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CORTICOSTEROIDS AND LUNG SURFACTANT LEVELS IN ADULT MALE RATS

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Summary—Following lung instillation in adult male rats of 3.4 μmol hexavalent chromium ($K_2Cr_2O_7$) dissolved in 0.5 ml of 0.9% NaCl, increased levels of lung surfactant could be detected after 48 h. The blood serum concentration of corticosterone was elevated in these animals. Blood serum thyroxine and triiodothyronine showed an initial increase after lung instillation of hexavalent chromium followed by a decline. Metabolism of testosterone by the alveolar macrophages to 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α,17β-diol was reduced 6 and 12 h after the $K_2Cr_2O_7$ instillation, which was also associated with damage of lung cell function and decreased uptake by the alveolar macrophages of *Candida albicans* particles. As early as 12 h after s.c. administration of 400 μg dexamethasone/100 g body wt, increased levels of lung surfactant could be measured. At this time the lungs showed no signs of cellular damage, and metabolism of testosterone as well as uptake of *Candida albicans* particles by the alveolar macrophages were normal. Lower s.c. doses of dexamethasone did not result in raising the levels of lung surfactant in 12 h. Within 12 h after s.c. administration of large doses of testosterone, dihydrotestosterone or dehydroepiandrosterone no measurable effects on the levels of lung surfactant could be measured. Since animals treated with dexamethasone (200 μg/100 g body wt) or long-acting synthetic ACTH (100 μg i.m. Synacthen Depot/100 g body wt) for 5 days after lung instillation of $K_2Cr_2O_7$ had extremely high levels of lung surfactant, it is concluded that the corticosteroids in adult rats may help to create augmented surfactant levels following lung intoxication. This could proceed via stimulation of surfactant production and reduction of surfactant removal. Different aspects of lung surfactant metabolism are discussed.

INTRODUCTION

The inner surface of the lung alveoli is lined with surfactant, a lipoprotein-rich material which lowers surface tension, reduces the work necessary for breathing and prevents lung collapse on expiration [1, 2]. It is evident from experiments that the glucocorticosteroids are important for levels of lung surfactant before birth and in the immature animal, and this class of compounds has been used as therapy in human infants suffering from respiratory distress syndrome [3]. However, the regulatory role of glucocorticosteroids on lung surfactant levels in adult animals appears unsettled [1, 2, 4].

Type II alveolar epithelial cells (granular pneumocytes) are the source of the lung surfactant [1, 2]. These are the major lipogenic cells in the adult rat lung [5]. It is commonly held that the lamellar inclusion bodies in Type II alveolar epithelial cells promote secretion of the surfactant to the lung alveoli [1, 6]. The surfactant is removed from the alveoli via the mucociliary escalator [7], phagocytosis by the alveolar macrophages [8] and lymphatic drainage [9]. While reutilization of surfactant compounds can take place in the lung [10], the rate of surfactant metabolism is rapid. Turnover times of 4–11 h are recorded for adult rabbits [11] and rats [12].

For some time, our laboratories have been interested in the effects of welding fumes on the lung. We have found that the content of hexavalent chromium in fumes from welding of stainless steel with stainless steel electrodes contributes most to the toxic lung effects [13, 14]. Since lung instillation of a solution containing hexavalent chromium ($K_2Cr_2O_7$, called Cr VI in the following text) is followed by augmentation of lung surfactant levels in adult rats [24], we decided to investigate whether such instillation is associated with increased concentrations of circulating corticosterone and whether administration of corticosteroids to adult rats causes increases in lung surfactant levels.

MATERIALS AND METHODS

The experiments were conducted in adult male Wistar rats ranging in body wt from 200 to 250 g. The animals were kept under controlled light (14 h light and 10 h darkness) and temperature (19–21°C) conditions. Rat chow and tap water were provided *ad libitum*.

Steroids were dissolved in 0.9% NaCl and administered subcutaneously in 0.5 ml of vehicle. Long-acting synthetic ACTH (Synacthen Depot) was given intramuscularly in 0.2 ml of 0.9% NaCl.

