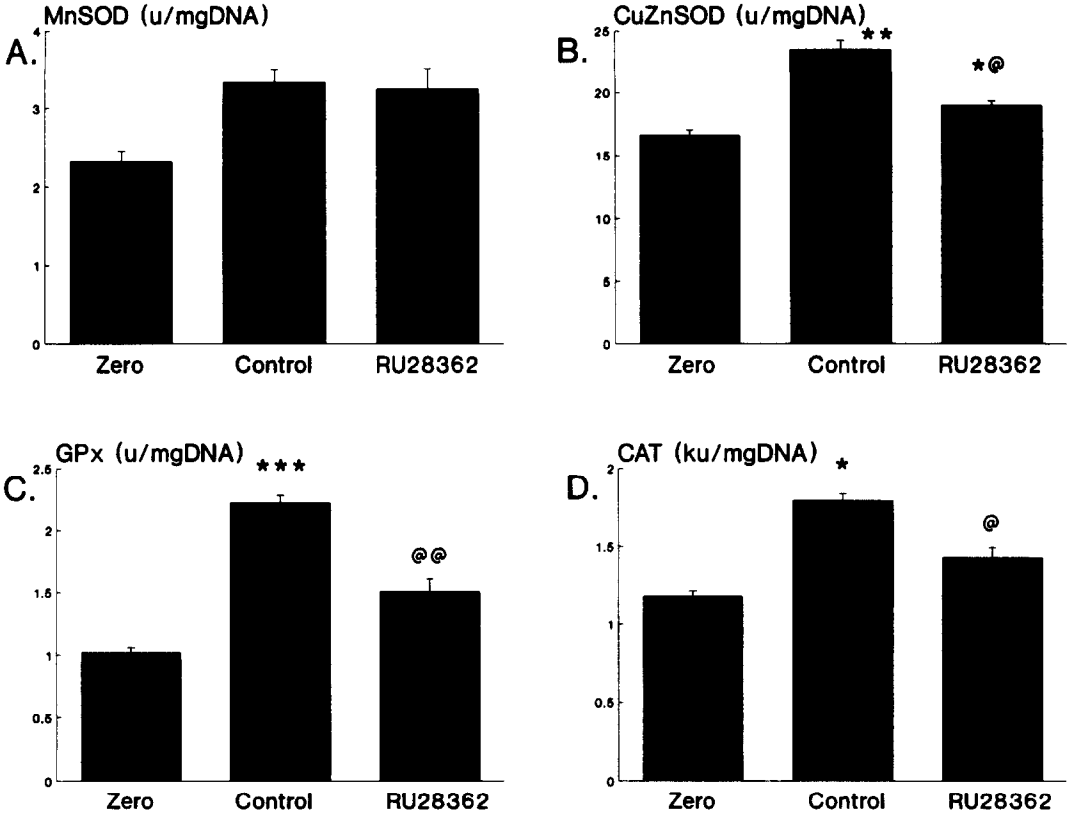

Fig. 3. The effects of RU28362 treatment on antioxidant enzyme activities of obese Zucker rat lung. (A) Mn SOD, (B) Cu/Zn SOD, (C) GPx, (D) CAT. RU28362 was injected s.c. at 9.00 a.m. each day for 10 days. Data shown are mean ± SEM for five zero time, seven control and five treated animals. *, **, *** Indicate statistically significant differences between control and RU28362 groups relative to zero time controls ($P < 0.05$, 0.01, 0.001, respectively). @ and @@ indicate statistically significant differences between controls and RU28362-treated animals ($P < 0.05$, 0.01, respectively).

this effect was not significant until the final day of the study.

Gonadal fat depot weight was reduced by 60% in RU486-treated animals (0.14 ± 0.06 vs 0.34 ± 0.09 g, $P < 0.05$). Lung weight (Fig. 5A) was significantly reduced (25%), and consistent with an inhibition of lung growth, lung protein (Fig. 5B) and lung DNA contents (Fig. 5C) were also decreased (25% and 35%, respectively).

