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Dexamethasone and methylprednisolone affect rat peritoneal phagocyte chemiluminescence after administration in vivo

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Abstract

Production of reactive oxygen compounds by peritoneal monocytes/macrophages was studied in rats exposed to dexamethasone or methylprednisolone in the drinking water. Luminol-amplified chemiluminescence was measured in preparations of peritoneal leukocytes activated ex vivo by serum opsonized zymosan, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) or phorbol 12-myristate 13-acetate (PMA). After dexamethasone administration for 1 day (~ 0.13 mg/kg per 24 h) a significant reduction in chemiluminescence was found in cells stimulated with serum opsonized zymosan, while responses to fMLP and PMA stimulation were significantly reduced after 2 days. The maximal inhibition obtained after 5–8 days of dexamethasone administration (plasma levels < 5 nM) was $92.0 \pm 1.2\%$, $87.6 \pm 0.2\%$ and $84.5 \pm 3.1\%$ in cells stimulated with serum opsonized zymosan, fMLP and PMA, respectively. Administration of dexamethasone or methylprednisolone for 48 h gave a dose-dependent reduction of chemiluminescence. ED_{50} values of dexamethasone were estimated at 0.06–0.15 mg/kg for the different stimulators (plasma concentrations 5–10 nM). Estimated ED_{50} values for methylprednisolone were 35–36 mg/kg. Since the percentage of mononuclear phagocytes in the peritoneal cell population did not change significantly with dose or time of dexamethasone exposure, this study indicates that glucocorticoids have a depressive effect on the monocyte/macrophage 'respiratory burst' in vivo. The results are consistent with the hypothesis that these effects are mediated by glucocorticoid receptors. Although the pathway activated by serum opsonized zymosan was more rapidly inhibited than the fMLP- and PMA-activated pathways, the responses induced by the different stimulators were similarly affected, suggesting a modulation of common components in the activation pathways, possibly protein kinase C or the NADPH-oxidase complex, after administration of low pharmacological doses of glucocorticoids in vivo.

Keywords: Glucocorticoid; Peritoneal leukocyte; Respiratory burst; Activation, ex vivo; (Rat)

1. Introduction

Cells of the monocyte/macrophage lineage are of prime importance in many specific and non-specific immune processes. The ability of these cells to generate highly reactive oxygen species, e.g. the superoxide anion ($O_2^-\cdot$) and hydrogen peroxide (H_2O_2), following activation of a membrane associated NADPH-oxidase, constitutes an important part of the host defence system against invading micro-organisms (Babior, 1984). Although initially recognised for their microbicidal properties, the oxidants produced in 'the respiratory burst' have also been implicated in a number of inflammatory diseases including arthritis (Halliwell et al., 1985), in ischemic tissue injury (Arroyo et al., 1987), atherosclerotic lesions (Cathcart et al., 1989) and asthma (Vachier et al., 1992). A better understanding of the activation and regulation of 'the respiratory burst' therefore provides a basis for the design of therapeutic measures against infection and inflammation.

Glucocorticoids are among the most potent and widely used anti-inflammatory agents and affect the formation, tissue distribution and functions of immune competent cells (Cupps and Fauci, 1982; Munck and Guyre, 1991). The effect of anti-inflammatory steroids on oxygen radical production by phagocytes is less extensively studied, and contradictory results have been reported. In vitro studies have demonstrated inhibitory effects of high concentrations of glucocorticoids on

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oxygen radical production in human granulocytes (Goldstein et al., 1976; Jones et al., 1983; Umeki and Soejima, 1990) and in guinea pig monocytes (Õyanagui, 1976). In contrast, dexamethasone treatment has been reported not to interfere with the secretion of reactive oxygen intermediates in human blood derived macrophages in culture (Schaffner and Schaffner, 1987), and even a stimulatory in vitro effect of glucocorticoids on chemiluminescence in human monocytes has been demonstrated (Haar and Nielsen, 1988). Little in vivo data are available, but no effect on superoxide anion production in murine peritoneal inflammatory cells has been reported after corticosterone administration (Fleming et al., 1991), or in human alveolar macrophages after local budesonide treatment (Bergstrand et al., 1990). In contrast, in vivo treatment with a single dose of cortisol or dexamethasone has been shown to cause reduced superoxide anion radical production in a mixture of peripheral blood leukocytes (Nelson and Ruhmann-Wennhold, 1978; Nelson et al., 1978).

