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Dexamethasone improves vascular hyporeactivity induced by LPS *in vivo* by modulating ATP-sensitive potassium channels activity

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1 Septic shock represents an important risk factor for patients critically ill. This pathology has been largely demonstrated to be a result of a myriad of events. Glucocorticoids represent the main pharmacological therapy used in this pathology.

2 Previously we showed that ATP-sensitive potassium (K_{ATP}) channels are involved in delayed vascular hyporeactivity in rats (24 h after *Escherichia coli* lipopolysaccharide (LPS) injection). In LPS-treated rats, we observed a significant hyporeactivity to phenylephrine (PE) that was reverted by glybenclamide (GLB), and a significant increase in cromakalim (CRK)-induced hypotension.

3 We evaluated the effect of dexamethasone (DEX 8 mg kg^{-1} i.p.) whether on hyporeactivity to PE or on hyperreactivity to CRK administration, *in vivo*, in a model of LPS (8×10^6 U kg^{-1} i.p.)-induced endotoxemia in urethane-anaesthetised rats.

4 DEX treatment significantly reduced, in a time-dependent manner, the increased hypotensive effect induced by CRK in LPS-treated rats. This effect was significantly ($P<0.05$) reverted by the glucocorticoid receptor antagonist RU38486 (6.6 mg kg^{-1} i.p.).

5 GLB-induced hypertension (40 mg kg^{-1} i.p.), in LPS-treated rats, was significantly inhibited by DEX if administered at the same time of LPS.

6 Simultaneous administration of DEX and LPS to rats completely abolished the hyporeactivity to PE observed after 24 h from LPS injection.

7 In conclusion, our results suggest that the beneficial effect of DEX in endotoxemia could be ascribed, at least in part, to its ability to interfere with K_{ATP} channel activation induced by LPS. This interaction may explain the improvement of vascular reactivity to PE, mediated by DEX, in LPS-treated rats, highlighting a new pharmacological activity to the well-known anti-inflammatory properties of glucocorticoids.

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Abbreviations: K_{ATP}, ATP-sensitive potassium channels; CRK, cromakalim; DEX, dexamethasone; GLB, glybenclamide; GTN, glyceryltrinitrate; LPS, lipopolysaccharide; MAP, mean arterial pressure; PE, phenylephrine; RU, RU38486

Introduction

Severe sepsis and resultant septic shock is a cumulative result of a myriad of events caused by microorganisms or their products (Bone, 1991) and it represents an important risk factor for patients critically ill. A characteristic of this pathological syndrome is the impaired oxygen supply to the tissues and organs caused by a reduced organ perfusion induced by the deep hypotension that contributes to the high mortality clinically observed. Indeed, by increasing the vascular tone and improving the ventricular function, it is possible to restore the mean arterial blood pressure (MAP), which is essential in order to increase the patient survival to septic shock syndromes (Metrangolo *et al*., 1995).

The complexity of cellular and molecular events involved in the vascular hyporeactivity that occurs in septic shock is far to be completely clarified. Intravenous administration of lipopolysaccharide (LPS) from *Escherichia coli* in animals causes a disorder similar to human septic shock, characterised by hypotension and vascular hyporeactivity. LPS activates many cell types and when administered to animals, a variety of factors are released, such as cytokines (Bentler, 1990), platelet activating factor (PAF) (Etienne *et al*., 1986), prostacyclin (Halushka *et al*., 1985), complement-derived C5a anaphylatoxin (Smedegard *et al*., 1989) and NO (Thiemermann & Vane, 1990; Kosaka *et al*., 1992). Moreover, LPS injection causes induction of expression of inducible enzyme isoforms, such as the group II extracellular phospholipase A2 (PLA_2), the inducible NO synthase (iNOS) and the cyclooxygenase-2 (COX2) (Nakano & Arita, 1990; Moncada *et al*., 1991; Nathan, 1992; Akarasereenont *et al*., 1995; see Mitchell *et al*., 1995 for reviews), all contributing to the hypotensive state in septic shock.