Lung instillation was done under halothane anaesthesia [13]. The animals were instilled either with 0.5 ml of 0.9% NaCl or with 0.5 ml of 0.9% NaCl containing 3.4 μmol of Cr VI.

The animals were sacrificed by CO_2 asphyxiation and blood was collected from the heart for the

estimation of corticosterone [15], total thyroxine (tT_4) and total triiodothyronine (tT_3) by published techniques [16, 17]. The lungs and trachea were removed intact. The lungs were lavaged 6 times, using 8 ml of 0.9% NaCl each time [13, 14], and the lavage fluid pooled. After the second lavage, 0.4 ml of the lung wash was removed, filtered (Millipore 0.2 μm) and the filtrate assayed for N-acetyl-β-glucosaminidase (β-NAG) and lactate dehydrogenase (LDH) activities [13, 14]. The cells in the lavage fluid from each animal were counted. Air-dried smears from this fluid were fixed in 1.25% glutaraldehyde, stained with Giemsa and the percentage of polymorphonuclear leucocytes (% PMN) determined from these slides.

The lavage fluid from at least 4 rats belonging to the same experimental group was pooled and centrifuged at 300 **g** for 20 min at 4°C. The supernatant layer was removed and centrifuged at 1000 **g** for 60 min at 4°C. The resulting small pellet was suspended in 21% NaCl and centrifuged at 1500 **g** for 25 min at 4°C. A three-phase separation resulted. The lipoprotein-like material floating on the top, was removed, dialyzed against distilled water for 24 h, freeze-dried and weighed. This fraction was designated pulmonary surfactant [18]. Changes in surfactant levels, β-NAG and LDH activities in lavage fluid were evaluated for significance as published [13, 14, 19–21].

Techniques published by our laboratories [15, 22] were used to measure the ability of pulmonary macrophages to convert testosterone to 17β-hydroxy-5α-androstane-3-one (5α-DhT) and 5α-androstane-3α,17β-diol (3α-ADIOL) *in vitro*. The formation of 5α-androstane-3β,17β-diol when incubating alveolar macrophages in this system is below the limits of detection [15, 22].

The phagocytic capacity of bovine and rat alveolar macrophages was measured by uptake of fluorescent *Candida albicans* particles [23]. Bovine alveolar macrophages were collected and cultured *in vitro* as published [24]. The cells were then exposed to either 30 nM Cr VI/ml incubation medium or to 10^{-4} M dexamethasone/ml incubation medium for 17 h. The incubation medium was then changed and fresh medium containing ^{125}I-labelled *Candida albicans* particles added, keeping the alveolar macrophages: *Candida albicans* particles relationship at 1 : 10. After incubation for 30 min the culture dishes were washed with 0.9% NaCl and fixed in 3.7% formaldehyde for 1 h. The number of *Candida albicans* particles inside the incubated cells could then be counted by use of a fluorescence microscope. Such experiments *in vitro* were also done on alveolar macrophages from rats either exposed *in vivo* to Cr VI instillation for 48 h or to dexamethasone (s.c., 400 μg/100 g body wt) for 12 h. No Cr VI or dexamethasone were added *in vitro* to these macrophages and the exposure to *Candida albicans* particles lasted for 1 h.

The sources for chemicals and reagents used in this work have been recorded elsewhere [15, 20, 21].

RESULTS

Following lung instillation of 3.4 μmol of Cr VI in 0.5 ml saline, no significant increase in levels of lung surfactant could be observed for the first 24 h (Table 1). Twelve h after this instillation, augmented activity of β-NAG and LDH activity could, however, be found in lung lavage fluid, showing increased activity of lysosomal enzymes [25] due to damage of the plasma membrane of lung cells [26]. Using these criteria for cell function, damaged lung cells were present for the ensuing 7 days following lung instillation of this dose of Cr VI [20]. Significant increase in the levels of lung surfactant could first be measured 48 h after the Cr VI instillation. For the following 5 days this level was elevated (Table 1).