The present study was performed to achieve a better characterisation of the in vivo effect of glucocorticoids on 'the respiratory burst' activity of mononuclear phagocytes. We administered synthetic glucocorticoids to rats in different doses and for various periods of time (1–8 days). In order to minimise stress responses in the experimental animals, which might affect oxygen radical production, the steroids were given in the drinking water. Monocytes and macrophages were obtained by peritoneal lavage, and smears were examined to study whether glucocorticoids affect the differential distribution of the peritoneal cells. The ability of the peritoneal cells to produce reactive oxygen metabolites was measured with luminol-amplified chemiluminescence, and three stimulators which induce 'the respiratory burst' by different activation pathways were used in an attempt to evaluate the cellular mechanisms underlying the observed effects of the glucocorticoids.

2. Materials and methods

2.1. Animals

Male Wistar Kyoto (WKY) rats, 200–300 g (10–14 weeks), were obtained from Møllegaard Breeding Centre A/S (Ejby, Denmark) and used after 2 weeks of acclimatisation to their housing conditions. Standard rat chow pellets were available ad libitum, and the animals were maintained under a 12–12 h light/dark cycle (lights on 7 a.m. to 7 p.m.) with temperature and humidity at 22°C and 40–60%, respectively. Each rat was housed individually two days before the beginning of the experiments.

2.2. Dose-response studies with dexamethasone and methylprednisolone

46 animals were assigned to dose-response studies and examined during 48 h. Controls ($n = 14$) got only tap water, while glucocorticoid-treated animals received water supply containing 0.4–500 μM dexamethasone 21-phosphate ($n = 18$), or 10–1000 μM 6α-methylprednisolone 21-hemisuccinate (Sigma Chem. Co., St. Louis, MO, USA) ($n = 14$).

2.3. Dexamethasone administration for various periods of time

The experiment was designed to allow the animals to receive a constant concentration of dexamethasone 21-phosphate (2 μM ~ 0.13 mg/kg per 24 h) in their water supply for a period of 1–8 days ($n = 24$). All the animals were killed after a total experimental period of 8 days, receiving only tap water in the 1–7 'glucocorticoid-free' days before dexamethasone administration. Controls ($n = 5$) got tap water during the whole experimental period.

2.4. Collection of peritoneal leukocytes

The rats were killed by decapitation at about 10 a.m. and trunk blood was collected in chilled tubes with 40.8 μmol EDTA. Care was taken to avoid stress during handling of the animals, and decapitation took place within 1 min after entry in the animal quarters. Peritoneal cells were obtained by washing the peritoneum with 30 ml of 0.9% NaCl and were stored on ice (4°C). Cells were pelleted, washed in 0.9% NaCl and resuspended in Earle's balanced salt solution (EBSS) supplied with 20 mM Hepes buffer and 5 mM glucose. Cell numbers were counted electronically, and smears for light microscopy differential counts were prepared.

2.5. Peritoneal leukocyte chemiluminescence

Peritoneal cells (1.3×10^6 cells/ml, kept on ice) were mixed with a prewarmed buffer solution (37°C) containing one of the three different activators: serum opsonized zymosan particles (0.5 mg/ml); *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP; 1.0 μM) or phorbol 12-myristate 13-acetate (PMA; 0.1 μM) (Sigma). All experiments included 80 μM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (Sigma) in a total volume of 0.25 ml. Luminol-amplified chemiluminescence was measured in duplicate samples every 5 min up to 25 min at 37°C in a LKB Wallace 1251 luminometer as described previously (Trush et al., 1978; Wiik, 1989): the maximal chemiluminescence responses induced by the different stimulators are presented. In this experimental design the peak values were recorded

20 min after stimulation with serum opsonized zymosan, and 10 min after stimulation with fMLP and PMA.

In a control experiment, dexamethasone and methylprednisolone up to 10 μM did not directly modulate luminol-amplified chemiluminescence. A direct interference of glucocorticoids with light emission in the luminol-amplified chemiluminescence response has been reported, but only by very high concentrations (mM) of betamethasone (Haar and Nielsen, 1988). The very low levels of steroids present in the peritonal cell suspension after two consecutive washes will therefore have negligible impact on the chemiluminescence response.