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Standen *et al*. (1989) have shown the presence of ATP-sensitive potassium (K_{ATP}) channels on vascular smooth muscle cells, ascribing to these channels a role in the regulation of vascular tone. Particular attention has been focused on the involvement of K_{ATP} channels in both hypotension and vascular hyporeactivity induced by endotoxemia. In this context, Landry & Oliver (1992) have shown the involvement of K_{ATP} channels in the hypotension that occurs in the early phase of LPS septic shock in dogs. Recently, we have demonstrated, in the rat, that an increase in K_{ATP} channel activity is implicated in the vascular hyporeactivity to contracting agents observed in the delayed phase (24 h from LPS challenge) of septic shock (Sorrentino *et al*., 1999). The involvement of K_{ATP} channels in septic shock has been confirmed by Czaika *et al*. (2000), who have demonstrated an upregulation of the u-K(ATP)-1 protein expression in this pathological condition.

The therapeutic use of glucocorticoids in septic shock remains one of the first-aid approaches for their anti-inflammatory properties and its efficacy seems to be related to the time of administration. In fact, as suggested by some authors, the earlier glucocorticoid administration in septic shock significantly improves the survival (Sprung *et al*., 1984; Annane, 2001a).

The aim of the present study was to investigate the effect of glucocorticoids on K_{ATP} channel activity in the delayed phase of septic shock in rats. We have evaluated the effect of glucocorticoid both on cromakalim (CRK)-induced hypotension and on hyporeactivity to phenylephrine (PE). In this study we have used dexamethasone (DEX), since it is the most frequently used glucocorticoid in animal models and this has allowed us to compare our data with the reported ones.

Methods

Animals and treatments

Male Wistar rats (200–300 g Charles River, Italy) were housed in an environment with controlled temperature (21–24°C) and 12 : 12 h light–darkness cycle. Standard chow and drinking water were provided *ad libitum*. A period of 7 days was allowed for acclimatisation before any experimental manipulation was undertaken. All the experiments were conducted following the principles of laboratory animal care (law N. 86/609/CEE), as well as the specific national law (N. 116/1992).

Rats were divided into two groups in a random block design and treated with saline (1 ml kg^{-1}; NaCl 0.09%, w v^{-1}; intraperitoneal (i.p.)) or LPS (8×10^6 U kg^{-1}; i.p.). Since the time frame in which glucocorticoids are administered seems to play a crucial key role, DEX treatment was performed at different times from LPS injection. In fact, DEX (8 mg kg^{-1}; i.p.) was administered contemporaneously to LPS or saline (DEX-0), 18 h (DEX-18) or 23 h (DEX-23) after LPS or saline injection (Figure 1). After 24 h from LPS or saline injection, we evaluated haemodynamic changes to administration of CRK (150 μg kg^{-1}; intravenous (i.v.)), a K_{ATP} channel opener, glybenclamide (GLB; 40 mg kg^{-1} i.p.), a K_{ATP} channel inhibitor, PE 30 μg kg^{-1}; i.v.), an α_1 adrenergic receptor agonist and glyceryltrinitrate (GTN; 500 μg kg^{-1}; i.v.), a nitric oxide donor, used as reference drug. Phenylephrine and GTN were evaluated only in the group DEX-0.

RU38486, a glucocorticoid receptorial antagonist, was used to assess if DEX effect was mediated by a receptorial mechanism. Animals were pretreated with RU38486 (6.6 mg kg^{1}; i.p.), 30 min prior to saline or DEX-0 administration, in both LPS- and saline-treated rats (Figure 1).

Measurement of the haemodynamic changes

Briefly, tracheotomy was performed in rats under urethane anaesthesia (1 g kg^{-1}; i.p.) and carotid artery and jugular vein were dissected. A polyethylene cannula (PE-50) was placed in the internal jugular vein and in the left carotid artery for drug administrations and blood pressure monitoring (Bentley 800 Trantec; Basile, Comerio, Italy), respectively. The carotid artery catheter was filled with heparinised saline (5 U ml^{-1}) to avoid its occlusion. Blood pressure was recorded using the Thermal Arraycorder WR 7400 recorder (Graphtec, Tokyo). After 30 min of stabilisation from surgery, drugs (i.e. CRK, PE, GLB or GTN) were administered via jugular vein or *via* i.p. injection.

The doses of CRK, GTN, LPS and GLB, which we used in the present study, are those previously described (Sorrentino *et al*., 1999). CRK was suspended in polyethyleneglycol (0.5 ml kg^{-1}; i.v.) and GLB was dissolved in dimethylsulphoxide (0.5 ml kg^{-1}; i.p.). All other drugs were dissolved in normal saline (NaCl 0.09% w v^{-1}).