Significant augmentation in the levels of lung surfactant was seen 12 h after s.c. administration of 400 μg dexamethasone/100 g body wt. At this time, damaged function of lung cells could not be detected in any of the dexamethasone-treated rats. High doses

Table 1. Changes in levels of lung surfactant and in activity of lactate dehydrogenase (LDH) and N-acetyl-β-glucosaminidase (β-NAG) in lung lavage fluid from male rats subjected to lung instillation or subcutaneous administration

	Pulmonary surfactant (mg/rat) (h after treatment)							β-NAG (% of control)	LDH (% of control)
Treatment	3	6	12	24	48	96	168	12 h after treatment	12 h after treatment
NaCl instillation	1.04	0.67	0.89	0.90	0.66	0.60	0.80	100	100
CrVI instillation	0.60	0.74	0.62	0.89	1.65**	1.31**	1.59**	149**	187**
NaCl s.c.	0.82	0.94	0.80					100	100
400 μg* Dex. s.c.	0.90	0.91	1.44**					93	111
200 μg* Dex. s.c.	0.82		1.15					89	72
100 μg* Dex. s.c.	0.82		1.08					73	69
10 μg* Dex. s.c.	0.82		0.98					81	88

*Per 100 g body wt.

**Significantly different from control animals by published criteria incorporating confidence limits of the experimental approach [13, 14, 19, 20].

Table 2. Mean concentration of corticosterone ± SD in blood serum of rats following instillation of 0.5 ml of 0.9% NaCl or 0.5 ml of 0.9% NaCl containing 3.4 μmol of Cr VI

H after instillation	Corticosterone (nmol/l)	
	NaCl	Cr VI
3	502 ± 603 (4)	363 ± 308 (4)
6	427 ± 243 (8)	****868 ± 297 (8)
12	648 ± 436 (4)	739 ± 461 (4)
24	426 ± 326 (12)	**1016 ± 418 (3)
48	757 ± 339 (11)	905 ± 601 (11)
96	390 ± 203 (3)	*951 ± 484 (3)
168	462 ± 191 (7)	437 ± 359 (6)

Number of animals is given in parentheses.
Significance levels by Student's *t*-test. $*P < 0.10$; $**P < 0.025$; $***P < 0.010$; $****P < 0.005$.

of testosterone, 5α-DhT or dehydroepiandrosterone did not change the levels of lung surfactant within 12 h after s.c. administration [15], and s.c. doses of dexamethasone below 400 μg/100 g body wt did not increase the levels of lung surfactant for 12 h after the administration (Table 1).

Lung instillation of 0.5 ml saline or of 0.5 ml saline containing 3.4 μmol of Cr VI resulted in increased concentrations of serum corticosterone (Table 2). Mean serum concentration of this steroid in 8 adult Wistar rats of the same age and subjected to sacrifice by CO_2 asphyxiation was 189 nmol/l ± 123 (SD). Addition of Cr VI to the instilled saline gave significantly higher concentrations of serum corticosterone 6, 24 and 96 h after the Cr VI instillation compared to rats instilled with 0.5 ml saline alone (Table 2). Three hours after the Cr VI instillation, serum tT_4 and tT_3 were already increased (Table 3). In contrast to the serum corticosterone levels, circulating tT_4 and tT_3 fell in the Cr VI instilled rats during subsequent days.

Alveolar macrophages removed from rats 6 and 12 h following Cr VI instillation showed reduced metabolism of testosterone to 5α-DhT and 3α-ADIOL *in vitro* (Table 4). No such effect could be seen in alveolar macrophages removed from rats 6 and 12 h after s.c. administration of 400 μg dexamethasone/100 g body wt (Table 4). Moreover, rats treated subcutaneously with high doses of testosterone, 5α-DhT or dehydroepiandrosterone for 12 h had alveolar macrophages metabolizing testosterone in a normal fashion [15]. In rats instilled with 3.4 μmol of Cr VI the ability of the alveolar macrophages to metabolize testosterone was depressed before an increase in percentage of PMN could be found in the lavage fluid [15]. At the time when s.c. administration of 400 μg dexamethasone/100 g body wt had produced a significant increase in the levels of lung surfactant, the uptake of *Candida albicans* particles by the alveolar macrophages from such rats was normal (Table 5). This uptake was decreased by alveolar macrophages from rats showing increased levels of lung surfactant 48 h after the Cr VI instillation (Table 5). When male rats were given s.c. doses (10, 100 or 200 μg/100 g body wt) of dexamethasone daily for 5 days following the Cr VI lung instillation, high levels of lung surfactant were found 7 days after the instillation. This effect could also be produced by i.m. administration of long-acting synthetic ACTH given for the same time (Table 6). Such administration of hormones did not

