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Equivalent Doses and Relative Drug Potencies for Non-genomic Glucocorticoid Effects: A Novel Glucocorticoid Hierarchy

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ABSTRACT. Glucocorticoids have three distinct therapeutically relevant effects (genomic, specific non-genomic, and unspecific non-genomic), raising the hypothesis that the relative potencies of non-genomic and genomic effects of glucocorticoids may differ. Therefore, we measured the unspecific non-genomic potencies of five clinically important glucocorticoids and compared them with the classical (genomic) potencies. We studied the immediate glucocorticoid effects on respiration, on protein synthesis, and on Na^+-K^+-ATPase and Ca^{2+}-ATPase in concanavalin A-stimulated rat thymocytes. We titrated the respiration of the cells with methylprednisolone, prednylidene, dexamethasone, prednisolone or betamethasone, and then interpolated the glucocorticoid concentrations needed to inhibit concanavalin A-stimulated respiration back to normal. These "equivalent doses" produced equal inhibition of respiration, of specific energy-consuming pathways, and of the concanavalin A effect on quiescent cells. The relative drug potencies were calculated as the inverse of the equivalent doses normalized to methylprednisolone and were: prednylidene (3.0) > dexamethasone (1.2) > methylprednisolone (1.0) > prednisolone (0.4) > betamethasone (0.2). This hierarchy is completely different from that for the classical effects. These new data are of crucial relevance for *in vitro* experiments and clinical use, especially in glucocorticoid high-dose therapy. Examples are the choice between methylprednisolone and prednisolone in pulse therapy, and the completely different clinical usage of dexamethasone and betamethasone, despite their similar affinities for nuclear receptors. BIOCHEM PHARMACOL **58**;2:363–368, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. genomic glucocorticoid effects; non-genomic glucocorticoid effects; ATP-consuming processes; thymocytes; equivalent doses; relative drug potencies

Glucocorticoids are important anti-inflammatory and immunosuppressive drugs with three distinct effects: *genomic*, *specific non-genomic*, and *unspecific non-genomic* [1]. The mechanism of the *genomic*, or nuclear receptor-dependent, actions of glucocorticoids has been fairly well investigated [2, 3]. As lipophilic substances, glucocorticoids pass very easily through the cell membrane into the cell, where they bind to the ubiquitously expressed cytosolic glucocorticoid receptor. The activated glucocorticoid–receptor complex is then translocated into the nucleus where it binds to specific DNA sites. This initiates or inhibits transcription of certain genes and hence the synthesis of various proteins. It is important to know that these genomic effects occur at any relevant therapeutic concentration and are observed not earlier then 30 min after the receptor binding.

There is no doubt that the therapeutic effects of glucocorticoids are mostly receptor-mediated. However, there is growing evidence that there are also rapid direct glucocorticoid effects. These *non-genomic* effects are not mediated by induction or repression of specific genes. In contrast to genomic effects, they are observed at higher concentrations within seconds or minutes. Much less is known about these non-genomic glucocorticoid effects, although their existence has been apparent from the very beginning of research into the physiology and pharmacology of steroids. Currently, we distinguish between *specific* and *unspecific non-genomic* effects. *Specific non-genomic* effects occur within a few minutes and are considered to be mediated by steroid-selective membrane receptors [1, 4]. *Unspecific non-genomic* effects occur within seconds and seem to result from direct interactions with biological membranes; we have proposed that it is these effects that are of greatest clinical relevance in high-dose glucocorticoid therapy [5, 6].

The mechanisms of the three effects are completely

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different, raising the clinically crucial possibility that the relative potencies of non-genomic and genomic effects of glucocorticoids may differ. In this article, we establish *in vitro* the relative potencies of five clinically important glucocorticoids for *unspecific non-genomic* effects to be: prednylidene > dexamethasone > methylprednisolone > prednisolone > betamethasone. This hierarchy is completely different from that for the classical effects. These new data are of crucial relevance for *in vitro* experiments and clinical use, especially in glucocorticoid high-dose therapy.