Hepatic TAT activity was not significantly changed by RU486 treatment (RU486; 24.50 ± 1.95 vs control; 19.70 ± 1.45 μmol/min/mg protein). Lung antioxidant enzyme activities are shown in Fig. 6. Mn SOD, Cu/Zn SOD, CAT and GPx activities all increased in both RU486-treated and control groups, relative to zero time controls (Fig. 3). Although activities of GPx, Cu/Zn SOD and Mn SOD were markedly higher in the controls of this study, relative to controls in the preceding agonist study, we are confident, through the use of a quality control, that this difference is not the result of interassay variation. RU486 treatment significantly elevated Mn SOD (23%) (Fig. 6A) and Cu/Zn SOD (12%) activities (Fig. 6B). GPx and CAT activities were unchanged relative to controls (Fig. 6C and D).

The effects of RU486 and RU28362 on lung phospholipid metabolism

The response of lung PC concentration and composition to treatment with the glucocorticoid agonists and antagonists was striking, especially in RU28362-treated animals (Table 1). Compared with the time zero control group, total lung PC concentration increased significantly in both experimental groups, whilst in saline-treated control animals there was no significant change. The increase was most marked for the RU28362-treated group, total lung PC concentration being 75% greater than in the control group. The molecular species composition of lung PC was similar for both control and RU486-treated rats, and did not significantly differ from the time zero control group. By contrast, the changes in PC content after treatment with RU28362 was highly selective in terms of molecular species composition. There was no agonist-induced accumulation of either of the monounsaturated species PC16:0/16:1 or PC16:0/18:1, while concentrations of the disaturated species PC14:0/16:0 and PC16:0/16:0 increased by 175% and 84%, respectively. The ratio of PC16:0/16:0 to PC16:0/