Table 4. Metabolism of testosterone to 17β-hydroxy-5α-androstane-3-one (5α-DhT) and 5α-androstane-3α,17β-diol (3α-ADIOL) by alveolar macrophages from male rats following instillation of 0.5 ml of 0.9% NaCl, instillation of 0.5 ml of 0.9% NaCl containing 3.4 μmol of Cr VI, s.c. administration of 0.5 ml of 0.9% NaCl or s.c. administration of 0.5 ml of 0.9% NaCl containing 400 μg dexamethasone/100 g body wt

Treatment	Time after treatment (h)		
	3	6	12
NaCl instillation	100 ± 11	100 ± 15	100 ± 31
Cr VI instillation	81 ± 24	^31 ± 4	^59 ± 23
NaCl s.c.	100 ± 11	100 ± 22	100 ± 12
Dexa. s.c.	94 ± 9	117 ± 22	116 ± 17

The sum of the steroids 5α-DhT and 3α-ADIOL, expressed as ng steroids/100 mg alveolar protein, was used as a measure for steroid 5α-reductase activity. Enzyme activity is given as percentage of controls.
A: significantly different from control.

Table 3. Mean concentration of total triiodothyronine (tT_3) and total thyroxine (tT_4) ± SD in blood serum of male rats following instillation of 0.5 ml of 0.9% NaCl or 0.5 ml of 0.9% NaCl containing 3.4 μmol of Cr VI

H after instillation	tT_3 (nmol/l)		tT_4 (nmol/l)	
	NaCl	Cr VI	NaCl	Cr VI
3	**0.78 ± 0.05	0.98 ± 0.15	****35.0 ± 3.4	49.0 ± 5.7
6	****1.12 ± 0.10	0.87 ± 0.12	54.3 ± 5.6	54.1 ± 8.8
48	****1.16 ± 0.07	0.95 ± 0.19	***60.0 ± 7.1	47.9 ± 9.6
168	*1.17 ± 0.14	1.02 ± 0.24	****64.4 ± 7.8	47.0 ± 8.6

Experiments 6 and 168 h after instillation were conducted in groups of 6 rats. The rest of the experiments were done in groups of 8 rats.
Significance levels by Student's *t*-test: $*P < 0.10$; $**P < 0.025$; $***P < 0.010$; $****P < 0.005$.

Table 5. Incorporation of *Candida albicans* particles in rat alveolar macrophages following instillation of 0.5 ml of 0.9% NaCl, instillation of 0.5 ml of 0.9% NaCl containing 3.4 μmol of Cr VI, s.c. administration of 0.5 ml of 0.9% NaCl or s.c. administration of 0.5 ml of 0.9% NaCl containing 400 μg dexamethasone/100 g body wt. Incorporation of *Candida albicans* particles was also investigated in normal bovine alveolar macrophages 17 h following exposure *in vitro* to either 30 nmol Cr VI/ml incubation medium or 10^{-4} M dexamethasone/ml incubation medium

Condition	Average number ± S.D. of *Candida albicans* particles inside a lung macrophage	No. of lung macrophages examined
12 h after 0.5 ml of NaCl s.c.	3.1 ± 2.7	263
12 h after dexamethasone s.c.	2.8 ± 2.4	300
48 h after NaCl instillation	4.0 ± 3.3	104
48 h after Cr VI instillation	A2.3 ± 2.0	92
Bovine alveolar macrophages		
17 h after 0.9% NaCl *in vitro*	2.0 ± 2.0	167
17 h after 10^{-4} M dexamethasone *in vitro*	2.3 ± 2.2	130
17 h after 30 nmol of Cr VI *in vitro*	A0.2 ± 0.5	82

A: significantly different from control.

alter lung damage caused by the Cr VI instillation [20]. Administration of dexamethasone or of long-acting synthetic ACTH for 5 days also gave elevated lung surfactant levels in animals instilled with 0.5 ml saline alone (Table 6).

