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Non-genomic glucocorticoid effects to provide the basis for new drug developments

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Abstract

Glucocorticoids act via genomic and non-genomic actions. The genomic glucocorticoid actions are well known and new details on processes of transactivation and transrepression have been reported recently. Here we describe the current knowledge on non-genomic glucocorticoid actions and discuss why these actions are considered to be of therapeutic relevance.

It is assumed that rapid non-genomic glucocorticoid effects are mediated by three different mechanisms: (1) physicochemical interactions with cellular membranes (non-specific non-genomic effects); (2) membrane-bound glucocorticoid receptor (mGCR)-mediated non-genomic effects; and (3) cytosolic glucocorticoid receptor (cGCR)-mediated non-genomic effects. With regard to the first mechanism, we discuss here lazaroids and the novel development of drug targeting with liposomes as the carrier system for glucocorticoids. The clinical use of the latter two mechanisms is still speculative, but intriguing ideas are being discussed in this regard.

Keywords: Glucocorticoids; Methylprednisolone; Lazaroids

1. Introduction

The mechanisms of glucocorticoid actions can be divided into genomic and non-genomic effects. Genomic effects are known to be mediated by transactivation and transrepression processes. These mechanisms have been recently reviewed more in detail (Schacke et al., 2004; Perretti et al., 2003; Song et al., 2005).

Here we discuss the therapeutic relevance of rapid non-genomic glucocorticoid effects which are assumed to be mediated by three mechanisms: (1) physicochemical interactions with cellular membranes (non-specific non-genomic effects) (Buttgereit and Scheffold, 2002; Buttgereit et al., 2004); (2) cytosolic glucocorticoid receptor (cGCR)-mediated non-genomic effects (Croxtall et al., 2000); and (3) membrane-bound glucocorticoid receptor (mGCR)-mediated non-genomic effects (Bartholome et al., 2004). The therapeutic relevance of non-genomic glucocorticoid actions is an issue of ongoing discussion. For example, very recently, beneficial rapid non-genomic glucocorticoid effects have been described for the first time in vivo in the treatment of seasonal allergic rhinitis (Tillmann et al., 2004).

1.1. Physicochemical interactions with cellular membranes (non-specific non-genomic effects)

Rapid glucocorticoid actions (which occur within seconds) are being considered as a result of physicochemical interactions with cellular membranes. How can we explain these non-specific non-genomic effects? Currently, the following hypothesis is favoured: Glucocorticoid molecules intercalate at high concentrations in cellular membranes (plasma and mitochondrial membranes) which alter cell functions by influencing cation transport through the plasma membrane and by increasing the proton leak of the mitochondria. The impaired cation cycling across and the compromised ATP production via oxidative phosphorylation are considered to result in immunosuppressive effects and to a reduced activity of inflammatory processes (Buttgereit and Scheffold, 2002; Buttgereit et al., 2004). In the following section, we would like to explain more in detail the experimental results that have led to this theory.

The background to explain the rapid non-specific non-genomic glucocorticoid effects is provided by considerations of the cellular energy metabolism. Every organism and every cell needs metabolic energy whereby ATP is the major source. Also immune cells produce and consume certain amounts of ATP for their housekeeping activities and for specific immune functions. ATP-dependent immune functions include cytokinesis, migration, phagocytosis, antigen processing and antigen presentation,

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signalling and effector functions such as the synthesis of antibodies, cytotoxicity and regulatory functions (Buttgereit et al., 2000). The major sources for ATP production are glycolysis and oxidative phosphorylation. The most important ATP-consuming pathways are identified to be the transport of cations and synthesis of macromolecules (Buttgereit et al., 2000). In the case of energy deficit, the functions of immune cells are known to be impaired (Meldrum et al., 1994; Karlsson and Nassberger, 1992; Sanchez-Alcazar et al., 1995).

Given this background, we have shown a clear hierarchy of energy-consuming pathways in case of reduced energy supply: Pathways of macromolecule biosynthesis (protein synthesis and RNA/DNA synthesis) are most sensitive to energy restriction whereas cation transport ATPases are much less affected. The mitochondrial proton leak is least sensitive to enery supply. These results were obtained by quantitating main ATP-consuming pathways under progressively restricted mitochondrial ATP production by using myxothiazol, a specific inhibitor of the electron transport chain. Obviously, processes not essential for the immediate needs of the cell will be given up before those that are more critical for ionic integrity (Buttgereit and Brand, 1995; Buttgereit et al., 1999).

