Eur Respir J 2003; 21: 862–865
DOI: 10.1183/09031936.03.00084503
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European Respiratory Journal
ISSN 0903-1936

Dehydroepiandrosterone sulphate reduces chronic hypoxic pulmonary hypertension in rats

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Dehydroepiandrosterone sulphate reduces chronic hypoxic pulmonary hypertension in rats. V. Hampl, J. Bíbová, V. Povýšilová, J. Herget. ©ERS Journals Ltd 2003.

ABSTRACT: Pathogenesis of pulmonary hypertension includes vascular smooth muscle cell membrane depolarisation and consequent calcium influx. Usually, calcium-gated potassium channels are activated under such conditions and repolarise the membrane. However, in pulmonary hypertension they are downregulated. The authors hypothesised that pharmacological augmentation of these channels would reduce pulmonary hypertension.

Dehydroepiandrosterone sulphate (DHEA-S, 0.1 mg·mL^{-1}), a recently characterised activator of calcium-gated potassium channels, was given to rats in drinking water.

Pulmonary arterial blood pressure, increased by 4 weeks of hypoxia (from 15±0.2 to 29.4±2.5 mmHg), was selectively attenuated in rats treated with DHEA-S for the whole duration of the hypoxic exposure (23.9±0.9 mmHg) and in rats given DHEA-S only after pulmonary hypertension had fully developed (last 2 weeks of hypoxia; 24.4±1.4 mmHg). Pulmonary vascular remodelling and right ventricular hypertrophy associated with pulmonary hypertension were also reduced by DHEA-S. Cardiac index and systemic arterial blood pressure did not differ among the groups.

The authors conclude that treatment with an activator of calcium-gated potassium channels, dehydroepiandrosterone sulphate, known to be well tolerated by humans, reduces hypoxic pulmonary hypertension in rats.

Eur Respir J 2003; 21: 862–865.

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Keywords: Antioxidants, hormones, hypertension, large-conductance calcium-activated potassium channel, potassium channels, pulmonary

Received: September 12 2002
Accepted after revision: November 20 2002

This study was supported by the grant agency of the Czech Republic, grant nos. 306/97/0854, 305/97/S070 and 305/00/1432.

Although the possibilities of therapeutic intervention in pulmonary hypertension have somewhat expanded recently, they still have important limitations, particularly unsatisfactory efficacy, severe side-effects and high cost [1–7]. Thus, a need remains for an effective, safe and easy-to-use therapy for chronic pulmonary hypertension.

The mechanism of pulmonary hypertension includes vasoconstriction and vascular wall remodelling [1]. Both of these processes are controlled, among other factors, by intracellular calcium (Ca) concentration ($[Ca^{2+}]_i$) [8, 9], which, in turn, is governed mostly by membrane potential [10]. In resistance pulmonary arteries, resting membrane potential of myocytes is controlled predominantly by voltage-gated potassium (K) channels [10, 11]. Conversely, Ca-gated K (KCa) channels are relatively inactive under resting conditions but open upon cell stimulation (*i.e.* depolarisation and increased $[Ca^{2+}]_i$) [10–13]. Their opening repolarises the cell membrane and thus functions as a negative feedback limiting depolarisation [10, 12, 13].

The authors therefore hypothesised that pharmacological potentiation of KCa channel activity may suppress the development of pulmonary hypertension. To test this hypothesis, a water soluble sulphate ester of a recently characterised KCa channel activator (dehydroepiandrosterone (DHEA) [14, 15]) was used, because it is well tolerated by humans [16–18]. The well established chronic hypoxic model of pulmonary hypertension in rats was utilised.

Methods

Experiments were performed on adult rats in accordance with the Helsinki convention and the European Community and National Institutes of Health guidelines for using experimental animals [19–21]. All procedures were approved by the Animal Studies Committee in the authors' institution.