2.6. Plasma dexamethasone

Plasma dexamethasone levels were determined by using a modification of the technique described by Dawson et al. (1984) and Gardiner et al. (1986). All plasma samples (1–1.5 ml) were deproteinised by the addition of methanol (2 ml/ml of plasma) which contained 0.5 μM of 11-deoxy-17-hydroxycorticosterone added as an internal standard, followed by vortex mixing for 10 s, and then centrifuging for 10 min at 4°C. The supernatant was separated and diluted with double-distilled, deionised water (2 ml/ml of plasma). Extraction columns (C_8, 3CC, Bond Elut, Analytichem International), connected to a suction apparatus, were activated with 3 ml of methanol and 2 ml of methanol in water [12.5 mol/l (40%, v/v)] before the diluted, deproteinised plasma samples were passed through the columns. The columns were then washed with 2 ml of water, 1 ml of methanol in water [3.12 mol/l (10%, v/v)] and 2 ml of acetone in water (4.54 mol/l). Methanol (0.2 ml) was drawn through the columns and discarded before the corticosteroids and internal standard were eluted with a further 2 ml of methanol. The extracts were lyophilised, reconstituted in 50 (or 100) μl of mobile phase (30% acetonitrile) and injected on to a high-performance liquid chromatography system (HPLC) for separation by reverse phase chromatography and quantification by u.v. absorption (260 nm), using a Supelco column (Supelcosil LC-18-DB, 25 cm × 4.6 mm ID, 5 μm). The mobile phase was acetonitrile/water (30%, v/v), pumped at 2.0 ml/min. Peak integral analyses of dexamethasone and internal standards in the samples and external standards were used to determine plasma levels of dexamethasone. The lower limit of detection was 5 nM.

2.7. Statistical methods

Data are given as means ± S.E.M. Multiple comparisons between groups were done with analysis of variance, followed by an unpaired *t*-test when only two groups were compared. Correlations are given as Pearson's product moment correlations. Differences among groups were considered significant if $P < 0.05$. Intake of water as well as concentration of glucocorticoids in the drinking water varied between the animals. To be able to evaluate possible statistically significant effects of dose for the different glucocorticoids, the intake of glucocorticoids (mg/kg) was categorised into nine groups where category 0 represents control animals. Glucocorticoid intake in the different categories (1–8) was: 0.0–0.1, 0.1–0.3, 0.3–1.0, 1.0–3.0, 3.0–10.0, 10–30, 30–56 and > 56 mg/kg. ED_{50} values for the dose-response relationships were estimated by non-linear regression analysis using the data program Sigma Plot (Jandel Scientific, Erkrath, Germany). A sigmoidal curve was fitted to the individual data according to a four-parameter logistic function. Logarithmic values for dexamethasone and methylprednisolone intake were used in the regression analysis, and log ED_{50} ± log S.E.M. were calculated. ED_{50} values are given with corresponding intervals (inverse logarithmic) in brackets.

3. Results

3.1. Chemiluminescence response after 1–8 days of dexamethasone treatment

The day to day variation in water volume intake, e.g. glucocorticoid dose, was small: mean water volume intake per day; 37.6 ± 0.9 ml. The effect of dexamethasone (~ 0.13 mg/kg per 24 h) administered for 1–8 days on the production of reactive oxygen compounds by peritoneal leukocytes is shown in Fig. 1. Significant differences in chemiluminescence response with time of dexamethasone exposure were found in cells stimulated with serum opsonized zymosan ($F(8,20) = 18.19$, $P < 0.0005$), fMLP ($F(8,20) = 6.14$, $P < 0.0005$) and PMA ($F(8,20) = 11.66$, $P < 0.0005$). After dexamethasone exposure for 1 day (24 h) a significant reduction in chemiluminescence compared to that of the control group was obtained in cells activated with serum opsonized zymosan ($t(7) = 2.39$, $P = 0.048$), while a significant reduction in the fMLP- ($t(7) = 3.61$, $P = 0.009$) and PMA- ($t(7) = 4.23$, $P = 0.004$) responses was observed after 2 days of dexamethasone administration. The maximal inhibition demonstrated was 92.0 ± 1.2%, 87.6 ± 0.2% and 84.5 ± 3.1% in cells stimulated with serum opsonized zymosan, fMLP and PMA, respectively. Little further inhibition of chemiluminescence was obtained after 5 days with dexamethasone exposure, and no significant differences in percent inhibition were found between animals exposed to dexamethasone for 5–8 days. Plasma concentration of dexamethasone was non-detectable (< 5 nM) in all animals irrespective of the duration (1–8 days) of treatment.

3.2. Chemiluminescence response after dexamethasone and methylprednisolone treatment for 48 h; dose-response relationships