MATERIAL AND METHODS

Preparation and Incubation of Cells

Thymocytes were prepared from 4-to-6-week-old female Wistar rats as described previously [5–7]. The thymus was disaggregated by pressing it through nylon mesh. The cells were centrifuged once at 1,000 *g* for 5 min and resuspended at (5–6) $\times$ 10^7 cells/mL at 37°. Isolation and incubation medium was RPMI 1640 with 10 mM glucose and 2 mM glutamine (obtained powdered from Flow Laboratories), buffered at pH 7.4 with 10 mM HEPES (Sigma) and 24 mM $NaHCO_3$ (Sigma), supplemented with 20 μg of gentamicin/mL (Sigma) and filtered through a 0.2-μm pore-size filter to remove undissolved particles. Cells were stored and incubated for up to 4 hr in plastic flasks at 37° with gentle shaking (80 cycles/min) under an atmosphere of CO_2/air (1:19). The viability of freshly isolated cells was greater than 95% as determined by trypan blue exclusion. Incubation of cells with steroids for measurements of respiration rate (see below) did not significantly change cell viability or cell number.

Measurements of Respiration Rate

Oxygen consumption was measured amperometrically in a 0.6-mL aliquot of cell suspension with a Clark electrode for up to 15 min as described previously [5–7]. The cell suspensions in the perspex incubation chamber of the electrode were magnetically stirred and thermostatically maintained at 37°. All inhibitors were dissolved in water. Concanavalin A (Sigma) was dissolved in water and in all experiments was added to cell suspensions at 25 μg/mL. The following water-solubilized glucocorticoids were used at final concentrations between 0.1 and 0.5 mg/10^7 cells (1–5 mM): methylprednisolone hemisuccinate-Na, METYPRED® (Orion Pharmaceutica) at 62.5 mg/mL; prednylidene-21-diethylaminoacetat-hydrochloride, DECORTILEN SOLUBILE®, at 60 mg/mL; prednisolone-21-hydrogensuccinat-Na, SOLU-DECORTIN®, at 50 mg/mL; and dexamethasone-21-dihydrogen-phosphate-Di-Na, FORTECORTIN®, at 8 mg/mL (all from Merck); and betamethasone-21-phosphate-Na at 62.5 mg/mL (Sigma). As described previously [5, 7], we applied inhibitors of protein synthesis (1 mM cycloheximide), Na^+K^+-ATPase (1 mM ouabain), and Ca^{2+}-ATPase (2 mM lanthanum(III) chloride 7-hydrate) (all from Sigma). Respiration rates were measured 1–3 min after each addition of concanavalin A or inhibitor.

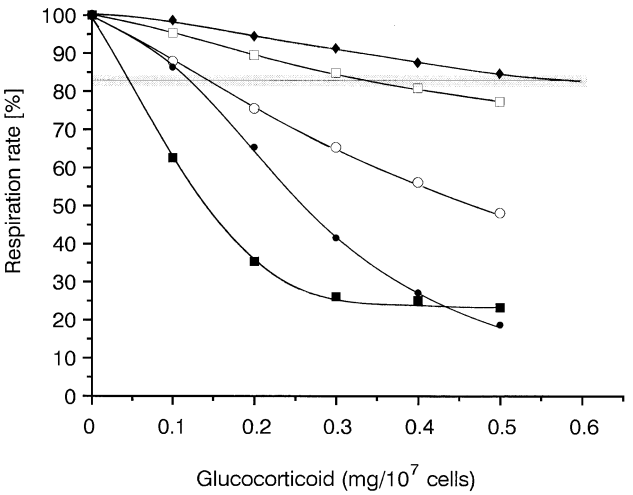

FIG. 1. Effects of different glucocorticoids on respiration of concanavalin A-induced thymocytes. Thymocytes were incubated in RPMI 1640 medium with 25 μg concanavalin A per mL, followed after 1–3 min by application of different concentrations of the glucocorticoids shown. Respiration rate was assayed as described in the Methods section. Results are means for 6 cell preparations. For clarity, SEM values are not shown; they were between 0.7 and 3.5%. Symbols: ■ prednylidene; ● dexamethasone; ○ methylprednisolone; □ prednisolone; ◆ betamethasone; ▤ basal respiration (mean ± SEM.)

