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Expression of the ACTH receptor, steroidogenic acute regulatory protein, and steroidogenic enzymes in canine cortisol-secreting adrenocortical tumors

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Abstract

Studies of human adrenocortical tumors (ATs) causing Cushing's syndrome suggest that hypersecretion of cortisol is caused by altered expression of steroidogenic enzymes and that steroidogenesis can only be maintained when there is expression of the ACTH receptor (ACTH-R). Here we report the screening for the mRNA expression of the ACTH-R, steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage enzyme, 3β-hydroxysteroid dehydrogenase, 21-hydroxylase (all in 38 cortisol-secreting ATs), 17α-hydroxylase, and 11β-hydroxylase (both in 28 cortisol-secreting ATs). Real-time PCR (RT-PCR) was applied in all samples and was compared with that in normal canine adrenal glands. Messenger-RNA encoding StAR, steroidogenic enzymes, and ACTH-R were present in both normal adrenal glands and cortisol-secreting ATs. The amounts of mRNA encoding StAR and enzymes of the steroidogenic cluster needed for cortisol production did not differ significantly between either adenomas or carcinomas and normal adrenal glands. The amount of mRNA encoding ACTH-R was significantly lower in carcinomas than in normal adrenal glands ($P = 0.008$). In conclusion, RT-PCR analysis revealed no overexpression of StAR and steroidogenic enzymes in canine cortisol-secreting ATs. Significant downregulation of ACTH-R in carcinomas might be associated with the malignant character of the AT.

Keywords: Cushing's syndrome; Hypercortisolism; Tumorigenesis; Adrenal cortex; Dog

1. Introduction

Adrenal or ACTH-independent hypercortisolism accounts for about 15% of cases of spontaneous hypercortisolism in dogs [1]. The mechanisms leading to autonomous hypersecretion of cortisol have not yet been defined. Under physiological conditions, steroidogenesis in an adrenocortical cell is initiated by the binding of ACTH to its receptor (ACTH-R) [2], which is a seven-transmembrane domain receptor belonging to a G-protein-coupled receptor subfamily. ACTH-R is called also MC2-R and is a member of the melanocorticotropin receptor subfamily [3,4]. These receptors are characterized by short NH_2-terminal extracellular domains, short intracellular COOH-terminal domains, short fourth and fifth transmembrane-spanning domains, and a small hydrophobic second extracellular loop [5]. The acute response to a steroidogenic stimulus is mediated by steroidogenic acute regulatory protein

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(StAR). This mitochondrial phosphoprotein enhances cholesterol transport from the outer to the inner mitochondrial membrane [6,7]. Consequently, cholesterol will be converted to pregnenolone by the cholesterol side-chain cleavage enzyme (CYP11A), the rate-limiting step in steroidogenesis [7,8].

In humans and mice it has been demonstrated that regulation of the ACTH-R gene is unique, in that it is upregulated by its own ligand in a time- and dose-dependent manner [2]. Plasma ACTH concentration is suppressed in cortisol-secreting adrenocortical tumors (ATs). Hence the expression of ACTH-R would be expected to be low and the role of ACTH and its receptor in initiating steroidogenesis would be expected to be small. The expression of ACTH-R in human cortisol-secreting carcinomas and nonfunctional ATs has indeed been found to be significantly downregulated [9,10], but ACTH-R mRNA expression in cortisol-secreting adenomas did not differ significantly from that in normal adrenals and was independent of plasma ACTH concentration [11]. Administration of ACTH to patients with cortisol-secreting adenomas resulted in a significant increase in plasma cortisol concentration [12]. This and the finding that the expression of ACTH-R in adenomas is correlated with expression of CYP11A led to the conclusion that ACTH-R appears to be functional in cortisol-secreting adenomas [11,12].

A recent study on the expression of enzymes in the cortisol pathway of steroidogenesis provides evidence that hypersecretion of cortisol by human ATs can be explained by alterations in steroidogenesis. In comparison with normal adrenals, human cortisol-secreting adenomas overexpressed mRNA encoding CYP11A, 3β-hydroxysteroid dehydrogenase (HSD3B2), 17α-hydroxylase (CYP17), and 11β-hydroxylase (CYP11B1) [13].

There is as yet no information on the expression of ACTH-R and enzymes of the steroidogenic cluster needed for cortisol production in canine ATs, but there is indirect evidence of the functionality of ACTH-R. In approximately 60% of dogs with hypercortisolism resulting from AT, the cortisol response to ACTH administration is exaggerated [14].

The aim of this study was to investigate the mRNA expression of ACTH-R, StAR, and the steroidogenic enzymes of the cortisol pathway—CYP11A, HSD3B2, CYP17, 21-hydroxylase (CYP21), and CYP11B1 (Fig. 1) and—by real time-PCR (RT-PCR) in canine cortisol-secreting ATs.

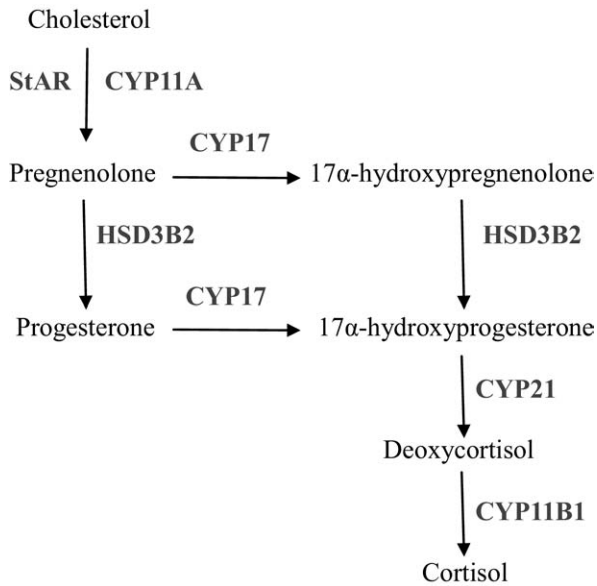

Fig. 1. Steroidogenic acute regulatory protein (StAR) and steroidogenic enzymes involved in the cortisol pathway: steroidogenic cholesterol side-chain cleavage enzyme (CYP11A), 3β-hydroxysteroid dehydrogenase (HSD3B2), 17α-hydroxylase (CYP17), 21-hydroxylase (CYP21), and 11β-hydroxylase (CYP11B1).

2. Materials and methods

2.1. Animals and tissues

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Utrecht University. Permission to use adrenal gland tissue for this study was obtained from the owners of the dogs. The ATs were obtained from 38 dogs with ACTH-independent hypercortisolism that underwent unilateral adrenalectomy at the Department of Clinical Sciences of Companion Animals. Their ages at the time of surgery ranged from 6 to 14 yr (mean, 9 yr). Twelve dogs were mongrels and the others were of 10 different breeds. There were 18 males (8 castrated) and 20 females (15 neutered).

Suspicion of hypercortisolism was based on the history, physical examination, and routine laboratory findings. The diagnosis was considered confirmed by the finding of a urinary corticoid:creatinine ratio (UCCR) above the upper limit of the reference range (8.3×10^{-6}) in two consecutive morning urine samples collected at home [15]. After collection of the second urine sample the dog received three doses of 0.1 mg dexamethasone/kg body weight orally at 8-h intervals; the third urine sample was collected the following morning. ACTH-independent hypercortisolism was diagnosed when the UCCR of the third sample was sup-