Pulmonary hypertension was elicited in three groups of adult male Wistar SPF rats by chronic exposure to hypoxia (normobaric 10% oxygen, 4 weeks) [22]. They were compared to two groups without pulmonary hypertension (*i.e.* kept in normoxia) (table 1). All rats were purchased from Anlab, Prague, Czech Republic, when weighing 250 g.

In order to test the ability of DHEA sulphate (DHEA-S) to prevent the development of pulmonary hypertension, one group was treated with DHEA-S for all 4 weeks of the hypoxic exposure ("preventive" administration, group HD4). To see whether DHEA-S can reverse an already established pulmonary hypertension, another group was given DHEA-S for 2 weeks starting from the 3rd week of the hypoxic exposure, when pulmonary hypertension is fully developed [22] ("therapeutic" administration, group HD2). The remaining hypoxic rats did not receive any treatment (group H). To exclude adverse effects of DHEA-S on normal, healthy pulmonary circulation, one group of normoxic rats was given DHEA-S for 4 weeks (group ND4), while the remaining normoxic rats received no treatment (group N). There was no mortality in any group. DHEA-S (Sigma-Aldrich, Prague, Czech Republic) was administered in drinking water (0.1 mg·mL^{-1}). The average daily DHEA-S intake was 9.0–9.7 μg·g body weight^{-1}.

On completion of the hypoxic exposure and DHEA-S treatment, the rats were anesthetised with thiopental (40 mg·kg body·weight^{-1}, *i.p.*). Systemic arterial blood pressure (SAP; *via* a carotid artery cannula) and pulmonary arterial blood pressure (PAP; by a catheter introduced through a jugular vein and right ventricle) [22] were measured while the rat was

Table 1. – Experimental groups

Group	Environment	DHEA-S treatment	Subjects n
N	21% O_2 all the time	None	9
ND4	21% O_2 all the time	Last 4 weeks before measurements	10
H	10% O_2 4 weeks	None	10
HD2	10% O_2 4 weeks	Last 2 weeks of hypoxic exposure	9
HD4	10% O_2 4 weeks	All 4 weeks of hypoxic exposure	10

DHEA-S: dehydroepiandrosterone sulphate; O_2: oxygen.

spontaneously breathing room air. After obtaining stable values, the rat was ventilated through a tracheostomy with room air at 60 breaths·min^{-1} and peak inspiratory pressure of 6–8 cmH_2O. After thoracotomy, blood flow in the ascending aorta was measured with an ultrasonic flowmeter (T106+2.5 mm SS-series flowprobe with J-reflector; Transonic Systems, Ithaca, NY, USA) as an estimate of cardiac output [23]. This value relative to body weight is referred to as cardiac index.

After sampling left ventricular blood for haematocrit determination, the heart was removed and the right ventricle to left ventricle plus septum weight ratio (RV/LV+S) was determined as a measure of right ventricular hypertrophy associated with pulmonary hypertension. To assess the morphological remodelling of the pulmonary vasculature underlying pulmonary hypertension, the percentage of thick-walled to all peripheral vessels ($\leqslant$300 μm) (%TWPV) was calculated on a slide of formaldehyde-fixed lung [22].

The results are presented as mean±SEM. The differences between the groups were assessed using one-factor analysis of variance followed by Fisher's protected least significant difference *post hoc* test. A p-value of <0.05 was considered significant.

Results

At the end of the exposure to chronic hypoxia, the rats had lower body weight than rats of the same age living in room air. DHEA-S treatment had no effect on body weight (table 2).

As expected, chronic exposure to hypoxia elicited pulmonary hypertension: PAP was significantly higher in the H group than in the N group (fig. 1a). Importantly, PAP was significantly lower in both of the chronically hypoxic groups treated with DHEA-S (HD4 and HD2) than in the untreated hypoxic group (H), although it was still higher than in the normoxic groups (N and ND4) (fig. 1a). In normoxia, DHEA-S had no effect on PAP.