Materials and statistical analysis

LPS (from *Escherichia coli*, serotype 0127:B8), DEX, CRK, GLB and PE were purchased from Sigma-Aldrich (Milan, Italy). GTN (Venitrin®) was purchased from ASTRA, Italy. RU38486 was a gift from Professor Mauro Perretti (The William Harvey Research Institute, London).

Changes in MAP were expressed as per cent of mean variation of basal value (% ± s.e.m.). The value of MAP before drug administration was taken as basal value. Data were analysed by Student's *t*-test, whereas for multiple comparison, one- or two-way analysis of variance (ANOVA) followed by Bonferroni as post-test was used. Values of $P < 0.05$ were taken as significant. Means and statistics were performed using a computerised statistical package (GraphPad Prism 3.0, U.S.A.).

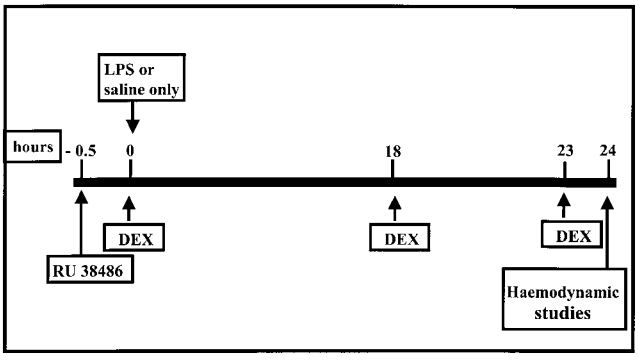

Figure 1 DEX (8 mg kg^{-1}; i.p.) was administered together with LPS or saline (DEX-0), 18 h (DEX-18) or 23 h (DEX-23) after LPS or saline injection. In another set of experiments, RU38486 was administered 0.5 h before LPS or LPS + DEX or saline only. At 24 h after LPS or saline haemodynamic studies were performed, at this same time point, we analysed CRK- or GTN-induced hypotension and PE or GLB increase in MAP.

Results

Effect of DEX on hypotension induced by CRK in LPS-treated rats

At 24 h after LPS (8×10^6 U kg^{-1}; i.p.) or saline treatment, animals did not show any significant ($P > 0.05$) change in basal MAP (99.8 ± 2.1 mmHg, $n = 31$ for saline- and 104.3 ± 2.1 mmHg, $n = 29$ for LPS-treated rats). DEX (8 mg kg^{-1}; i.p.), administered at different time intervals (0, 18 or 23 h) after LPS or saline, did not modify MAP basal values in both saline- and LPS-treated rats. Indeed, MAP values were 102.4 ± 6.0, 111.4 ± 5 and 103.3 ± 4.3 mmHg ($n = 6$) for saline-treated rats and 104.4 ± 3.0, 104.1 ± 3.0 and 99.9 ± 4.7 mmHg ($n = 6$) in LPS-treated rats for DEX-0, DEX-18 and DEX-23, respectively. The dose of DEX that we have used in our study has been shown to significantly prolong the mean survival time (Ottosson *et al*., 1982).

CRK administration (150 μg kg^{-1}; i.v.) in saline- and LPS-treated rats, as shown previously (Sorrentino *et al*., 1999), caused hypotension, which was significantly ($P < 0.05$) increased in LPS-treated rats ($n = 5$) compared to saline-treated rats ($n = 8$).

In saline DEX-18 and DEX-23 groups, CRK-induced hypotension was not statistically modified. Conversely, when the corticosteroid was given together with saline, that is, saline DEX-0 group, a significant ($P < 0.05$) reduction of CRK-induced hypotension was observed compared to saline group (Figure 2a).

DEX, administered simultaneously with LPS (DEX-0) or 18 h (DEX-18) after LPS, significantly ($P < 0.005$ and $P < 0.05$, respectively) and markedly reduced the increase in CRK-induced hypotension observed in LPS-treated rats. In contrast, DEX administration 23 h after LPS (DEX-23) did not modify the increase in CRK-induced hypotension (Figure 2a).

To assess if DEX results were due to a nonspecific vasorelaxant effect on smooth muscle cells, we tested the response to GTN in rats treated with DEX coadministered with LPS or saline. The hypotension induced by GTN (500 μg kg^{-1}; i.v.) was not modified by DEX treatment (DEX-0; $n = 6$; Figure 2b) both in LPS- or saline-treated rats.