RESULTS

The basal respiration rate of quiescent thymocytes was 8.68 ± 0.53 nmol O_2/min/5 $\times$ 10^7 cells. Concanavalin A stimulated this within seconds by 21% to 10.49 ± 0.58 nmol O_2/min/5 $\times$ 10^7 cells (mean ± SEM, 17 preparations), in agreement with previous results [5–7]. We titrated the respiration of concanavalin A-stimulated cells with methylprednisolone, prednylidene, dexamethasone, prednisolone, or betamethasone (Fig. 1). Statistical analysis revealed betamethasone to be significantly less potent than prednylidene, dexamethasone, and methylprednisolone at all concentrations applied ($P < 0.05$). Furthermore, at 0.2–0.5 mg/10^7 ($P < 0.05$), prednylidene and dexamethasone were found to be significantly stronger than methylprednisolone, but prednisolone and betamethasone were found to be significantly weaker than methylprednisolone (*t*-test). The results for methylprednisolone replicate those found previously [5]. From Fig. 1, we interpolated the glucocorticoid concentrations needed to inhibit concanavalin A-stimulated respiration back to basal. These concentrations were "equivalent doses" because they produced the same effect on respiration (Table 1). A separate set of four experiments (not shown) confirmed that direct addition of each of the equivalent doses listed in Table 1 had the same inhibitory effect.

TABLE 1. Equivalent doses and relative potencies of different glucocorticoids

Glucocorticoid	Unspecific non-genomic effects		Classical (genomic) effects*	
	Equivalent dose (mg per 10^7 cells)	Potency relative to methylprednisolone	Potency relative to cortisol	Potency relative to methylprednisolone
Prednylidene	0.045	3.0	3.5	0.7
Dexamethasone	0.115	1.2	25	5
Methylprednisolone	0.135	1.0	5	1.0
Prednisolone	0.335	0.40	4	0.8
Betamethasone	0.600	0.23	25	5

The equivalent doses for unspecific non-genomic effects on respiration were taken from Fig. 1. Potency relative to methylprednisolone was calculated as the inverse of the equivalent dose normalised to the value for methylprednisolone.

*Classical (genomic) effects were from [8, 9] and were scaled to the value for methylprednisolone as shown.

We determined whether these equivalent doses also cause equal inhibition of different ATP-consuming reactions. We incubated control cells or cells incubated with equivalent doses of the various glucocorticoids and then quantified the rates of different processes by measuring the effects on respiration rate of completely inhibiting them one by one with specific inhibitors. For example, if we inhibit ATP consumption by Na^+K^+-ATPase using ouabain, the oxygen consumption will decrease in proportion to the rate of ATP use by this enzyme. This technique using specific inhibitors has been used successfully in several different model systems and has been tested for validity in different ways [5, 7]. Table 2 shows that plasma membrane sodium and calcium cycling in 3–4 different cell preparations was very sensitive to these glucocorticoids, as previously found for methylprednisolone [5], and equivalent doses of the different glucocorticoids caused similar inhibitions. Statistical analysis of these data shows that the rates of ouabain-sensitive oxygen consumption in pretreated cells do not differ significantly between the different glucocorticoids (95% confidence interval), but are significantly different from the controls (t-test, $P < 0.001$). The same statistical results were obtained for the rates of lanthanum-sensitive oxygen consumption. As with methylprednisolone [5], protein synthesis was not significantly inhibited by the different glucocorticoids and may have been slightly stimulated (data not shown). Equivalent doses of the glucocorticoids were also equally potent at preventing 28–35% of the concanavalin A stimulation of quiescent cell respiration (5 cell preparations, data not shown). Thus, equivalent doses of these glucocorticoids cause equal inhibition of concanavalin A-stimulated respiration, equal inhibition of specific energy-consuming pathways, and equal inhibition of the concanavalin A effect of quiescent cells.

We calculated relative drug potencies for the different glucocorticoids from this data by taking the inverse of the equivalent doses found. We divided the potencies by the value for methylprednisolone to allow better comparison with classical relative glucocorticoid potencies. Table 1 shows that prednylidene had the most potent unspecific non-genomic effects, followed by dexamethasone and methylprednisolone, and then by prednisolone and betamethasone. There were significant differences in potencies: prednylidene was more than one order of magnitude more effective than betamethasone. Note that the formula weights of the different glucocorticoid preparations used here are similar, so this hierarchy of potencies also applies to molar concentrations. The 5-fold difference in the potencies of dexamethasone (1.2) and betamethasone (0.23) must be due to the beta position of the 16-methyl group in betamethasone but its alpha position in dexamethasone. This contrasts strongly with their classical (genomic) action, where the potencies are identical.