The effect of DHEA-S was selective for the pulmonary circulation, since SAP was not altered (fig. 1b). The reduction of PAP by DHEA-S was not secondary to any alterations in cardiac output or haematocrit, since neither differed among the hypoxic groups (table 2). A surprising reduction of haematocrit by DHEA-S in normoxia (ND4 *versus* N, table 2) was noted, for which the authors have no explanation, especially as DHEA-S had no such effect in chronic hypoxia. The authors are unaware of reduced haematocrit being reported in humans ingesting DHEA or DHEA-S. Cardiac output and SAP were not altered by DHEA-S treatment in normoxia (table 2 and fig. 1b).

Thickening of the peripheral pulmonary vessels associated with chronic hypoxic pulmonary hypertension was evident in the study as a higher %TWPV in the H compared to the N and ND4 groups. DHEA-S markedly reduced this increase in a manner dependent on the duration of treatment (fig. 1c).

Chronic hypoxic pulmonary hypertension was associated with right ventricular hypertrophy: both the weight of the right ventricular wall and RV/LV+S were significantly higher in the H group than in the N group (fig. 1d, table 2). DHEA-S administration from the beginning of the hypoxic exposure limited the development of right ventricular hypertrophy, as documented by a lower RV/LV+S in the HD4 group than in the H group (fig. 1d). In addition, the weight of the right ventricular wall did not differ significantly between the HD4 and N groups (table 2). However, the HD2 and H groups did not differ in right ventricular weight or RV/LV+S (table 2, fig. 1d). Left ventricle plus septum weight was unaffected by DHEA-S (table 2).

Discussion

The results show that both preventive and therapeutic application of DHEA-S selectively reduces pulmonary hypertension elicited in rats by chronic exposure to hypoxia. This finding might be of clinical interest since a considerable experience with long-term DHEA or DHEA-S ingestion by humans attests to its safety [16–18].

DHEA (3 beta-hydroxy-5-androsten-17-one) and its sulphated form are quantitatively the most abundant steroids in mammals, including humans [24]. They are secreted by the adrenal cortex in response to adrenocorticotrophin stimulation [25]. Although they have been known for more than half a century, their physiological role and mechanism of action are still

Table 2. – Body weight, cardiac index, haematocrit and wet ventricular weights

Group	Body weight g	Cardiac index mL·min^{-1}·kg^{-1}	Haematocrit %	Right ventricle weight mg	Left ventricle plus septum weight mg
N	413±8	117±7	49.3±0.8	192±11	802±28
ND4	427±12	134±5	39.5±0.8$^{\#}$	200±10	796±25
H	240±6*	138±12	73.4±1.0*	251±16*	590±20*
HD2	231±9*	137±7	74.4±1.2*	240±20*	600±29*
HD4	220±8*	126±8	74.2±1.3*	213±14	577±22*

Data are means±SEM. N: 21% oxygen (O_2) all the time, no dehydroepiandrosterone sulphate (DHEA-S); ND4: 21% O_2 all the time, DHEA-S last 4 weeks before measurements; H: 10% O_2 4 weeks, no DHEA-S; HD2: 10% O_2 4 weeks, DHEA-S last 2 weeks of hypoxic exposure; HD4: 10% O_2 4 weeks, DHEA-S all 4 weeks of hypoxic exposure. *: $p<0.05$ hypoxic groups differ from normoxic groups; $^{\#}$: $p<0.05$ DHEA-S treated group differs from a corresponding nontreated group.