Effect of RU38486 in DEX-treated rats

Since DEX has shown the maximal activity when administered together with LPS (DEX-0), we tested RU38486, a glucocorticoid receptor antagonist, in this experimental condition. Pretreatment with RU38486 in saline-treated animals did not modify CRK-induced hypotension, while in LPS-treated rats the glucocorticoid receptor antagonist totally reverted the effect of DEX coadministered with LPS ($P < 0.01$, $n = 5$). Furthermore, it is noteworthy that RU38486 administration, in the absence of DEX, in LPS-treated rats, caused a significant ($P < 0.05$, $n = 5$) increase in CRK-induced hypotension when compared to LPS alone (Figure 3).

Effect of DEX on the increase of MAP induced by GLB in LPS-treated rats

Previously we have shown that GLB (40 mg kg^{-1}; i.p.) administration, to LPS-treated rats, significantly ($P < 0.01$) increased the basal value of MAP (Sorrentino *et al*., 1999). The increase in MAP induced by GLB in LPS-treated animals was significantly reduced by DEX-0 treatment ($P < 0.05$, $n = 6$). This effect was not statistically ($P > 0.05$, $n = 6$) different from the effect of GLB observed in saline group. In contrast, the administration of DEX at 18 or 23 h after LPS (DEX-18 and DEX-23) did not statistically modify ($P > 0.05$, $n = 6$) the increase of MAP induced by GLB in LPS-treated rats (Figure 4).

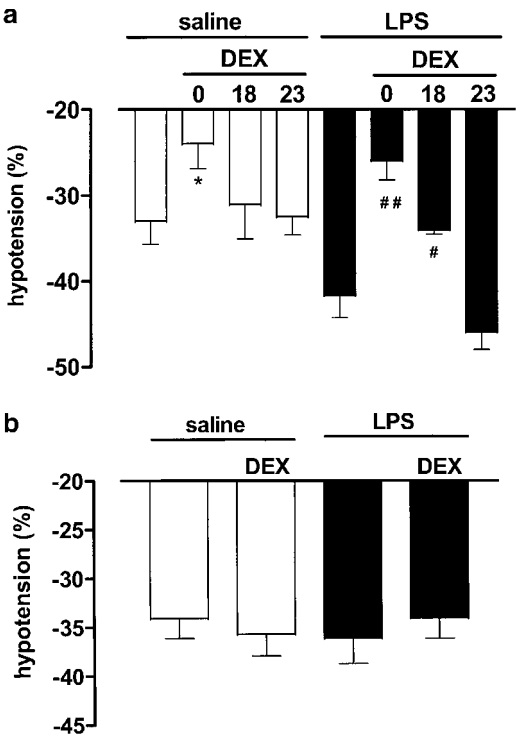

Figure 2 (a) Time-dependent effect of DEX (8 mg kg^{-1}; i.p.) on CRK (150 μg kg^{-1}; i.v.)-induced hypotension in saline- or LPS-treated rats. DEX was administered at 0, 18 or 23 h after LPS or saline injection, (b) Effect of DEX-0 on GTN (500 μg kg^{-1}; i.v.)-induced hypotension in saline- or LPS-treated rats. Data are expressed as mean ± s.e.m. of five to eight separate experiments and calculated as percentage of hypotension of MAP *versus* each basal value. $^{*}P < 0.05$ *versus* saline-treated rats; $^{\#}P < 0.05$ *versus* LPS-treated rats; $^{\#\#}P < 0.005$ *versus* LPS-treated rats.

Effect of DEX on vascular hyporeactivity to PE in LPS-treated rats

The increase in MAP induced by PE (30 μg kg^{-1}, i.v.) in LPS-treated rats was significantly reduced compared to saline-treated rats ($P < 0.05$, $n = 5$). This vascular hyporeactivity was completely prevented when DEX was administered together with LPS (DEX-0; $P < 0.05$, $n = 6$). On the other hand, DEX-0 treatment did not modify ($P > 0.05$, $n = 6$), the increase in MAP induced by PE in saline-treated rats (Figure 5).

