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SHORT COMMUNICATION

THE ANTIGLUCOCORTICOID RU486 INHIBITS THE ETHANOL-INDUCED INCREASE OF TRYPTOPHAN OXYGENASE

HALLVARD GJERDE, JØRG MØRLAND and HARALD OLSEN
National Institute of Forensic Toxicology, Sognsvannsveien 28, N-0372 Oslo 3, Norway

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Summary—The effect of the glucocorticoid-antagonist RU486 (Roussel-Uclaf, France) on the increased activity of hepatic tryptophan oxygenase (TO) after administration of corticosterone and ethanol in rats was studied. RU486 (40 mg/kg *per os*) inhibited completely the effect of corticosterone (5–15 mg/kg injected intraperitoneally) on TO. Ethanol (4 g/kg) given intraperitoneally is followed by peak corticosterone concentrations comparable to those seen after the administration of 5–10 mg exogenous corticosterone, and increased the TO activity 3-fold 4 h after the injection. RU486 inhibited completely the ethanol-induced increase of TO, indicating that this increase is mediated by corticosterone.

INTRODUCTION

It has been shown that administration of a single large dose of ethanol increases the activity of hepatic tryptophan oxygenase (TO; EC 1.13.11.11) [1, 2]. This enzyme is the rate-limiting enzyme in the most important pathway for tryptophan degradation in the body and changes in its activity can alter tryptophan metabolism in other tissues, such as the synthesis of the neurotransmitter serotonin in brain, which could be involved in some of the central nervous system effects of ethanol [3, 4].

Well known factors increasing liver TO activity are glucocorticosteroids [5, 6], which increase synthesis of the enzyme, and tryptophan [7], which decreases the rate of degradation of TO. Acute ethanol administration has been shown to increase the blood level of both these factors [3, 8].

This study was undertaken to clarify the role of glucocorticoid hormones in the ethanol-induced increase of liver TO. This was done by administering the glucocorticoid antagonist RU486 to rats in addition to ethanol and measure the effects on liver TO. RU486 is the first antiglucocorticoid with *in vivo* activity [9–11] and was a gift from Roussel-Uclaf.

EXPERIMENTAL

Adult male Wistar rats (Møllegaard, Ejby, Denmark) weighing 200–300 g were used. The animals were starved 20–24 h before the experiments, but had free access to water. RU486 (Roussel-Uclaf, Roumainville, France; 40 mg/kg) was intubated intragastrically as a suspension in water (10 mg/ml). The crystalline antagonist was dissolved in a small amount of ethanol (100 mg/ml)before water was added. This amount of ethanol is too low to have any effect on TO [12]. Corticosterone (Sigma Chemical Co., St Louis, MO, U.S.A.) was injected intraperitoneally as a suspension in saline (0.9% NaCl w/v). Ethanol (4 g/kg) was injected intraperitioneally as a 12% (w/v) solution in saline.

The animals were anaesthetized using Nembutal (Abbott, St Remy-sur-Avre, France; 60 mg/kg, intraperitoneally). Livers were purged of blood by perfusing an ice-cold saline-heparin mixture (10 IU heparin per ml saline) through the portal vein for 30 s, and liver samples were frozen in liquid nitrogen $3\frac{1}{2}$ min after the perfusion and stored at −80°C [13]. TO activities were determined spectrophotometrically, measuring the formation of kynurenine from tryptophan during the incubation of crude liver homogenates in presence (total enzyme activity) and in the absence (holoenzyme activity) of added hemoglobin as a source of heme cofactor [14].

RESULTS AND DISCUSSION

In order to examine the ability of RU486 to antagonize the effect of corticosterone on TO, different doses of RU486 were administered to rats in addition to corticosterone (10 mg/kg). Neither 15 mg/kg (injected i.p. 15 min prior to the injection of corticosterone) nor 20 mg/kg (*per os*, intubated 30 min prior to corticosterone injection) inhibited completely the induction of TO. However, 40 mg/kg produced a complete inhibition when intubated 30–40 min prior to the injection of corticosterone. The effect of RU486 (40 mg/kg) on the induction of TO by different doses of corticosterone is presented in Fig. 1. Figure 1 shows that 5–15 mg corticosterone per kg induced TO by 86–200% respectively (total enzyme activity), this was completely inhibited by RU486.