For a fuller comparison, Table 1 lists the classical (genomic) glucocorticoid potencies relative to cortisol [8,

TABLE 2. Effects of different glucocorticoids on cation transport in concanavalin A-stimulated thymocytes

	Na^+K^+-ATPase		Ca^{2+}-ATPase	
	Rate nmol O_2/min/10^7cells ± SEM	Inhibition % ± SEM	Rate nmol O_2/min/10^7 cells ± SEM	Inhibition % ± SEM
Control	0.93 ± 0.09	–	0.86 ± 0.09	–
Pretreatment with equivalent doses of				
Methylprednisolone	0.14 ± 0.03	85.3 ± 3.3	0.27 ± 0.04	68.9 ± 4.5
Prednylidene	0.19 ± 0.03	79.9 ± 3.7	0.18 ± 0.05	78.6 ± 6.6
Dexamethasone	0.15 ± 0.04	83.5 ± 4.2	0.13 ± 0.09	84.4 ± 10.5
Prednisolone	0.10 ± 0.03	89.7 ± 3.6	0.18 ± 0.03	79.0 ± 3.3
Betamethasone	0.21 ± 0.04	77.2 ± 4.1	0.14 ± 0.03	84.0 ± 4.0

Rates of ouabain-sensitive or lanthanum-sensitive oxygen consumption were measured in 3 or 4 preparations in the absence of glucocorticoid (control) or the presence of the glucocorticoid concentrations in Table 1.

9], recalculated to methylprednisolone. There is clearly no relationship between the hierarchy for unspecific non-genomic effects found in the present work and the classical hierarchy. It should be noted that classical potencies mainly reflect genomic potencies, but there could also be an unknown contribution from (specific, but not unspecific) non-genomic effects of a given glucocorticoid. For genomic effects, the different potencies of the glucocorticoids result from different binding to the cytosolic receptor and other pharmacokinetic and pharmacodynamic parameters. The difference in relative potencies implies that genomic and unspecific non-genomic effects are mediated by completely different mechanisms. This reinforces our previous proposal, based on rapidity of action and range of effective concentrations, that unspecific non-genomic effects cannot be mediated by cytosolic glucocorticoid receptors [5].

Current experiments with human peripheral blood mononuclear cells show the same principal feature.* From 3–5 cell preparations, we have already determined preliminary glucocorticoid concentrations needed to inhibit concanavalin A-stimulated respiration back to basal. The results show the same hierarchy and similar absolute results (all in mg/10^7 cells) as found for thymocytes: prednylidene (0.10) > dexamethasone (0.16) > methylprednisolone (0.24) > prednisolone (0.81) > betamethasone (>1.1).

DISCUSSION

Glucocorticoids have been known for five decades to have profound anti-inflammatory and immunosuppressive actions when used therapeutically. We recently developed a new modular glucocorticoid concept that is currently being discussed [1]. The key message is that very low dosages produce exclusively genomic effects [2, 3], but as dosage is increased, there are additional non-genomic effects that may contribute to the therapeutic benefits. These effects are considered to be mediated by membrane-bound receptors [4] or by physico-chemical interactions with cellular membranes [1]. For the latter termed unspecific non-genomic effects we showed that clinically relevant concentrations of methylprednisolone (the glucocorticoid most commonly used for high-dose therapy) inhibit calcium and sodium cycling across the plasma membrane and decrease intracellular free calcium concentrations, but have little effect on protein synthesis [5, 10]. Therefore, we have chosen to consider these processes for this comparative study. Recently, we showed that the inhibition of cation cycling in concanavalin A-stimulated thymocytes by the glucocorticoid is caused by direct effects and not by a reduction in ATP production [5], even though methylprednisolone reduces ATP availability to some extent by inhibiting the reactions of substrate oxidation and by increasing mitochondrial proton leak [6, 11]. It is assumed that methylprednisolone dissolves in membranes and affects physico-chemical membrane properties and the activities of membrane-associated proteins. The resulting inhibition of calcium and sodium entry across the plasma membrane would explain the decrease in ATP use for plasma membrane ion cycling and the drop in cytosolic free calcium, while a direct effect on the mitochondrial inner membrane would explain the observed increase in proton permeability and the consequent partial uncoupling of oxidative phosphorylation. These effects are seen *in vitro* at concentrations that may be experienced by cells of the immune system during clinical use with bolus infusion therapy [5]. They could diminish or prevent the acute immune response by interfering with processes, such as the rise in intracellular Ca^{2+} concentration, that are essential for the immediate and sustained activation of lymphocytes. In addition, glucocorticoids at high doses could also influence cytoplasmic components of signal transduction pathways within seconds or minutes of administration in a non-genomic fashion.