Dose-response curves for the suppressive effect of dexamethasone and methylprednisolone treatment for 48 h on serum opsonized zymosan-, fMLP- and PMA-evoked chemiluminescence are shown in Figs. 2 and 3. Significant overall differences in chemiluminescence were found between the control animals (0 mg/kg), the dexamethasone group and the methylprednisolone group for peritoneal leukocytes stimulated with serum opsonized zymosan ($F(2,42) = 20.74$, $P < 0.0005$), fMLP ($F(2,42) = 9.23$, $P < 0.0005$) and PMA ($F(2,42) = 15.68$, $P < 0.0005$). Dexamethasone inhibited chemiluminescence dose dependently in cells activated with serum opsonized zymosan ($F(8,23) = 9.25$, $P < 0.0005$), fMLP ($F(8,23) = 5.10$, $P = 0.0010$) and PMA ($F(8,23) = 4.79$, $P = 0.0015$). Maximal inhibition was obtained with 3–10 mg dexamethasone/kg (giving plasma concentrations of 70–130 nM) ($n = 4$), where a 97.6 ± 0.4%, 93.6 ± 2.3% and 93.3 ± 1.6% reduction in chemiluminescence response compared to control animals was observed in cells stimulated with serum opsonized zymosan, fMLP and PMA, respectively. ED_{50} values of dexamethasone were 0.15 [0.12–0.19], 0.17 [0.12–0.25] and 0.06 [0.03–0.11] mg/kg with plasma dexamethasone levels of about 5–10 nM. A significant dose-dependent inhibition of chemiluminescence was also found with increasing doses of methylprednisolone in cells activated by serum opsonized zymosan ($F(5,21) = 5.27$, $P = 0.0027$), fMLP ($F(5,21) = 6.31$, $P = 0.001$) and PMA ($F(5,21) = 4.54$, $P = 0.0058$), but here the maximal inhibition observed with dexamethasone was not obtained even in the highest dose-category (> 56 mg/kg). Assuming that the maximal effects of the two glucocorticoids are similar, the ED_{50} values for methylprednisolone were estimated to 35 [10–112], 36 [21–60] and 36 [14–93] mg/kg, illustrating the pronounced

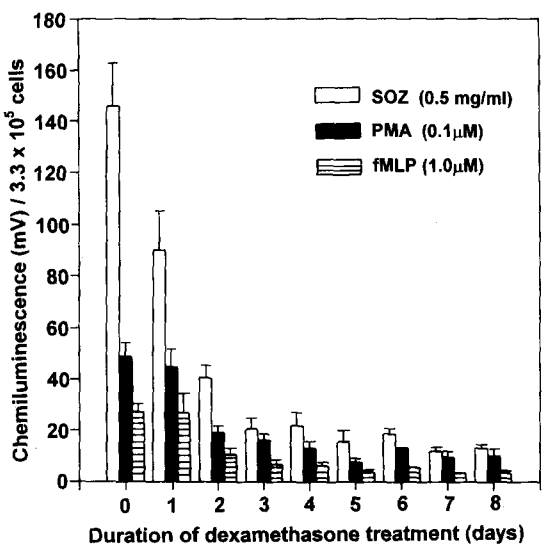

Fig. 1. Effect of dexamethasone 21-phosphate administration of various durations on the chemiluminescence response in rat peritoneal leukocytes. Dexamethasone (2 μM ~ 0.13 mg/kg per 24 h) was administered in the drinking water for 1–8 days. Animals were killed after an experimental period of 8 days, and received only tap water in the 1–7 'glucocorticoid-free' days before dexamethasone exposure. Peritoneal leukocytes were stimulated ex vivo by serum opsonized zymosan (SOZ), *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) or phorbol-myristate-acetate (PMA). Data are means with S.E.M. and represent the peak chemiluminescence response measured 20 min after addition of serum opsonized zymosan and 10 min after addition of fMLP and PMA. $n = 5$, 4 and 2 for control animals (day 0), days 2–4 and days 5–8, respectively.