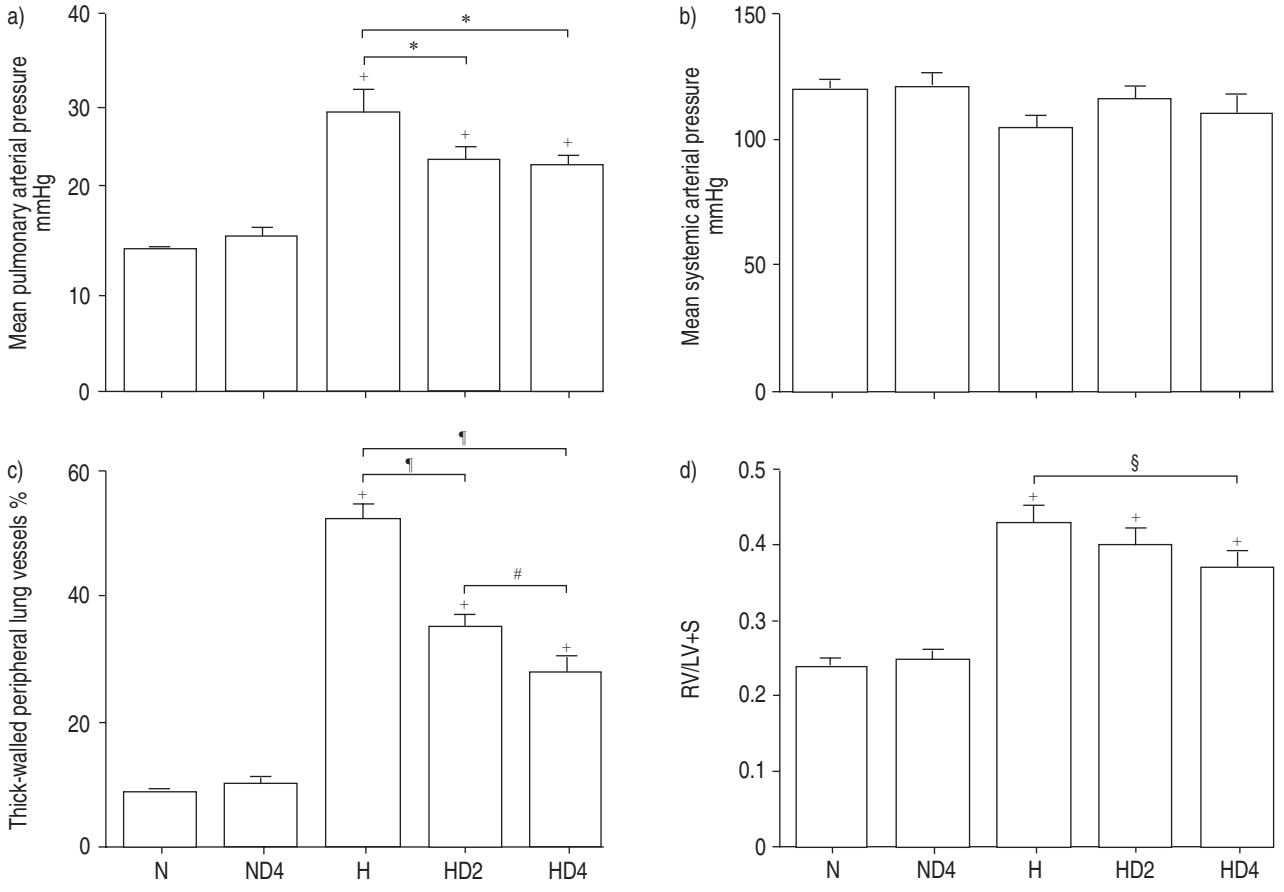

Fig. 1. – Dehydroepiandrosterone sulphate (DHEA-S) selectively reduces chronic hypoxic pulmonary hypertension in rats. Chronic hypoxia-induced increases in pulmonary arterial blood pressure (a), percentage of thick-walled peripheral pulmonary vessels (c), and right ventricle to left ventricle plus septum weight ratio (RV/LV+S; d) are all reduced by DHEA-S treatment. Systemic arterial blood pressure (b) is unaltered by chronic hypoxia or DHEA-S. N: 21% oxygen (O_2) all the time, no DHEA-S; ND4: 21% O_2 all the time, DHEA-S last 4 weeks before measurements; H: 10% O_2 4 weeks, no DHEA-S; HD2: 10% O_2 4 weeks, DHEA-S last 2 weeks of hypoxic exposure; HD4: 10% O_2 4 weeks, DHEA-S all 4 weeks of hypoxic exposure. Data are presented as mean±SEM. §: $p<0.05$; *: $p<0.01$; $^{\#}$: $p<0.005$; ¶: $p<0.0001$; $^{+}$: $p<0.0005$ hypoxic groups differ from normoxic groups.

poorly defined [24–26]. DHEA is a precursor of biologically active androgens and estrogens, but that does not account for its multiple anticancerogenic, antisclerotic, antidiabetic, anti-obese and immunoprotective effects [24–26]. These actions are not mediated through specific intracellular receptors. The proposed mechanisms of DHEA action include antiglucocorticoid activity for some of the effects (especially immunoprotective), antioxidant properties [24–26] and, most recently, activation of KCa channels [14]. Each of the latter two mechanisms is capable of underlying the reduction of pulmonary hypertension by DHEA-S observed in this study. The antiglucocorticoid effect is unlikely to play any role in this case because adrenalectomy does not affect the development of chronic hypoxic pulmonary hypertension [27].

DHEA-S was initially tested in this study to see the effect on KCa channel activation. While KCa channels are hardly active in the resistance pulmonary arteries under resting conditions, they open upon membrane depolarisation and increased $[Ca^{2+}]_i$ [10–13]. In this situation, KCa channel opening repolarises the cell membrane and, thus, functions as a servomechanism limiting the extent of depolarisation and consequent Ca influx, vasoconstriction, and proliferation [10, 12, 13]. Although pulmonary vascular smooth muscle depolarisation and increased $[Ca^{2+}]_i$ are present