DISCUSSION

Lung instillation of 0.5 ml of 0.9% NaCl in adult rats is evidently a "stress" since circulating concentrations of corticosterone will increase and remain increased for some time afterwards. The levels of lung surfactant in these animals are, however, unaltered [20, 21] compared with non-instilled rats. Considerable individual variations can be seen in the corticosterone response following saline instillation. Addition of 3.4 μmol of Cr VI to the instilled saline raises further the levels of circulating corticosterone as when a "stress" is added to a "stress" [27], but 48 h after the Cr VI instillation, increased levels of lung surfactant can first be measured. At this time the ability of the alveolar macrophages to take up *Candida albicans* particles is reduced, the lung has undergone rather severe cellular damage [20, 21] and the rate of surfactant removal via the alveolar macrophages is probably impaired. Since the corticosterone levels in the Cr VI instilled rats are increased, one may ask whether this increase has given cytotoxic effects on the alveolar macrophages [28] and/or decreased the alveolar macrophage population [28]. A single dose of dexamethasone will not influence the number of alveolar macrophages in Swiss mice within 12 h [29]. Subcutaneous administration of a large dose of dexamethasone produces increased levels of lung surfactant in 12 h. Since the metabolic function of the alveolar macrophages and of other lung cells is normal at this time, the effects on surfactant levels could be due to increased production and/or secretion. In light of the fact that lower s.c. doses of dexamethasone showed no effects on surfactant levels within 12 h, the physiological importance of glucocorticosteroids in controlling surfactant levels in adult animals remains dubious. The adult lung has, however, specific receptors for this class of steroid hormones [30].

Increased concentrations of glucocorticosteroids could, however, aid the intoxicated lung to raise the levels of lung surfactant, thus helping to achieve a more adequate situation for the breathing processes in a damaged organ. Illustrations of this view can be found in Table 6. Long-acting synthetic ACTH has an effect on lung surfactant levels in the NaCl and the Cr VI instilled animals similar to that of dexamethasone. This shows that the endogenous adrenocortical capacity in the adult rat can enhance the levels of lung surfactant provided that the adrenal gland is stimulated for several days. During conditions of continuous stimulation of the adrenal cortex, as seen in rats following Cr VI instillation (Table 2), the adrenocortical capacity of the adult rat can augment the levels of lung surfactant. Seven hours and 1 day after i.v. administration of $Na_2{}^{51}CrO_4$ in female rats, retention of ^{51}Cr in the adrenal gland has been noted [31]. Whether this retention affects adrenal function is, however, unknown. It is of interest that animals given dexamethasone post Cr VI instillation produced higher levels of lung surfactant than achieved by Cr VI instillation alone (Table 6). The upper limit in surfactant levels has not been reached in the intoxicated lung but surfactant removal may be impaired following Cr VI instillation [28, 29]. Dexamethasone or long-acting ACTH administration for 5 days gave increased levels of lung surfactant in rats instilled with saline only (Table 6). The "stress" of saline instillation (Table 1) will not increase the levels of lung surfactant unless aided by dexamethasone or continual increased adrenocortical activity.

