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RESEARCH ARTICLE

In vitro effects of some anabolic compounds on erythrocyte carbonic anhydrase I and II

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Abstract
The *in vitro* effects of the anabolic compounds, zeranol, 17 β-estradiol, diethylstilbestrol (DES), and trenbolone, on the activity of purified human carbonic anhydrase I and II were evaluated. *In vitro* CA enzyme activity was determined colorimetrically using the CO_2 hydration method of Maren. IC_{50} values of the compounds that caused inhibition were determined by means of activity percentage diagrams. The IC_{50} concentrations of zeranol, 17 β-estradiol, DES and trenbolone on hCA I were 94, 55, 10, 898 μM and for hCA II 89, 159, 439 and 101 μM, respectively.

Keywords: Carbonic anhydrase, anabolic compounds, inhibition

Introduction

The metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) catalyzes a very simple but critically important physiological reaction: the involvement of the CA enzyme family, which catalyzes the physiological hydration of CO_2 to yield bicarbonate and a proton, in many physiological/pathological processes open up widespread opportunities for the development of diverse, specific inhibitors for clinical application[1,2].

The use of anabolic steroids for growth promotion purposes in meat producing animals results in an improvement in muscle growth, more lean meat and a higher feed efficiency. However, toxicological/epidemiological studies show that there are harmful effects to consumers; as a result, the public health is placed at risk. As a consequence, the use of anabolic steroids for fattening purposes has been banned in the European Union since 1986[3]. These anabolic agents are used for increasing the rate of weight gain, improving the feed efficiency, storing protein and decreasing fatness[4–6]. However, depending on the use of anabolic in animal feed, anabolic residues that may occur in meat and meat products present risks to human health[7].

Zeranol is a resorcylic acid lactone and a synthetic oestrogenic derivative of the mycotoxin zearalenone, which is produced by Fusarium moulds. It is a weak oestrogen and is currently used to improve feed conversion efficiency and promote growth rates in livestock production. It has been widely used since 1969 as a growth promoter in the USA to improve the fattening rates of cattle[8]. In cattle, zeranol is discharged 65 days after implantation with a rate of 96.3% and zeranol level decreases in all organs and tissues below 2 ppb (μg/kg)[9].

Trenbolone acetate (TBA), a kind of 19-nortestesteron, is a synthetic steroid with anabolic properties[10–12]. TBA decreases the rate of both protein synthesis and degradation, and when the rate of degradation is less than the rate of synthesis, muscle protein rate increases[13]. Diethylstilbestrol (DES) is a synthetic estrogenic compound with carcinogenic and anabolic effects (3). Its most important effect is to improve the growth rate by increasing the quantity of digestible feed in livestock. As DES is a carcinogenic compound, its use has been banned in animal production in European Union countries[10]. Estradiol (ES) is a sort of natural anabolic steroid hormone. It exists as 17α- and 17β-isomers. Estradiol-17β has been widely applied in clinics for its most potent biological effects of the endogenous estrogens[3], and has also been used to promote unisexualization, improve feed

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conversion efficiency and increase the rate of weight gain in aquaculture because of its protein synthesis stimulation and sex reversal effects. However, chronic exposure of humans to ES-17β through food chain can cause toxic effects as caused by other steroid hormones on public health, such as children precocious puberty, teratogenicity and carcinogenesis. Ingestion of ES-17β residues in treated aquatic animal tissues may be potentially hazardous to consumers. Therefore, monitoring the residual content of this compound in fishery products is necessary for controlling the illegal use of such substances to ensure public health and trade contacts[14]. Oxytocin is a posterior pituitary hormone that acts directly on smooth muscle to produce rhythmic contractions and with this ability it is closely involved in lactation[15]. Because of this property, oxytocin is often injected into cattle that are under stress or unable to produce much milk to enhance milk production[16].

