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The effect of long-term exposure to combinations of growth promoters in Long Evans rats Part 1: Endocrine adrenal function

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Abstract

The aim of the study was to investigate whether the chronic administration (45 days) of clenbuterol (CB) at a growth promoting dose (1 mg kg^{-1} bw) and/or dexamethasone (DEX: 0.1 mg kg^{-1} bw) may cause the disruption of rat endocrine adrenal function. Blood samples were taken weekly during the whole experiment (S0–S7), and at different days of withdrawal (W0, W5, W10, W15 and W20). Hormone profiles were determined by RIA (ACTH) or EIA (corticosterone and catecholamines). ACTH showed significantly elevated concentrations from S1 until W5 ($p < 0.05$) with CB administration. It began to decrease the day of DEX and CB-DEX administration. DEX showed significantly lowered ACTH concentrations from the day of drug injection ($p < 0.05$). Corticosterone showed significantly elevated levels until W10 ($p < 0.01$) with CB and CB + DEX. DEX showed lowered levels of corticosterone during the whole withdrawal period. Epinephrine presented significantly elevated plasma levels until W5 with CB and CB + DEX. With DEX, epinephrine was also elevated from W5 to W15 ($p < 0.05$). Norepinephrine also presented significantly elevated plasma levels until S7 with CB and CB + DEX ($p < 0.001$). With DEX no differences were found. *Conclusion*: Long-term administration of CB and/or DEX causes an endocrine adrenal disruption with changes in ACTH, glucocorticoid and catecholamine secretion.

Keywords: Growth promoters; Clenbuterol; Dexamethasone; Adrenal endocrinology; Corticotropin (ACTH); Corticosterone; Catecholamines; Rats

1. Introduction

Several growth promoting substances have been illegally used in animal production. Among them, β-agonists and corticosteroids have been reported to still be used in several European countries [1,2]. Clenbuterol (CB), a β-adrenergic agonist, acts as a repartitioning agent to improve performance of meat producing animals. Some metabolic and endocrine studies of the effects of clenbuterol at growth promoting doses have been performed in laboratory animals. It has been stated that the enhancement of growth efficiency is produced by stimulation of β-adrenergic receptors located on cell surfaces. In the muscle clenbuterol promotes protein synthesis and cell hypertrophy while in adipose tissue it promotes lipolysis. The involvement of different hormones in the repartitioning effect of clenbuterol is under discussion but the final effect is the increase of lean mass and the reduction of carcass fat up to 40% accompanied by a remarkable effect on the growth rate [3]. Serious concern exits on the possibility that CB could cause hyperstimulation of hypothalamus–pituitary–adrenal axis (HPA) accompanied by the increase of serum levels of glucocorticoid hormones, and compromising animal wellbeing [4]. However, most of these studies have been restricted to short-term treatments [5,6]. Our previous results, after a long-term treatment with clenbuterol, have shown an increase in plasma and tissue content of corticosterone even 20 days after withdrawal [4].

Dexamethasone (DEX) is a potent synthetic glucocorticoid and although at large doses it reduces growth rates and leads to muscle atrophy, it is frequently used as an illegal promoter in livestock production because it has been stated that low doses of

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glucocorticoids improve feed intake and increase growth rate, at the expense of reducing nitrogen retention and increasing water retention and fat content [1,7]. On the other hand, the suppressive actions of synthetic glucocorticoids on the HPA axis are well known [8,9].

More recently, analysis of individuals from several species revealed the administration of certain combinations of growth promoters in illegal practice [1,10], including β-adrenergic agonists, anabolic steroid hormones, thyreostatics and corticosteroids, more specifically dexamethasone.

The combination of β-agonists and dexamethasone has been used in order to prevent β-adrenergic down-regulation and tolerance in the animal caused by β-agonists (clenbuterol) [11]. Combination of these growth promoters also improves meat quality by compensating/diminishing undesirable side effects of both groups of promoters [1,12]. Finally, the combination of dexamethasone with β-adrenergic agonists (called "*erasers*") seems to reduce the content of these drugs in several organic matrices such as liver and plasma while the growth promoting effect of the β-agonist is maintained. The effects of the combination of illegal substances are poorly studied and at present no evidence has been reported for the underlying mechanism that causes the decrease of β-agonists levels [13].