In the present study, we again used rat thymocytes mitogenically activated by the plant lectin concanavalin A, but this time to compare the immediate effects of different glucocorticoids on cellular energy metabolism. For this aim, mitogen-induced thymocytes were especially convenient since, first, they provide a model for the short-term therapeutic effects of glucocorticoids in patients with activated states of autoimmune diseases and, second, more than 80% of ATP consumption in these cells can be accounted for. The major ATP-consuming processes of concanavalin A-stimulated thymocytes are protein synthesis and Na^+K^+-ATPase with approx. 20% each of total oxygen consumption, while Ca^{2+}-ATPase and RNA/DNA synthesis contribute about 10% each. These pathways can be monitored by measuring the effects of specific inhibitors on oxygen consumption, a technique that has been used successfully in several model systems and tested for validity in different ways [5].

In this study, we have revealed huge differences in non-genomic potencies between the glucocorticoids investigated as well as between the genomic and non-genomic potencies of a given glucocorticoid. These differences should be taken into account in *in vitro* experiments and, moreover, have important consequences for clinical medicine. There will be two separate beneficial effects of glucocorticoids in acute phases of immunologically mediated diseases:

1) The immediate effects on cellular cation transport could make a crucial contribution to the therapeutic effect in acute phases or particularly severe forms of rheumatic diseases [12–15]. High-dose glucocorticoid therapy ("pulse therapy") results in termination or in regression of a severe form of disease in a high proportion of cases and with a relatively low rate of side effects. The glucocorticoid concentrations likely to be achieved in clinical practice [5] are represented by about the first third of the curves given in Fig 1. For pulse therapy with

*Schmid D, Tripmacher R, Burmester G-R and Buttgereit F, unpublished work.

glucocorticoids at high doses, the new relative drug potencies should be most relevant for clinical use.

2) The immediate high-dose effects would be additive to the slower, low-dose genomic effects. For these, the classical hierarchy of drug potency will be clinically relevant. The choice and dose of glucocorticoid should reflect the relative desirability of the two modes of action in a particular case.

Much of the empirical use of glucocorticoids for high-dose therapy can be rationalised by our data as shown in the following examples. For pulse therapy, methylprednisolone is often preferred to prednisolone in exacerbated immunologically mediated disorders. Both genomic and immediate effects produced by high doses are probably therapeutically important. The two drugs have similar genomic potency, but in high-dose therapy the unspecific non-genomic effect of methylprednisolone is 2.5-fold stronger (Table 1). This may explain the empirical clinical preference for methylprednisolone. We stress that we are extrapolating from *in vitro* data to empirical usage; we know of no controlled clinical study showing the superiority of methylprednisolone to prednisolone (at the same concentrations) in this respect. The same arguments can be used to rationalise the preference for high doses of methylprednisolone to treat patients with acute spinal cord trauma [16, 17] and high doses of dexamethasone in patients with brain disorders where edema and elevated intracranial pressure is involved [18, 19]. Another example is the effect of high-dose glucocorticoid therapy in multiple sclerosis [20–22]. These initial effects of high-dose therapy occur rapidly, but are only evident in the presence of high doses and probably fade away if the dose is tapered. Therefore, we propose that unspecific effects in general may be most clinically relevant in a temporary and time-limited fashion. This may be therapeutically helpful, for example, in spinal cord injury to prevent the early edema or in acute immunologically mediated disorders to produce additional effects and/or to bridge the gap until genomic effects are maximal.

Another example is prednylidene. This drug is recommended for status asthmaticus and other situations of severe dyspnoea and in severe allergic reactions. These conditions have a special need for glucocorticoid membrane effects, so the use of prednylidene here can be rationalised by the high relative potency of prednylidene for unspecific non-genomic effects. In contrast, prednylidene is not commonly used for typical indications of low-dose therapy, which may be explained by its relatively low genomic potency. In the same way, the low non-genomic potency of betamethasone may be one reason why this drug is rarely used systemically, although it has the same genomic potency as dexamethasone.

In general, our results suggest that simply in terms of potency, prednylidene may be the glucocorticoid of choice when immediate effects are required, but dexamethasone and betamethasone should be preferred when genomic effects are more important. In intermediate situations, glucocorticoids that show both effects sufficiently strongly could be considered, or the overall concentration of glucocorticoid required could be minimized by judicious combination of drugs to give both effects. However, it should be stressed that pharmacodynamic and pharmacokinetic considerations will clearly also have a strong effect on the choice of which glucocorticoids to use clinically. The compromise between all three factors may explain the empirical preferences discussed above.

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