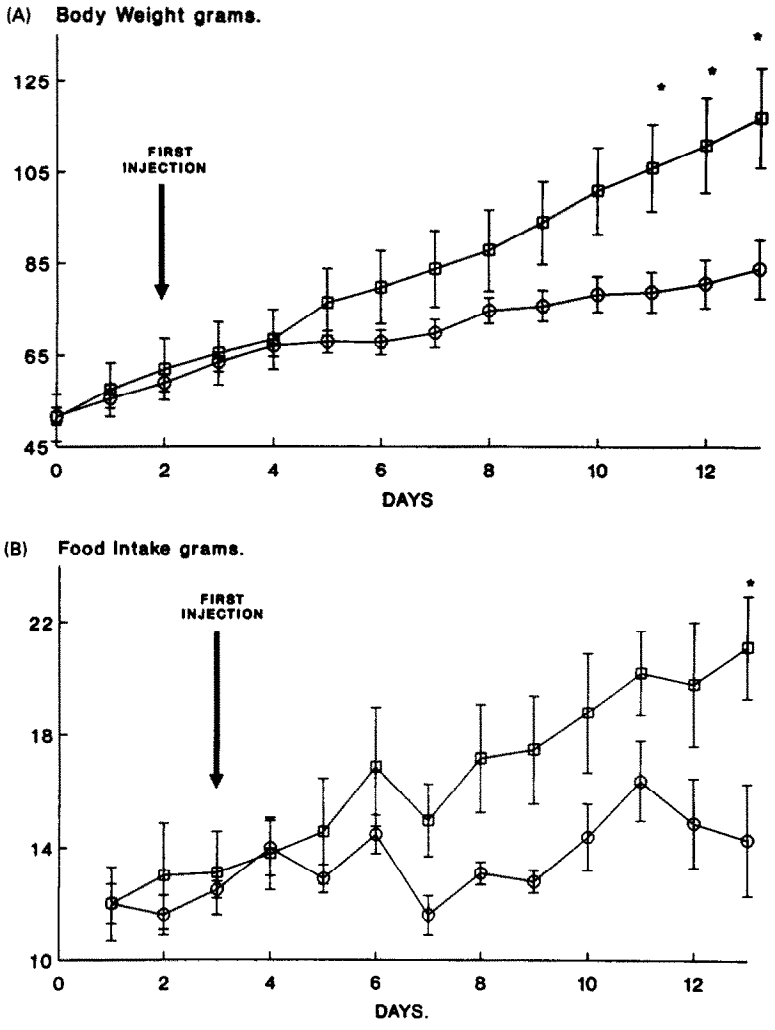

Fig. 4. The effect of RU486 treatment on the body weight (A) and food intake (B) of obese male Zucker rats. RU486 was injected s.c. at 9.00 a.m. each day for 11 days. Data shown are mean ± SEM for five control and five treated animals. * Indicates statistically significant differences ($P < 0.05$).

18:1 (P/O ratio), which we have previously used as an indicator of surfactant maturity in the newborn guinea pig [29, 30], changed from 2.03 ± 0.09 in control to 3.11 ± 0.20 in RU28362-treated rats, an increase of 53%. Intriguingly, in direct contrast to the unaltered concentrations of monounsaturated species, all of the three polyunsaturated species identified (PC16:0/22:6, PC16:0/20:4 and PC16:0/18:2) were present at increased concentrations (Table 1) in the RU28362-treated animals (increases of 173%, 44% and 119%, respectively).

DISCUSSION

Effects of RU486 and RU28362 on the obese state

Our previous studies have shown that the obese Zucker rat is highly sensitive to glucocorticoids by virtue of its increased numbers and activity of glucocorticoid receptors in the brain [21] and possibly in the periphery [22, 23]. In the present study we have confirmed our previous finding that RU486 attenuates the weight gain and reduces the food intake of obese Zucker rats [18].

RU28362, being an agonist of the glucocorticoid receptors, may have been expected to promote the development of a more extreme obesity in the Zucker rat. However, treatment with this powerful glucocorticoid agonist had the opposite effect and abolished weight gain and hyperphagia of the obese rats more effectively than RU486. The reduction of food intake by RU28362 was consistent with a previous report of hypophagia induced by synthetic glucocorticoids in rats [31]. The effects of the drug on body weight would appear to be due to the induction of a catabolic effect. Hypophagia alone would not adequately explain the cessation of weight gain and loss of fat observed. Lifelong food restriction of obese rats fails to attenuate the obese condition [32]. High-dose glucocorticoids are known to exert catabolic effects via the type II receptors [13]. The