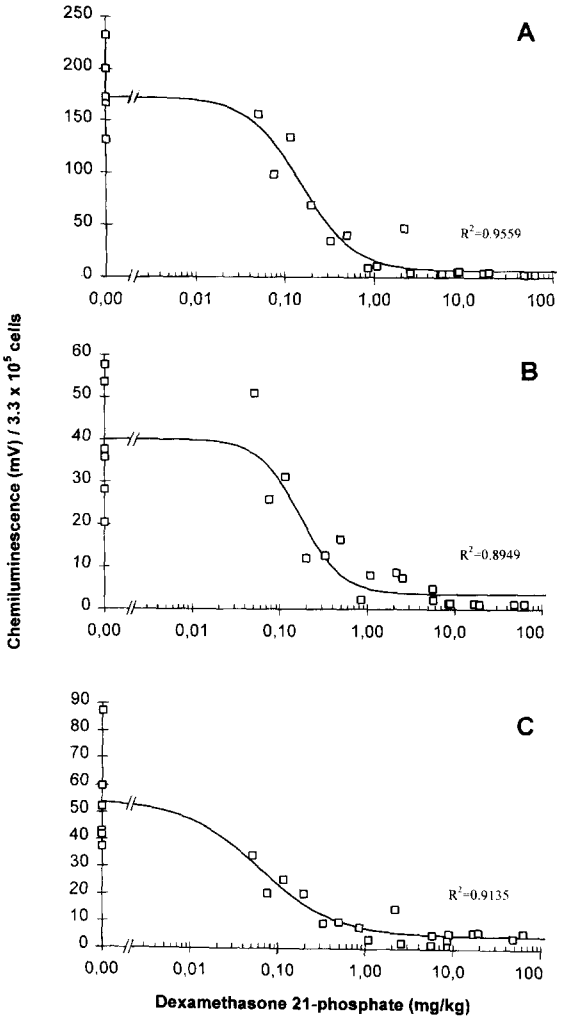

Fig. 2. Effect of dexamethasone 21-phosphate administered in the drinking water (0.4–500 μM) for 48 h on the chemiluminescence response in rat peritoneal leukocytes. Data represent individual peak chemiluminescence values measured 20 min after addition of serum opsonized zymosan (0.5 mg/ml) (A), and 10 min after addition of fMLP (1.0 μM) (B) and PMA (0.1 μM) (C). Half-maximal effective doses were estimated to 0.15 [0.12–0.19] (A), 0.17 [0.12–0.25] (B) and 0.06 [0.03–0.11] mg/kg (C). R^2 = coefficient of determination (n = 24).

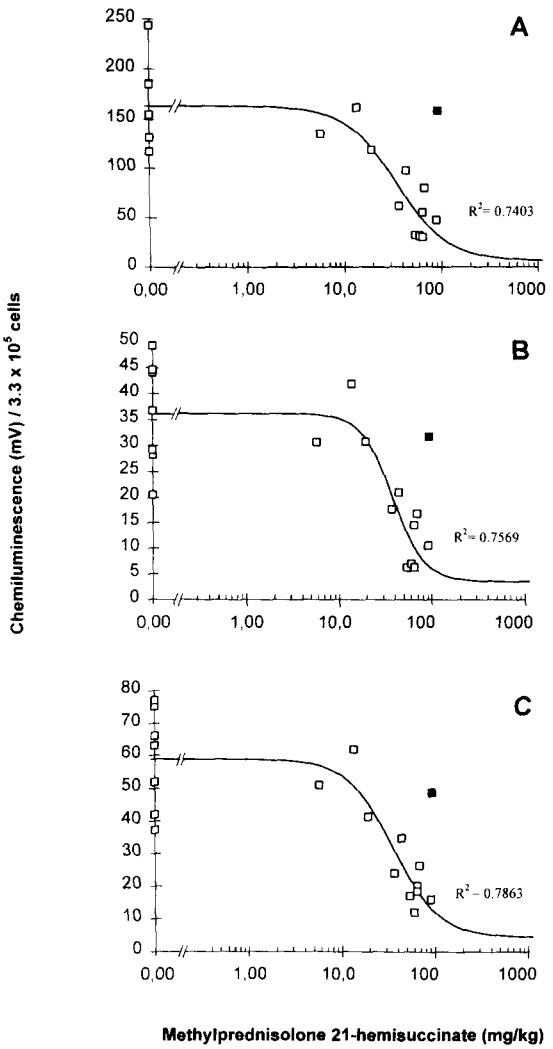

Fig. 3. Effect of methylprednisolone 21-hemisuccinate administered in the drinking water (10–1000 μM) for 48 h on the chemiluminescence response in rat peritoneal leukocytes. Data represent individual peak chemiluminescence values measured 20 min after addition of serum opsonized zymosan (A) and 10 min after addition of fMLP (B) and PMA (C). Half-maximal effective doses were estimated to 35 [10–112] (A), 36 [21–60] (B) and 36 [14–93] (C) mg/kg. ■, values are not included in the regression fitting. R^2 = coefficient of determination (n = 19).

differences in potency of the two glucocorticoids after per oral administration. A significant positive correlation between ingested dose of dexamethasone 21-phosphate and plasma concentration of dexamethasone was observed ($r = 0.7514$, $P < 0.0005$, $n = 32$). The calculated intake of dexamethasone 21-phosphate correlated better with the reduction in chemiluminescence response ($r = -0.8562$, $P < 0.0005$, $n = 32$) than the analysed plasma levels of dexamethasone ($r = -0.4673$, $P = 0.007$, $n = 32$).