In recent years, hormones and hormone like compounds have been used frequently in vegetables and livestock production to obtain a high performance in a short time. These anabolisants are used for increasing the rate of weight gain, improving the feed efficiency, storing protein and decreasing fatness. But, depending on the use of anabolic in animal feed, residues which may occur in meat and meat products present human health risks. Thus, the determination of the effects of these compounds on human CA I and II activity are vital. The aim of this study is to define the effects of DES **(1)**, trenbolone **(2)**, Estradiol-17β **(3)**, zeranol **(4)**, on erythrocyte CA I and II and thus evaluate the toxicological affects of these drugs *in vitro*.

Materials and methods

Materials

Sepharose 4B, L-tyrosine, sulphonamide, protein assay reagents and chemicals for electrophoresis were obtained from Sigma Chem. Co. (R-Biopharm pharmacy, Darmstadt, Germany). All other chemicals used were of analytical grade and obtained from either Sigma or Merck. All hormones were provided by the local pharmacy.

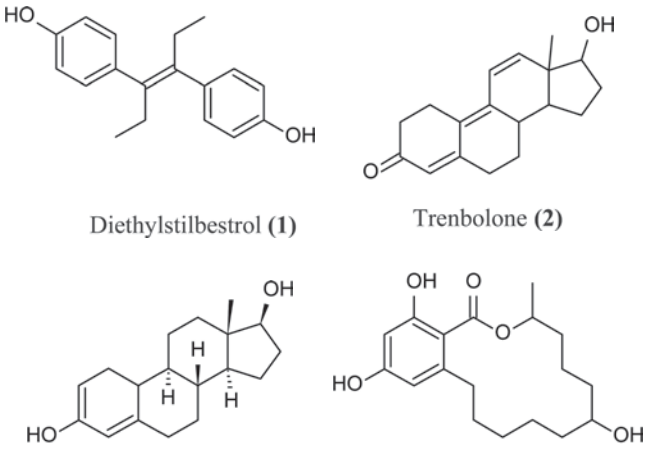
CA enzyme assay

CA activity was measured by the Maren method which is based on determination of the time required for the pH to decrease from 10.0 to 7.4 due to CO_2 hydration[17]. Human CA I and II were purified from red blood cells according to the method of Ozensoy et al. (2004[18]).

In vitro inhibition studies

For the inhibition studies of anabolisants different concentrations of these compounds were added to the enzyme activity. Activity % values of CA for different concentrations of each anabolisant were determined by regression analysis using Microsoft Office 2000 Excel. CA enzyme activity without an anabolisant solution was accepted as 100% activity. For the anabolisants having an inhibition affect, the inhibitor concentration causing up to 50% inhibition (IC_{50} values) was determined from the graphs.

Table 1. The IC_{50} values of anabolisant compounds.



Compound	hCA I	hCA II
1	94 μM	89 μM
2	55 μM	159 μM
3	10 μM	439 μM
4	898 μM	101 μM

Results and discussion

In this study, CA I and II isoenzymes from human erythrocytes were purified by a simple one step procedure by using Sepharose 4B-L-tirozin-sulfanilamide affinity column. The activity of the eluents was determined as described in material and methods. The inhibitory affects of some anabolisants on human cytosolic CA I and II activity were investigated. Different inhibition effects of the applied anabolisants were obtained and showed in Table 1. Approximately, hCA I and hCA II exhibit the same manner effect to the anabolisants tested. As shown in Table 1, estradiol-17β **(3)** has been shown to be the strongest inhibitor against the hCA I activity while DES **(1)** causes the strongest inhibition on hCA II activity.

We have determined the IC_{50} values of 94–898 μM for the inhibition of hCA I activity. Durdagi reported that the phenol showed an inhibition constant of 10.4 μM, whereas 3,5-dihydroxy-benzoic acid is a much more effective CAI compared to all other known classes of inhibitors (Ki of 0.55 μM). The least effective inhibitor is 1,2-dimethoxy-benzene (Ki of 10.4 μM) which is, however, rather similar with that of phenol. Anabolisants, therefore, have weak CA I inhibitory potencies to that of these phenols[19].

Declaration of interest

The authors report no conflicts of interest.

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