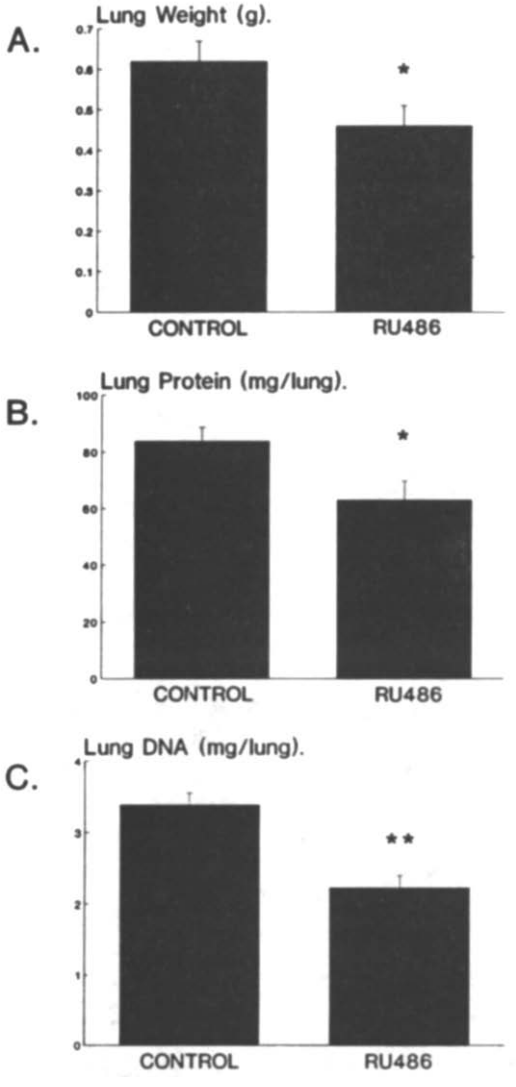

Fig. 5. The effects of RU486 treatment on lung weight (A), lung protein content (B) and lung DNA content (C) of obese male Zucker rats. RU486 was injected s.c. at 9.00 a.m. each day for 11 days. Data shown are mean ± SEM for five control and five treated animals. *, ** Indicate statistically significant differences between control and RU486-treated groups ($P < 0.05$, 0.01, respectively).

increased activity of glucocorticoid-sensitive hepatic TAT demonstrates this type II effect.

Effects of RU486 and RU28362 on pulmonary antioxidant enzyme activities

RU486 and RU28362 had pronounced effects on lung phospholipid metabolism and antioxidant enzyme activities in the young *fa/fa* rat. Whilst there is direct evidence for increased glucocorticoid receptor numbers only in the brain of the obese rat, elevated activity has been demonstrated in the liver [22], and CNS-mediated effects on the lung would seem improbable. In both experiments, control antioxidant enzyme activities increased over and above the activities seen in zero time controls. This suggests that in rats aged 4–6 weeks the maturational development of the pulmonary antioxidant defences is still proceeding. RU28362 blocked this developmental trend, while RU486 treatment mediated slight increases in Mn SOD and Cu/Zn SOD activities. These results are consistent with a role for the type I glucocorticoid receptors in the regulation of antioxidant enzyme activity by glucocorticoids. RU486 blockades the type II sites, and in the hypothalamus this disrupts the hypothalamic–pituitary–adrenal axis, consequently increasing circulating corticosterone concentrations. Corticosterone levels were not determined in the present study, but have previously been shown to rise by 470% in the RU486-treated obese Zucker rat [18]. Elevation of corticosterone concentrations in RU486-treated rats, with fully blockaded type II receptors, would be expected to increase binding to the type I sites. The observed concomitant rise in SOD activities may suggest a role for type I receptor sites in the lung, in the regulation of antioxidant enzyme activities. Whether these effects are mediated directly at the gene level, or through changes in lung cell populations is unclear.

RU28362 would be expected to decrease corticosterone synthesis due to increased hypothalamic feedback. However, such a decrease was not observed (control 64 ± 19, RU28362 68 ± 19 ng/mL in plasma). RU28362 has a half life of 16 hr at the receptor, which may have allowed some remission from its effects on each day of the experiment. As no injection was given on the last day of the study, the final corticosterone concentration may not have been representative of levels during the majority of the study. Assuming that endogenous steroid synthesis was inhibited, corticosteroid binding to type I sites would be insignificant. Under such circumstances, any glucocorticoid stimulus of antioxidant activity via the type I sites may be removed, and accordingly RU28362 was observed to inhibit developmental changes in antioxidant enzyme activities.

This hypothesis that corticosteroid binding to type I receptors promotes increased antioxidant activities, is not consistent with the observations of other workers [7, 8, 33] who reported that synthetic steroids enhance the antioxidant enzyme activities of foetal tissues. Changes in receptor subpopulation distribution and function during the development of the rat from foetus to "adolescent" might explain the inconsistency. Additionally, changes in the cell populations of the lung during RU28362 treatment, indicated by lung protein/DNA ratios may account for some of the differences between the findings of this, and previous studies.