3.3. Effects of dexamethasone and methylprednisolone on peritoneal leukocyte numbers

Significant differences in total cell number were found between animals treated with dexamethasone and methylprednisolone ($t(30) = -4.59$, $P < 0.0005$), with dexamethasone showing a significantly higher potency concerning the reduction of total peritoneal cell number (Fig. 4). A significant dose-dependent reduction of the total peritoneal cell number was found after dexamethasone intake for 48 h ($F(8,23) = 8.56$, $P < 0.0005$). The effects of increasing doses of dexamethasone on the individual peritoneal cell numbers are shown in Table 1. Mononuclear phagocytes constituted 71.5 ± 1.5% (n = 9) of the total peritoneal cell population, and no significant differences were found in the percentage of mononuclear phagocytes with increasing doses of dexamethasone administered for 48 h. A significant dose-dependent reduction in the number of eosinophils was observed with increasing dose of dexamethasone ($F(6,17) = 4.97$, $P = 0.041$). In contrast to the strong effect on chemiluminescence, no significant reduction in total cell count or in monocyte/macrophage numbers was obtained after 5–8 days exposure to low doses (~ 0.13 mg/kg per 24 h) of dexamethasone. Furthermore, the percentage of mononuclear phagocytes did not change significantly with the duration of dexamethasone treatment (78.6 ± 1.4%, n = 20)

Table 1
Effect of increasing doses of dexamethasone administered for 48 h on the number of different peritoneal cells

Dexamethasone intake		n	Monocytes	Macrophages	Lymphocytes	Eosinophils	Mast cells
Category	mg/kg						
0	0	9	5.58 ± 1.16	4.77 ± 0.58	2.51 ± 0.61	1.29 ± 0.22	1.76 ± 0.12
1	0.0– 0.1	2	1.66 ± 0.37	7.74 ± 1.15	1.90 ± 0.60	1.13 ± 0.59	1.16 ± 0.08
2	0.1– 0.3	2	4.95 ± 2.93	4.46 ± 1.11	0.79 ± 0.17	0.57 ± 0.19	0.54 ± 0.13
3	0.3– 1.0	3	1.69 ± 0.92	3.28 ± 0.76	0.72 ± 0.10	0.13 ± 0.05	0.82 ± 0.25
4	1.0– 3.0	3	2.18 ± 1.22	3.87 ± 0.22	0.53 ± 0.17	0.18 ± 0.18	0.92 ± 0.17
5	3.0–10	4	2.96 ± 0.93	3.66 ± 0.65	0.88 ± 0.15	0.02 ± 0.02	1.11 ± 0.24
6	10 –30	2	3.28 ± 0.01	2.70 ± 0.60	0.84 ± 0.06	0.02 ± 0.02	0.91 ± 0.29

Cells were isolated by washing the peritoneum with 30 ml of saline. Cell number (millions) are given as means with S.E.M. Category 0 represents control animals. n = number of animals.

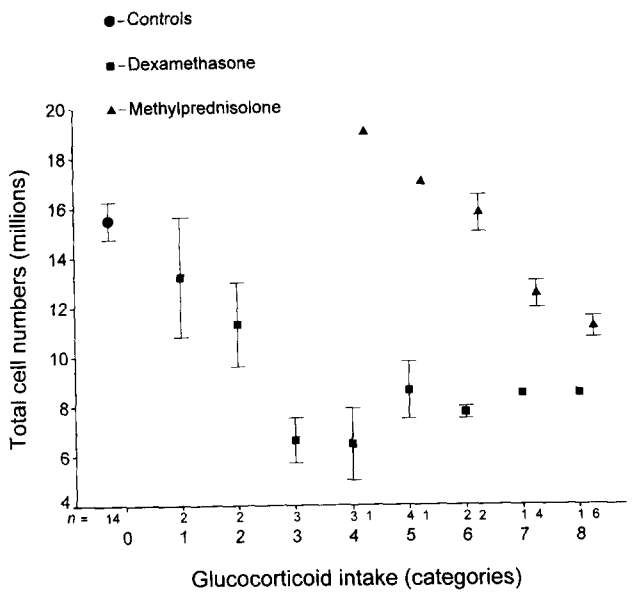

Fig. 4. Total cell number obtained by washing the peritoneum with 30 ml of saline in control animals and animals exposed to methylprednisolone or dexamethasone for 48 h. Cell numbers are given as millions with S.E.M. Glucocorticoid intake was categorised into the following 9 groups (0–8): 0, 0.0–0.1, 0.1–0.3, 0.3–1.0, 1.0–3.0, 3.0–10, 10–30, 30–56, > 56 mg/kg. n = number of animals.

(data not shown). Neutrophil granulocytes could not be detected in the peritoneal lavages.