Discussion

Sepsis is the most common cause of death in medical and surgical intensive care units, and the incidence of this disease has increased over 135% in the past decade. This shock syndrome can be defined as a progressive failure of circulation with a reduction of oxygen to vital organs. Pathophysiological

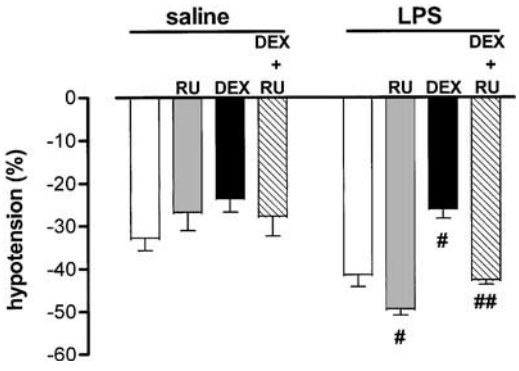

Figure 3 Effect of RU 38486 (6.6 mg kg^{-1}; i.p.) on CRK (150 μg kg^{-1}; i.v.)-induced hypotension in the presence of DEX-0 (8 mg kg^{-1}; i.p.), in saline- and LPS-treated rats. Data are expressed as mean ± s.e.m. of five to eight separate experiments and calculated as percentage of hypotension of MAP *versus* each basal value. $P<0.05$ *versus* LPS-treated rats; $^{\#\#}P<0.005$ *versus* LPS-treated rats.

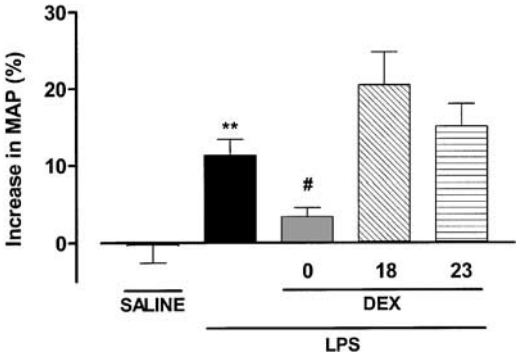

Figure 4 Effect of DEX-0 (8 mg kg^{-1}; i.p.), DEX-18 or DEX-23 on GLB (40 mg kg^{-1}; i.p.) increase in MAP in LPS-treated rats. Data are expressed as mean ± s.e.m. of 6–8 separate experiments and calculated as percentage of increase in MAP *versus* each basal value. $^{\#}P<0.05$ *versus* LPS alone; $^{**}P<0.01$ *versus* saline.

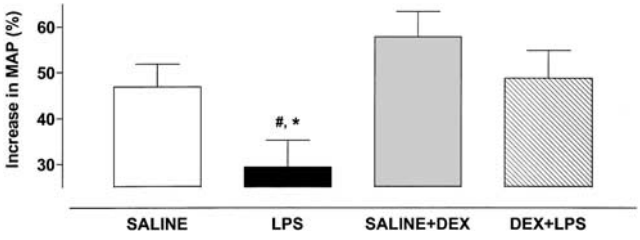

Figure 5 DEX-0 (8 mg kg^{-1}; i.p; simultaneously with LPS) effect on the hyporeactivity to PE (30 μg kg^{-1}, i.v.) in LPS-treated rat. Data are expressed as mean ± s.e.m. of five to six separate experiments and calculated as increase in MAP (%) *versus* basal value. $^{\#}P<0.05$ *versus* saline-treated rats; $^{*}P<0.05$ *versus* LPS + DEX-0-treated rats.

changes, associated with septic shock in human, result from a sophisticated interplay of mediators, leading to an impairment of cardiovascular system haemodynamics. Efficacy of therapies using receptor antagonists or antibodies for TNFα or interleukin-1 (Groeneveld *et al*., 2001), monoclonal antiendotoxin antibody (Angus *et al*., 2000) or high doses of antithrombin III (Warren *et al*., 2001) resulted ineffective, in randomised clinical trials, confirming that sepsis and shock remain a fatal disorders (Parillo *et al*., 1990; Suffredini, 1994). Steroids have been the first, among anti-inflammatory drugs, to be tested in large randomised controlled trials in septic shock, but their use is still controversial. Recently, findings highlighting the role of the hypothalamic–pituitary–adrenal axis to respond appropriately to a septic insult have led to a reappraisal of the use of steroids in sepsis (Annane, 2001b). Randomised controlled trials strongly suggest that corticosteroid therapy reduces the morbidity effect of septic shock and may favourably affect survival from sepsis (for a review see Annane, 2002). Thus, glucocorticoids represent the elective pharmacological therapeutic approach, in particular, if patients are treated within 4 h from the onset of shock (Sprung *et al*., 1984; Han *et al*., 1999). The efficacy of glucocorticoid administration has been attributed to several mechanisms, such as reduction of extracellular PLA_2 levels and inhibition of PAF release, complement activation, iNOS and COX-2 expression (Imai *et al*., 1982; Vadas *et al*., 1986; Thiemermann, 1997; Leach *et al*., 1998; Han *et al*., 1999; Minghetti *et al*., 1999).