In a separate experiment we tested if RU486 could have a nonspecific inhibitory effect on protein synthesis, manifesting itself by decreased TO-synthesis. Labelled valine dissolved in saline (L-[U-^{14}C]valine was obtained from New England Nuclear, Boston, MA, U.S.A.) was injected intraperitoneally to some rats (10 μCi per kg). In addition RU486 (40 mg/kg) or water was given to the rats *per os* 60 min prior to the injection of labelled valine. The rats were killed 3 h after the injection, and liver samples were homogenized in water. The incorporation of labelled valine into liver protein was determined by precipitating the protein in the liver homogenates with ice cold trichloroacetic acid, and washing the precipitated protein with ice-cold 6% (w/v) trichloroacetic acid containing 1% (w/v) tungstophosphoric acid and 1.3% (w/w) cold valine prior to scintillation counting. The results indicated that RU486 did not nonspecifically inhibit protein synthesis (results are not shown).

RU486 thus proved to be an excellent inhibitor of the induction of TO by corticosterone. Therefore it was of interest to examine whether RU486 could inhibit the

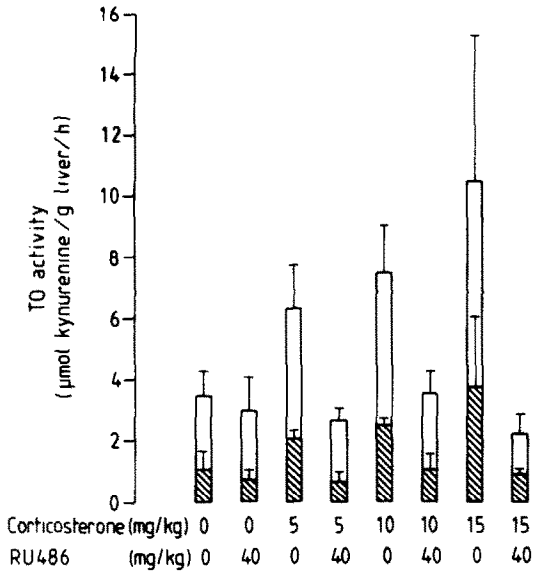

Fig. 1. Effects of corticosterone on TO activity (holoenzyme activity and total activity) without and in presence of RU486. Columns and vertical bars represent means and SD ($n = 4$), respectively. Hatched columns represent holoenzyme. RU486 or water was intubated *per os* 30–40 min prior to the injection of corticosterone, and the rats were killed 4 hours after the injection of corticosterone.

ethanol-induced increase of TO. The administration of 4 g ethanol increases plasma corticosterone 5-fold after 30 min [8]. This is comparable to the increase obtained after administration of 5–10 mg exogenous corticosterone by the intraperitoneal route [13]. As shown in Table 1, administration of ethanol (4 g/kg) increased the TO activity by 170% (total enzyme activity). When RU486 was administered 60 min prior to the injection of ethanol, the increase in TO activity was completely inhibited (both total and holoenzyme activity). This indicates that the induction is mediated by corticosterone.

It was suggested by Mørland [15] that the ethanol-induced increase in liver TO was due to a rise in plasma corticosterone. Badawy and Evans [2, 3] suggested, however, that this increase of TO was not mediated by corticosterone, but rather by free tryptophan. Stowell and Mørland [13] later measured the effect of ethanol on plasma corticosterone and tryptophan, concluding that the corticosterone increase was high enough to induce TO, while the increase in free tryptophan was not. They also studied the effect of the antilipolytic drug propranolol and the protein synthesis inhibitor cycloheximide on the ethanol-induced increase of TO, and the results indicated that this increase was not mediated by tryptophan but probably by corticosterone which induces synthesis of TO [16].

The observations presented in this report confirm that the ethanol-induced increase in liver TO is due to an increased plasma corticosterone concentration.

Table 1. The effect of RU486 on the ethanol-induced increase of tryptophan oxygenase

	TO enzyme activity (μmol kynurenine/g liver/h)	
	Holoenzyme	Total enzyme
Controls	0.9 ± 0.6	3.3 ± 0.7
Ethanol	4.4 ± 1.1	9.0 ± 1.6
Ethanol + RU486	1.3 ± 0.4	3.0 ± 0.5

RU486 (40 mg/kg) or water was administered 1 h prior to the administration of ethanol (4 g/kg), and the rats were killed 4 h after ethanol administration. Values are means ± SD of 6 rats.

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