4. Discussion

In the present study, in vivo administration of dexamethasone or methylprednisolone was shown to give a dose-dependent inhibition of rat peritoneal leukocyte chemiluminescence. The ability to generate a significant 'respiratory burst' has been considered a unique feature of granulocytes and monocytes (Borregaard, 1988). However, chemiluminescence activity, i.e. production of superoxide anion and hydrogen peroxide, has been demonstrated in preparations of B-lymphocytes, but this capacity is rather low in comparison with that of monocytes (i.e. 12–50 times less, dependent on type of stimuli) (Schopf et al., 1982; Leca et al., 1991). Quantitative differences in the chemiluminescence response between blood monocytes and granulocytes have been documented with a peak maximum for monocytes of only 30% of that of neutrophils and eosinophils (Lindena et al., 1987). However, less than 8% of the peritoneal cell population was identified as eosinophil granulocytes, and therefore the chemiluminescence recorded is mainly the response of monocytes/macrophages.

A reduction in total leukocyte counts with increasing doses of dexamethasone was observed and can be ascribed to either an inhibition of stem cell proliferation, or to a redistribution of leukocytes between bone marrow and tissues (Cupps and Fauci, 1982). The percentage of mononuclear phagocytes did not change significantly with the dose or duration of glucocorticoid exposure, which implies that the reduced chemiluminescence cannot be due to diminished numbers of mononuclear phagocytes in the peritoneal cell preparations. No significant change in the ratio of monocytes compared to macrophages was deteced in the present study, as judged by light microscopy. However, during prolonged treatment with dexamethasone a tendency towards an increase in the number of monocytes versus macrophages was observed. Earlier we have reported that administration of corticosterone to rats gave a reduced proportion of macrophages to immature monocytes in cells isolated from the peritoneum (Wiik et al., 1995). This is consistent with in vitro studies demonstrating inhibitory effects of glucocorticoids on monocyte to macrophage differentiation (Rinehart et al., 1982; Baybutt and Holsboer, 1990).

The luminol-amplified chemiluminescence response is a sensitive indicator for the ability of phagocytes to produce reactive oxygen metabolites following activation of 'the respiratory burst' (Allen and Loose, 1976; Allen, 1982). The precise mechanism of light emission, e.g. the oxygen species involved, is still a matter of debate, and the chemiluminescence reaction or the estimated ED_{50} values can not be linearly correlated with the production of a single type of oxygen metabolite. Furthermore, the $O_2^-\cdot/H_2O_2$ generated by the NADPH-oxidase is not sufficient to cause a chemiluminescence response of any magnitude; the presence of the granule enzyme, myeloperoxidase (MPO), is also required for this light-generating reaction (Johansson and Dahlgren, 1989; Albrecht and Jungi, 1993). The extracellular peroxidase is thought to be released through a degranulation process, and the amount of extracellular peroxidase as a limiting factor in extracellular chemiluminescence can be investigated by adding saturating levels of the enzyme (Johansson and Dahlgren, 1989). Addition of saturating levels of peroxidase (horseradish peroxidase) was shown to give a pronounced increase in PMA-evoked chemiluminescence, but the differences between glucocorticoid-treated animals and control animals were not reduced (unpublished results). Therefore, it does not seem likely that the reduced chemiluminescence response in glucocorticoid-treated animals can be due to a modulation of extracellular myeloperoxidase degranulation. However, the possibility remains that the steroids affect the intracellular myeloperoxidase activity, or its distribution, required for the intracellular chemiluminescence response. A progressive reduction of myeloperoxidase activity during in vitro monocyte differentiation has been reported (Seim, 1983; Jungi and Peterhans, 1988), and a reduced chemiluminescence response in mature

macrophages cultured in vitro has been related to the decreased myeloperoxidase activity of these cells (Nyberg and Klockars, 1990; Johansson and Dahlgren, 1992). It does not appear likely that altered myeloperoxidase activity due to changes in the proportion of mature macrophages contributed significantly to the reduction in chemiluminescence, since the glucocorticoids did not significantly alter the ratio of monocytes compared to macrophages in this study.

A time-dependent reduction in chemiluminescence was observed after dexamethasone administration for 1–8 days. This time dependency may theoretically in part be due to dose accumulation obtained with increasing days of glucocorticoid exposure, but the plasma analyses performed did not confirm any accumulation of dexamethasone (< 5 nM, irrespective of duration of dexamethasone treatment). It was, however, difficult to determine minor differences in the plasma analyses because of limitations in the sensitivity of the HPLC method.