in pulmonary hypertension [28–33], and, thus, KCa channel activity would be expected to rise, it has actually been repeatedly shown to be reduced [31, 33], possibly due to downregulation of the channel protein expression. The cause and mechanism of this KCa channel downregulation is unknown, but it probably contributes to the sustained depolarisation and increased $[Ca^{2+}]_i$ of pulmonary hypertension. Thus, it is likely that the pharmacological KCa channel stimulation by DHEA-S compensated for the chronic hypoxia-induced loss of KCa channels' ability to limit vascular smooth muscle membrane depolarisation. It is interesting to note that nitric oxide, useful for clinical management of several forms of pulmonary hypertension, also causes pulmonary vasodilation by activating KCa channels [34].

DHEA-S could also reduce pulmonary hypertension *via* its antioxidant capacity. Oxidative stress appears important in the mechanism of pulmonary hypertension [35]. Treating rats with an antioxidant, *N*-acetylcysteine, during chronic hypoxia blunts pulmonary hypertension to a similar extent as seen in the present study with DHEA-S [36].

An unexpected observation of the present study was a dissociation between the effects of the therapeutic (2 week) DHEA-S administration on PAP and right ventricular hypertrophy. In the HD2 group, PAP was significantly lower than in the untreated hypoxic rats, whereas RV/LV+S was not. As right ventricular hypertrophy is thought to be a consequence of the increased afterload due to elevated PAP [37], it is possible that there was insufficient time for the right ventricular hypertrophy to significantly regress after reduction of PAP.

In summary, the calcium-gated potassium channel activator and antioxidant, dehydroepiandrosterone sulphate, reduced pulmonary hypertension induced in rats by a chronic exposure to hypoxia. In this respect, dehydroepiandrosterone sulphate was equally effective if given from the beginning of the hypoxic exposure or when it was started from mid-exposure, when pulmonary hypertension is fully developed. As dehydroepiandrosterone sulphate is well known to be tolerated by humans, it might prove useful for clinical management of at least some types of chronic pulmonary hypertension.

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