Effects of RU486 and RU28362 on pulmonary PC metabolism

The effects of glucocorticoid agonists and antagonists on lung PC metabolism have largely been studied in relation to the maturation of pulmonary surfactant in late foetal development. Prenatal steroid treatment, in rats, results in accelerated lung maturation and increased foetal lung content of both total and disaturated PC [34].

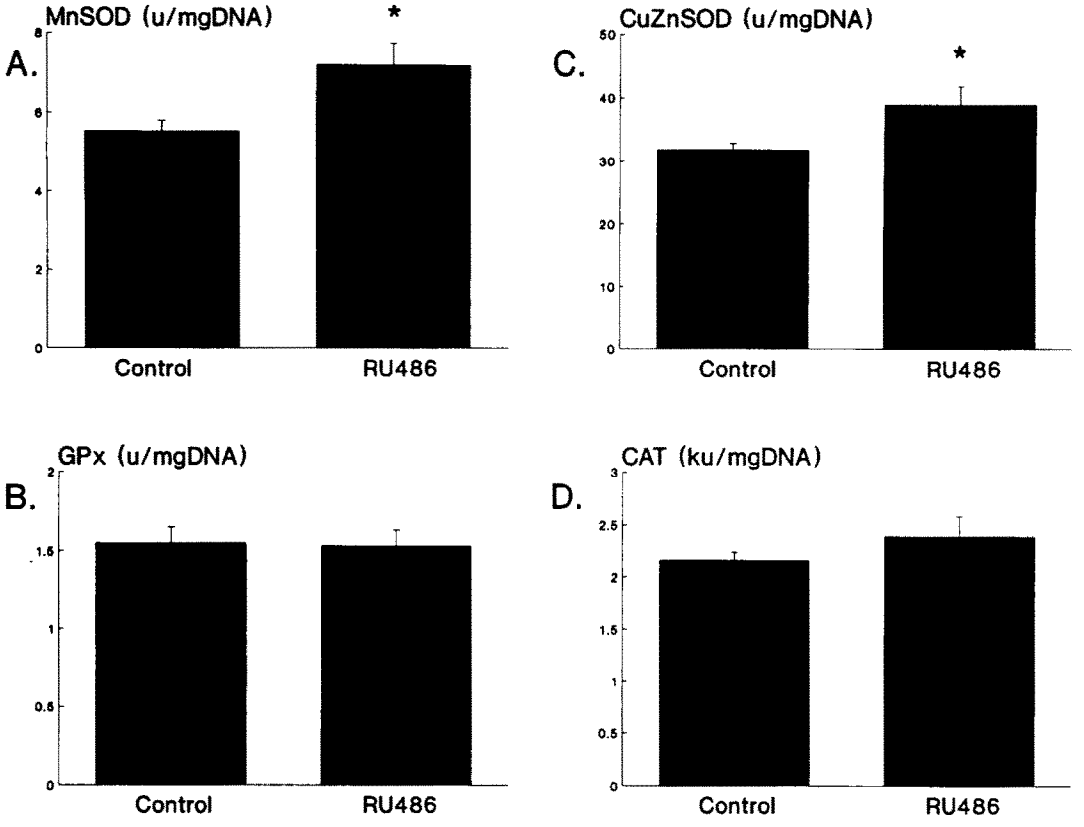

Fig. 6. The effects of RU486 treatment on antioxidant enzyme activities of obese Zucker rat lung. (A) Mn SOD, (B) Cu/Zn SOD, (C) GPx, (D) CAT. RU486 was injected s.c. at 9.00 a.m. each day for 11 days. Data shown are mean ± SEM for five control and five treated animals. * Indicates statistically significant differences between control and RU486-treated groups ($P < 0.05$).