Several authors have shown the involvement of K_{ATP} channels in the early phase (within 5 h of LPS infusion) of endotoxic shock, in anaesthetised (Wu *et al*., 1995) and in conscious rat (Gardiner *et al*., 1999). We have previously shown, both *in vitro* and *in vivo*, an involvement of K_{ATP} channels 24 h after LPS injection. Indeed, an increase was observed in CRK-induced hypotension in LPS-treated rats when compared to saline-treated rats. Furthermore, GLB increased MAP basal value in LPS-treated rats but not in saline-treated rats, indicating a hyperactivity of K_{ATP} channels in LPS-induced endotoxemia (Sorrentino *et al*., 1999).

Our results demonstrate that DEX administration, in LPS-treated rats, inhibits the increase in CRK-induced hypotension. DEX effect is time-dependent as demonstrated by other authors who have stressed that a timely glucocorticoid administration is the key to achieving the maximal beneficial therapeutic effect of DEX treatment (Sprung *et al*., 1984; Annane, 2001a). A nonspecific effect of DEX on vasorelaxant properties of smooth muscle cells can be ruled out since DEX does not modify the hypotension induced by GTN in both saline- and LPS-treated rats. Furthermore, the increase in MAP basal value mediated by GLB in LPS-treated rats, as previously shown, is significantly reduced by DEX coadministered with LPS, implying either a possible modulation of K_{ATP} channel activity or an inhibition of protein expression. Recently it has been shown, by RT–PCR and by Western blotting analysis, that sepsis upregulates the u-K(ATP)-1 channel expression (Czaika *et al*., 2000) and that glucocorticoid receptor agonists inhibit the expression of calcium-dependent potassium channel protein in primary vascular smooth muscle cell cultures (Brem *et al*., 1999). Hence, it seems more reasonable to hypothesise that DEX effect could be ascribed to the inhibition of potassium channel protein expression and/or to the synthesis of a mediator that could regulate the K_{ATP} channel expression. This hypothesis could also justify the time-dependent effect of DEX.

Next we used RU 38486, a glucocorticoid receptorial antagonist, to investigate whether DEX effect was mediated through a receptorial mechanism. An equimolar dose of the antagonist significantly restored the hypotension mediated by CRK in the presence of DEX in LPS-treated rats. Further, the effect of RU-38486 treatment, in LPS-treated rats, produced a significant increase in hypotension mediated by CRK. This

result could be a consequence of the receptor unavailability to the effect of endogenous glucocorticoids, as also suggested by Fan *et al*. (1994) who demonstrated that glucocorticoid receptor blockade by RU38486 exacerbates the pathological changes of endotoxemia in rats. A role for endogenous glucocorticoids has also been demonstrated in the endotoxin-induced cardiovascular tolerance (Szabò *et al*., 1994).

Our data also demonstrate that DEX treatment, in LPS-treated rats, improves the vascular hyporeactivity to PE. This effect could be ascribed, at least in part, to a direct or indirect inhibitory mechanism of DEX on K_{ATP} channel activity that ameliorates the vascular response to α_1-adrenoceptor agonist. This result fits with the clinical observation that hydrocortisone treatment in septic shock patients improves the response to PE i.v. infusion (Bellissant & Annane, 2000). Recently, another type of potassium channel, the calcium-activated potassium channel, has been shown to have a role in *in vitro* and *in vivo* models of hyporesponsiveness to PE (Chen *et al*., 1999; Terluk *et al*., 2000).

In conclusion, the beneficial effect of glucocorticoids in human septic shock could be linked not only to the well-known anti-inflammatory properties, but also to an improvement of vascular reactivity to vasoconstrictor agents by acting on K_{ATP} channels.

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