Our previous studies on the ovarian endocrine function and morphology showed that the long-term administration of CB caused the disruption of ovarian endocrine function and morphology but those changes were reverted after 20 days of withdrawal and reproductive function was recovered. However, when DEX is administered alone or combined with CB, the disruption of endocrine function, and the morphological changes observed were not reversed [13,14].

There is a lack of information regarding the effect of growth promoters on adrenal function in the above mentioned cases, that should be investigated. The presence of β-adrenergic receptors in the two structures of the adrenal gland (cortex and medulla) is known, the adrenal medulla is responsible for catecholamine secretion, and the cortex synthesizes glucocorticoids and other steroid hormones.

The aims of the study were to investigate whether the chronic administration of clenbuterol (for 45 days), at a growth promoting dose, alone and combined with dexamethasone, may cause the disruption of rat endocrine adrenal function (cortex and medulla), in terms of plasma levels of corticotropin (ACTH), corticosterone, epinephrine and norepinephrine. In order to elucidate if the effects of growth promoters could be reversed during a withdrawal period (20 days), plasma hormone concentrations will be also determined at 0, 5, 10, 15 and 20 days of this period.

2. Experimental

2.1. Animals

The experimental protocols adhered to the council of the EU rules [22] and were approved by the Institutional Animal Care and Use Committee of the Veterinary Faculty of Madrid at UCM (Spain). Treatments were previously described [15].

Briefly, the study was conducted in 100 adult female rats weighing 250–300 g of the Long Evans strain (LE/CppHsd, Harlan Iberica S.A., Barcelona, Spain). Rats were housed in Meraclon cages of dimensions 25 cm × 47.5 cm × 20 cm, divided in groups of five animals/cage, and maintained in a temperature, humidity and light controlled room: 20 ± 2 °C; 45% relative humidity; 12-h light:12-h dark cycle (from 08:00 a.m. to 08:00 p.m.). All rats were fed a standard laboratory pellet commercial diet (Harlan Iberica S.A.) and water ad libitum. In order to avoid stressful conditions during the experiment, rats were weighed daily, and saline solution was administered orally by stomach tube for an 8-day pretreatment period to allow them to become accustomed to handling and dosage. Rats were randomly allocated into 4 groups of 25 animals each. All animals were weighed weekly beginning before the first administration and ending at sacrifice.

2.1.1. Control group

Twenty-five rats were dosed orally by stomach tube every day for 45 days with 1 mL of saline solution (SS).

2.1.2. Treatment I-clenbuterol

Twenty-five rats were daily dosed orally by stomach tube for 45 days with an anabolic dose of CB-SS (1 mL at a final concentration of 1 mg CB kg^{-1} body weight) (Clenbuterol hydrochloride, Sigma Co., St. Louis, MO, USA).

2.1.3. Treatment II-clenbuterol + dexamethasone

Twenty-five rats were daily dosed orally by stomach tube for 45 days with the same dose of CB-SS administered in treatment I. Ten days before the end of treatment rats were injected subcutaneously with 0.1 mg kg^{-1} of DEX (Decadrán, Merck Sharp & Dohme de España, SA).

2.1.4. Treatment III-dexamethasone

Twenty-five rats were daily dosed orally by stomach tube for 45 days with 1 mL of SS. Ten days before the end of treatment rats were injected subcutaneously with 0.1 mg kg^{-1} of DEX.

2.2. Sampling

Rats were anaesthetized with a mixture of ketamine (90 mg kg^{-1} i.m.), xylazine (10 mg kg^{-1} i.m.) and atropine (0.04 mg kg^{-1} i.m.), and 2 mL of blood were collected as follows: S0, basal sample before administering any solution (SS or CB); S1, 1 h after the first solution (SS or CB) administration; S2–S7, weekly during the rest of the treatment.

Groups of 15 treated and 5 control rats were sacrificed after blood sampling at different days of withdrawal: the day of sacrifice (W0) or, 5 (W5), 10 (W10), 15 (W15) and 20 (W20) days by means of cervical dislocation, and adrenal glands removed and wet weighed.

All blood samples were obtained from jugular vein and placed in glass tubes with heparin (1%), they were then centrifuged (1300 × *g*) and plasma was obtained, aliquoted individually and stored frozen (−30 °C) until assayed.