Table 1. The effect of RU28362 and RU486 on the composition of individual molecular species of lung PC

	PC (μmol/mg DNA)			
Molecular species	Time zero N = 5	Saline control N = 12	RU28362 N = 5	RU486 N = 5
14:0/16:0	0.11 ± 0.01	0.16 ± 0.02	0.44 ± 0.03†	0.16 ± 0.01
16:0/16:1	0.16 ± 0.01	0.18 ± 0.02	0.16 ± 0.03	0.18 ± 0.02
16:0/22:6	0.07 ± 0.01	0.15 ± 0.01	0.41 ± 0.04†	0.13 ± 0.02
16:0/20:4	0.09 ± 0.01	0.09 ± 0.01	0.13 ± 0.01*	0.10 ± 0.01
16:0/18:2	0.17 ± 0.01	0.16 ± 0.01	0.35 ± 0.01†	0.19 ± 0.02
16:0/16:0	0.44 ± 0.04	0.61 ± 0.04	1.12 ± 0.06†	0.69 ± 0.04
16:0/18:1	0.21 ± 0.02	0.30 ± 0.02	0.36 ± 0.01	0.26 ± 0.02
Total PC	1.25 ± 0.09	1.65 ± 0.13	2.92 ± 0.17†	1.82 ± 0.06‡
P/O ratio	2.09 ± 0.06	2.03 ± 0.09	3.11 ± 0.20†	2.65 ± 0.15‡

*, † Denote RU28362 significantly different to saline control $P < 0.01$, $P < 0.001$.
‡ Denotes RU486 significantly different to zero time control.
Values shown are mean ± SEM.

In the present study, while there was an effect of RU486, treatment with RU28362 clearly had the most pronounced effect on lung PC composition. Elevated total PC content, PC16:0/16:0 content and an increased P/O ratio are consistent with an enhancement of lung maturation. The observation that the concentration of the other major disaturated species, PC14:0/16:0, was also increased demon-

strated, however, that the response of lung PC to agonist treatment, was not specific for the surface active PC16:0/16:0. No specific functional role for PC14:0/16:0 has been described in the lung.

The enhancement of levels of the polyunsaturated PC species in response to RU28362 was both dramatic and unexpected. While disaturated PC species are formed from fatty acids that can be synthesized endogenously within the lungs, fatty acids incorporated into polyunsaturated species must be supplied to the lung from the circulation. There is a well recognised differential response of lipid metabolism to a number of glucocorticoid agonists, with increased lipolysis in muscle and adipose tissue [35], and stimulated lipogenesis and glycerolipid synthesis in liver [36, 37], which is dependent on glucocorticoid-induced hyperinsulinaemia, which is observed in the Zucker rat without agonist treatment [38]. Polyunsaturated fatty acids are selectively esterified into PC species for preferential export into lipoprotein from rat liver [39], and act to supply polyunsaturated fatty acids to other tissues [40, 41]. One plausible scheme of action for the effect of RU28362 on obese rat lung PC metabolism, could thus involve the enhanced supply of substrate polyunsaturated fatty acids to the lung, secondary to stimulated adipose tissue and muscle lipolysis and liver lipoprotein secretion. Although RU486 elevates circulating corticosterone levels, and promotes lipolysis, similar effects on lipid metabolism would not be observed if these were mediated via the type II receptors.

Conclusion

The working model is, therefore, that pulmonary antioxidant enzyme activities are regulated by glucocorticoids binding to type I receptors, in the lung. Hence, RU486 treatment, which channels an increased endogenous steroid level towards these sites, enhances antioxidant defences. Pulmonary phospholipid synthesis and uptake from the circulation are under glucocorticoid control at the level of type II sites, and are, therefore, stimulated by the pure agonist RU28362. This model could be further investigated using type I agonists and antagonists.