In addition to high levels of serum corticosterone, lung instillation of Cr VI is also associated with increment in serum tT_4 and tT_3. This increase is, however, of short duration and a fall in the circulating concentration of these hormones is subsequently seen. It could be that the high concentration of serum

Table 6. On day 1, 0.5 ml of 0.9% NaCl or 0.5 ml of 0.9% NaCl containing 3.4 μmol of Cr VI were instilled, followed by daily administration of different doses of dexamethasone or of ACTH (Synacthen Depot) for the ensuing 5 days

Pulmonary surfactant	Control animals (NaCl instilled and NaCl s.c.)	10 μg Dex.s.c.*		100 μg Dex.s.c.*		200 μg Dex.s.c.*		100 μg ACTH i.m.*	
		NaCl	Cr VI	NaCl	Cr VI	NaCl	Cr VI	NaCl	Cr VI
(mg/rat)	0.54	0.66	2.04†	1.24†	2.99‡	1.13†	1.96†	1.30†	1.65†

Lung surfactant was examined 7 days after the lung instillation. Cr VI refers to Cr VI instilled animals, NaCl refers to 0.9% NaCl instilled animals.

*Per 100 g body wt.

†Significantly different from control animals by published criteria incorporating confidence limits of the experimental approach [13, 14, 19, 20].

‡Significantly different from animals instilled with Cr VI and receiving s.c. 0.5 ml of 0.9% NaCl daily for the following 5 days. An average concentration of 1.22 mg pulmonary surfactant/rat was found in such animals. This concentration is significantly different [13, 14, 19, 20] from that of the control animals of this table.

corticosterone in the Cr VI instilled animals depresses the peripheral conversion of T_4 to T_3 between the 3rd and the 6th hour after the Cr VI instillation [32]. The observed pattern in serum tT_4 and tT_3 has also been noted in young men following prolonged physical strain though tT_3 did not increase before it fell significantly in blood [17]. The fall in serum concentration of the thyroid hormones may reduce the turnover time of the lung surfactant [12] and thus help build up the surfactant levels in the Cr VI intoxicated lung. While thyroid hormones will stimulate these levels in immature animals [1, 2], their possible effect on surfactant levels in mature animals is more controversial. Specific receptors for the thyroid hormones can, however, be found in the adult lung [33].

Metabolism of testosterone to 5α-DhT and 3α-ADIOL was already reduced in alveolar macrophages 6 h after lung instillation of Cr VI. Since the steroid 5α-reductase in alveolar macrophages from rats can use different Δ^4-steroids as substrate [22], the presence of increased concentrations of such steroids in the surfactant could reduce the metabolism of the added substrate. One day following burn injury in male guinea-pigs the concentration of cortisol in the lung surfactant is unchanged though the blood plasma concentration of this hormone is significantly elevated [34]. Moreover, alveolar macrophages from rats 12 h after s.c. administration of 400 μg dexamethasone/100 g body wt show normal testosterone metabolism. It is remarkable how fast metabolism of testosterone by the alveolar macrophages is reduced following instillation of Cr VI. This metabolism is also reduced in whole lung tissue from the 6th to the 24th hour following Cr VI instillation [15]. During the early phase of Cr VI instillation more of the glucocorticosteroid hormones delivered to the lung is present in an unmetabolized state in this organ and should be available to act on lung production and/or secretion of lung surfactant making the breathing easier during the initial phase of lung intoxication with Cr VI. Following lung injury, the alveolar macrophages may change the concentration of receptors for the glucocorticosteroids [35] as well as reduce the metabolism and inactivation of these hormones. Of some interest is our observation that depression of testosterone metabolism can be measured in the alveolar macrophages from Cr VI instilled rats before the percentage of PMN is increased in such animals [15]. Increases in percentage of PMN is one of the more commonly used indexes for lung intoxication [14]. 5α-Reduction of Δ^4-steroid hormones by the alveolar macrophages should thus be explored as an early index for lung exposure to a toxicant.

If the "stress" of lung instillation also is associated with increased activity from the adrenal medulla, adrenergic stimulation of the lung surfactant level

may occur [36]. The alveolar wall of the rat lung shows virtual absence of nerves [37]. Fluid instillation in the lung is undoubtedly followed by increased respiration. This would involve changes in the activities of the adrenergic and cholinergic nerves to this organ [38]. Type II alveolar epithelial cells have receptors for both cholinergic and β-adrenergic agonists, but only the latter receptors will stimulate surfactant levels in the lung [39].

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