It is probable that the effects of glucocorticoids are due to a specific interaction with glucocorticoid receptors and consequently to a change in transcription/translation of glucocorticoid-responsive genes. The glucocorticoid concentrations needed for receptor-mediated effects are low, e.g. the K_d values for binding of dexamethasone to the glucocorticoid receptor in mononuclear leukocytes of WKY are in the nM range (Laux et al., 1989). Non-genomic physiochemical membrane effects of corticosteroids have also been presumed to affect leukocyte function, and these effects are thought to be of importance in some of the in vitro studies where cells are exposed to steroids in very high concentrations (> 1 μM) (Munck et al., 1984). Receptor-mediated effects of glucocorticoids commonly occur after a latency period, i.e. de novo protein synthesis needs a certain time to proceed. The delayed occurrence of effects (days), and the potency difference between dexamethasone and methylprednisolone should not be present if these non-specific actions of glucocorticoids were of importance in the present study. Furthermore, a significant inhibition of chemiluminescence was obtained with plasma levels of dexamethasone in the lower nM range, supporting that the effects are more likely to be mediated by glucocorticoid receptors.

The reduction in chemiluminescence could be due to a direct effect of glucocorticoids on peritoneal phagocytes, but it cannot be excluded that the glucocorticoids also have an indirect effect on phagocyte function. Glucocorticoids are presumed to affect phagocyte function through inhibition of cytokines produced by cells outside the mononuclear cell lineage. Interferon-γ in T-lymphocytes has been shown to give activation and increased chemiluminescence in mononuclear phagocytes in vitro (Jungi et al., 1989), and part of the reduced phagocyte activation can possibly be ascribed to inhibition of lymphocyte interferon-γ production by glucocorticoids (Munck and Guyre, 1991). Physiological parameters like glucose metabolism and insulin secretion can be modulated by glucocorticoids, sometimes inducing a diabetic state (West, 1959). A reduced chemiluminescence response has been observed in phagocytes isolated from hyperglycemic rats (Sato et al., 1992). In the present investigation, a heightened blood glucose (at most, 94% increase) was observed with intake of the highest doses of dexamethasone. However, no significant increase in glucose levels after administration of low doses (0.13 mg/kg per 24 h, giving a highly significant reduction in chemiluminescence) for up to 8 days was obtained. Therefore, the induction of a diabetic state seems to be of minor importance for the effects on chemiluminescence observed in the present study.

Activation of 'the respiratory burst' involves several steps and can be induced by a variety of stimuli. Serum opsonized zymosan activates the $O_2^-\cdot$ generating enzyme by interactions mainly with phagocyte complement receptors type 3 (CR3), which recognize the zymosan-bound complement fragment iC3b (Lindena et al., 1987; Rosen and Law, 1990). The signal transduction initiated by the C3 receptor is not completely elucidated. However, the iC3b-CR3 interaction increases membrane protein kinase C activity (Goldman et al., 1994), which is essential for activation of the NADPH-oxidase (Zor et al., 1993). The tumour promoter PMA binds to and activates protein kinase C, which is then translocated to the cell membrane, where it activates the NADPH-oxidase (Lehrer and Cohen, 1981). The bacterial peptide fMLP binds to chemoattractant receptors with concomitant cleavage of phosphatidyl-inositoldiphosphate (PIP_2) into diacylglycerol and inositoltriphosphate (IP_3). Diacylglycerol activates protein kinase C, and IP_3 elevates free Ca^{2+}, both mechanisms contributing to the activation of the NADPH-oxidase (Babior, 1984). The response obtained by serum opsonized zymosan was significantly inhibited after dexamethasone exposure for 1 day only, while the chemiluminescence evoked by fMLP and PMA was significantly inhibited after intake of dexamethasone for 2 days. Thus, modulation at an 'early step' in the pathway activated by serum opsonized zymosan (e.g. C3 receptors) could account for some of the reduction in chemiluminescence. This is in accordance with earlier in vitro studies, where glucocorticoids in very high concentrations (100–1000 μM) have been shown to inhibit the expression of C3 receptors (Schreiber et al., 1975, Wilkinson et al., 1991). The responses to all three stimulators were similarly affected by the glucocorticoids, suggesting a modulation of some of the common components in the activation pathways, possibly protein kinase C or the NADPH-

oxidase complex, due to a direct interaction of glucocorticoids with chemiluminescence producing cells, and/or to an indirect effect on phagocyte function. A direct effect of dexamethasone on phagocytes has been demonstrated in vitro, giving a dose-dependent reduction of mRNA coding for a component of the NADPH-oxidase complex (Amezaga et al., 1992).

In conclusion, this study demonstrated that synthetic glucocorticoids in low pharmacological doses have a depressive effect on the phagocyte 'respiratory burst' in vivo. The results are consistent with the hypothesis that these effects are mediated via glucocorticoid receptors, possibly resulting in modulation of some common components in the different activation pathways, e.g. protein kinase C or the NADPH-oxidase complex.

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