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REFERENCES

1. Ballard P, Glucocorticoids and differentiation. In: *Glucocorticoid Hormone Action* (Eds. Baxter JG and Rousseau GG), pp. 493–516. Springer, Berlin, 1979.
2. Guettari N, Marin L, Bourbon J, Dufour E, Rieutort M and Tordet C, Effects of the antiglucocorticoid RU486 on the inactivation of fetal rat ' ng surfactant. *Exp Lung Res* **15**: 151–166, 1989.
3. Kotas RV and Avery ME, Accelerated appearance of pulmonary surfactant in the fetal rabbit. *J Appl Physiol* **30**: 358–361, 1971.
4. Nelson GH, Eguchi K and McPherson JC, Effects of gestational age, dexamethasone and metopirone on lecithin concentrations in foetal lung tissue and amniotic fluid in rats and guinea pigs. *Gynecol Invest* **7**: 337–343, 1976.
5. Smith BT and Sabry K, Glucocorticoid-thyroid synergism in lung maturation: A mechanism involving epithelial-mesenchymal interaction. *Proc Natl Acad Sci USA* **80**: 1951–1954, 1983.
6. Wang NS, Kotas RV, Avery ME and Thurlbeck WM, Accelerated appearance of osmiophilic bodies in fetal lungs following steroid injection. *J Appl Physiol* **30**: 362–365, 1971.
7. Frank L, Lewis PL and Sosenko IRS, Dexamethasone stimulation of fetal rat lung antioxidant enzyme activity in parallel with surfactant stimulation. *Pediatrics* **75**: 569–574, 1985.
8. Randhawa P, Hass M, Frank L and Massaro D, Dexamethasone increases superoxide dismutase activity in serum-free rat fetal lung organ cultures. *Pediatr Res* **20**: 895–898, 1986.
9. McEwan BS, DeKloet ER and Rostene W, Adrenal steroid receptors and actions in the central nervous system. *Physiol Rev* **66**: 1122–1188, 1986.
10. Funder JW, Pearce PT, Smith R and Smith AI, Mineralocorticoid action: target tissue specificity is enzyme not receptor mediated. *Science* **242**: 583–585, 1988.
11. Beaumont K and Farnestil DD, Characterisation of rat brain aldosterone receptors reveals high affinity for corticosterone. *Endocrinology* **113**: 2043–2051, 1984.
12. Reul JMHM and DeKloet ER, Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology* **117**: 2505–2511, 1985.
13. Devenport L, Knehans A, Sundstrom A and Thomas T, Corticosterone's dual metabolic actions. *Life Sci* **45**: 1389–1396, 1989.
14. Philibert D and Moguilewsky M, RU28362, a useful tool for the characterisation of glucocorticoid and mineralocorticoid receptors. *Endocrine Soc* **1018**: (Abstract), 1983.
15. Moguilewsky M and Philibert D, RU38486: potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. *J Steroid Biochem* **20**: 271–276, 1984.
16. Baulieu E-E, RU486 (an anti-steroid hormone) receptor structure and heat shock protein mol. wt. 90 000 (hsp90). *Human Rep* **3**: 541–547, 1988.
17. Hardwick AJ, Linton EA and Rothwell NJ, Thermogenic effects of the antiglucocorticoid RU486 in the rat: involvement of corticotropin-releasing factor and sympathetic activation of brown adipose tissue. *Endocrinology* **124**: 1684–1688, 1989.
18. Langley SC and York DA, Effects of antiglucocorticoid RU486 on development of obesity in obese fa/fa Zucker rats. *Am J Physiol* **259**: R539–R544, 1990.
19. Freedman MR, Castonguay TW and Stern JS, Effect of adrenalectomy and corticosterone on meal patterns of Zucker rats. *Am J Physiol* **249**: R584–R594, 1985.
20. Freedman MR, Horwitz BA and Stern JS, Effect of adrenalectomy and corticosterone replacement on the development of obesity. *Am J Physiol* **249**: R595–R607, 1985.
21. Langley SC and York DA, Increased type II glucocorticoid-receptor numbers and glucocorticoid-sensitive enzyme activities in the brain of the obese Zucker rat. *Brain Res* **533**: 268–274, 1990.
22. Shargill NS, Al-Baker I and York DA, Normal levels of serum corticosterone and hepatic glucocorticoid receptors in obese (fa/fa) rats. *Biosci Rep* **7**: 843–851, 1987.
23. Langley SC and York DA, Glucocorticoid receptor numbers in the brain and liver of the obese Zucker rat. *Int J Obesity* **16**: 135–143, 1992.