Why are these experimental results important to understand rapid glucocorticoid effects? If immune cells are treated with high, but clinically relevant concentrations of methylprednisolone (the glucocorticoid most commonly used for high-dose therapy), then the following effects on bioenergetics are observed within seconds (Buttgereit and Scheffold, 2002; Buttgereit et al., 1997, 2004):

1. The glucocorticoid instantly inhibits respiration of Con A-stimulated thymocytes and human immune cells (Fig. 1) at concentrations that leave quiescent cells unaffected.
2. Methylprednisolone not only reverses but also prevents the Con A-effect on respiration in a dose dependent manner.
3. Con A is known to produce a dramatic increase of cytoplasmic calcium concentration. This effect is clearly reduced in the presence of therapeutically relevant drug concentrations or even abolished at suprapharmacological doses.
4. Methylprednisolone inhibits calcium and sodium cycling across the plasma membrane but has little effect on protein synthesis. The inhibition of cation cycling in Con A-stimulated thymocytes by the glucocorticoid is caused by direct effects and not by a reduction in ATP production even though methylprednisolone reduces ATP availability to some extent by inhibiting the reactions of substrate oxidation and by increasing mitochondrial proton leak.

The comparison of these methylprednisolone effects with those of myxothiazol we have reported above leads to the following hypothetical explanation: Methylprednisolone dissolves in membranes and affects physicochemical membrane properties and the activities of membrane-associated proteins (Buttgereit and Scheffold, 2002; Buttgereit et al., 2004). The resulting inhibition of calcium and sodium entry across the plasma membrane would explain (i) the decrease in ATP use (and therefore oxygen consumption, see Fig. 1) for plasma membrane ion cycling and (ii) the drop in cytosolic free calcium. A direct effect on the mitochondrial inner membrane would explain the observed increase in proton permeability and the consequent partial uncoupling of oxidative phosphorylation. This is suggested to result in immunosuppression since immune cell function depends on proper functioning of these processes.

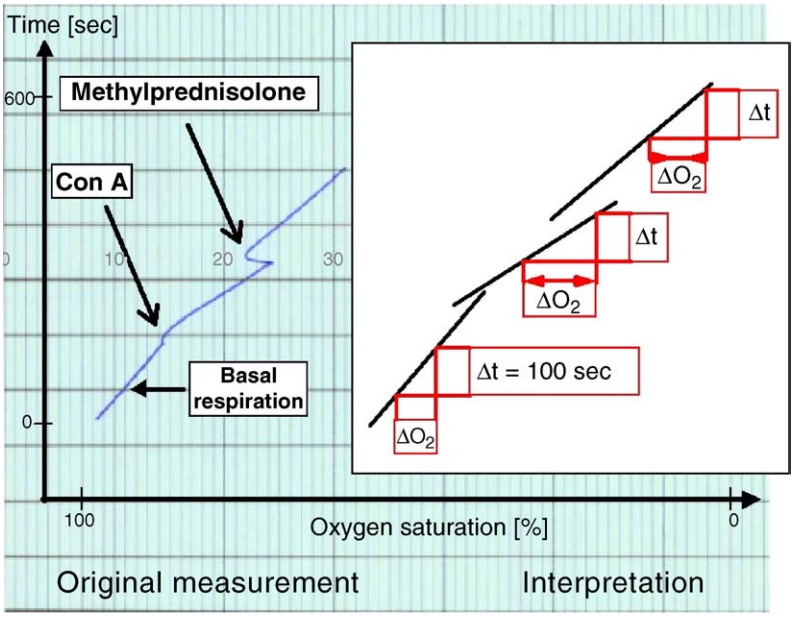

Fig. 1. Oxygen consumption of lymphocytes as stimulated by Con A and inhibited by methylprednisolone (original trace measured using a Clark electrode). This figure shows an original trace of oxygen consumption in thymocytes measured amperometrically with a Clark electrode. The first part of the curve reflects the basal rate of oxygen consumption, calculated by dividing the change in oxygen saturation (delta O2) by the time (delta t). Addition of concanavalin A (Con A) leads to a significant reater rate of oxygen consumption within seconds as reflected by the changed slope of the line. Additon of methylprednisolone leads to a reduction of oxygen consumption. This experiment was carried out by Robert Tripmacher from our laboratory.