24. Rickett GMW and Kelly FJ, Developmental expression of antioxidant enzymes in guinea pig lung and liver. *Development* **108**: 331–336, 1991.
25. Richards GM, Modifications of the dephenylamine reaction giving increased sensitivity and simplicity in the stimulation of DNA. *Anal Biochem* **57**: 369–376, 1974.
26. Bradford M, A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye binding. *Anal Biochem* **72**: 248–254, 1976.
27. Bligh EG and Dyer WS, A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–923, 1959.
28. Postle AD, A method for sensitive analysis of individual molecular species of phosphatidyl choline by high performance liquid chromatography using post column fluorescence detection. *J Chromatogr* **415**: 241–251, 1987.
29. Ashton MR, Postle AD, Hall MA, Smith SL, Kelly FJ and Normand ICS, The phosphatidylcholine composition of the initial endotracheal aspirate of neonates: implications for the development of subsequent disease. *Arch Dis Childh* **67**: 378–382, 1992.
30. Kelly FJ, Rickett GMW, Hunt AN, Town GI, Holgate ST and Postle AD, Biochemical maturation of the guinea pig lung and survival following premature delivery. *Int J Biochem* **23**: 467–471, 1991.
31. Simpson GW, Dicar LV and Wolf G, Glucocorticoid anorexia in rats. *Pharm Biochem Behav* **2**: 19–25, 1974.
32. Cleary MP, Vasselli JR and Greenwood MRC, Development of obesity in the Zucker obese rat (fa/fa) in the absence of hyperphagia. *Am J Physiol* **238**: E284–E292, 1980.
33. Tanswell AK, Tzaki MG and Byrne PJ, Hormonal and local factors influence antioxidant enzyme activity of rat fetal lung cells *in vitro*. *Exp Lung Res* **11**: 49–59, 1986.
34. Tsai MY, Josephson MW and Brown DM, Fetal rat lung phosphatidylcholine synthesis in diabetic and normal pregnancies. A comparison of prenatal dexamethasone treatment. *Biochim Biophys Acta* **664**: 174–181, 1981.
35. Fain JN and Czech MP, Glucocorticoid effects on lipid mobilisation and adipose tissue metabolism. In: *Handbook of Physiology, Section 7, Endocrinology 6, Adrenal Gland* (Eds. Greep RO and Astwood EB), pp. 169–178. American Physiological Society, Washington, 1975.
36. Diamant S and Shafrir E, Modulation of the activity of insulin dependent enzymes of lipogenesis by glucocorticoids. *Eur J Biochem* **53**: 541–546, 1975.
37. Glenny HP and Brindley DN, The effects of cortisol, corticotropin and thyroxine on the synthesis of glycerolipids and on the phosphate phosphohydrolase activity of rat liver. *Biochem J* **176**: 777–784, 1978.
38. Bray GA, York DA and Fisler J, Experimental obesity: a homeostatic failure due to defective nutrient stimulation of the sympathetic nervous system. *Vits Horms* **45**: 1–125, 1990.
39. Vance JE, The use of newly synthesized phospholipids for assembly into secreted lipoproteins. *Biochim Biophys Acta* **1006**: 59–69, 1989.
40. Scott BL and Bazan NG, Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. *Proc Natl Acad Sci USA* **86**: 2903–2907, 1989.
41. Zhang ZJ, Wilcox HG, Elan MB, Castellani LW and Heimberg M, Metabolism of n-3 polyunsaturated fatty acids by the isolated perfused rat liver. *Lipids* **26**: 504–511, 1991.