Influence of cannabinoids on immunoreactivity of regulatory peptides, produced in rat thyroid C cells; preliminary investigations

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Abstract

Mammalian tissues contain two types of cannabinoid receptors CB1 and CB2. The aim of our study was an evaluation of the influence of a single ip injection of a stable analogue of an endogenous cannabinoid anandamide - R-(+)-methanandamide (2.5 mg/kg) and CP 55,940 (0.25 mg/kg), which is an exogenous agonist of CB1 receptors, on the immunoreactivity of regulatory peptides, produced in rat thyroid C cells: calcitonin, CGRP, somatostatin and synaptophysin. This study indicates that a single injection of cannabinoids: R-(+)-methanandamide and CP 55,940 alters the immunoreactivity of regulatory peptides in thyroid parafollicular cells.

Key words: thyroid C cells, regulatory peptides, cannabinoids, rat.

Introduction

The psychoactive properties of the marijuana plant, *Cannabis sativa*, have been known for thousands of years. The pharmacological actions of its main active compound,

Δ^9-tetrahydrocannabinol (Δ^9-THC) have recently been documented, following the discovery of two distinct cannabinoid receptors: CB1 (expressed mainly in CNS) and CB2 (that occur mainly in the immune cells) [1]. The identification of naturally occuring ligands for these receptors, anandamide and 2-arachidonylglycerol (2-AG), has prompted a large research effort, aimed at investigating the physiological role of the endogenous cannabinoid system, as well as its potential use as a target for novel therapeutic interventions [1]. Recent studies suggest that endocannabinoids, among others, also play a role in the regulation of the activity of endocrine cells. It has been reported that Δ^9-THC and endocannabinoids exert an inhibitory influence on the regulation of reproduction. An administration of Δ^9-THC and anandamide decreased serum levels of luteinizing hormone and prolactin, while increased the levels of ACTH and corticosterone [1]. Further studies, performed with Δ^9-THC [2], and WIN 55,212-2 [3], a selective CB1 receptors agonist, while showing diminution of T_3 and T_4 plasma levels after a single injection of these compounds, confirmed their influence on the endocrine system. High levels of CB1 mRNA, observed during the late embryological stages of rat thyroid, and the presence of CB1 mRNA and protein in the adult rat thyroid, both in follicular and parafollicular (C) cells, may point to an involvement of cannabinoid receptors in the mediation of the thyroid gland activity [4].

The aim of this study was an evaluation of the influence of a single ip injection of a stable analogue of endogenous cannabinoid anandamide - R-(+)-methanandamide and CP 55,940, an exogenous agonist of CB1 receptors on the immunoreactivity of the regulatory peptides, produced in rat thyroid C cells: calcitonin (CT), CGRP, somatostatin (SS) and synaptophysin (SY).

The study was conducted on 30 male Wistar rats, weighing 180-185 g each, which were divided into 3 groups. All the animals were housed in plastic cages, four animals per cage, in temperature of 22^0 C and constant humidity, with a 12/12 light/dark cycle. Food and water were freely accessible. All the procedures were performed in compliance with the European Communities Council Directive of the 24th November 1986 (86/609/EEC) and were approved by the Local Ethics Committee in Białystok. R-(+)-Methanandamide and CP 55,940 (Tocris), dissolved in 19% solution of 2-hydroxypropyl-B-cyclodextrin (RBI) were injected once, at the intraperitoneal dose of 2.5 mg/kg or 0.25 mg/kg, respectively. The control rats

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Figure 1. CGRP-immunoreactivity: A) Thyroid gland of control rats (x 200); B) Thyroid gland of rats treated with R-(+)-methanandamide (x 200); C) Thyroid gland of rats treated with CP 55,940 (x 400).

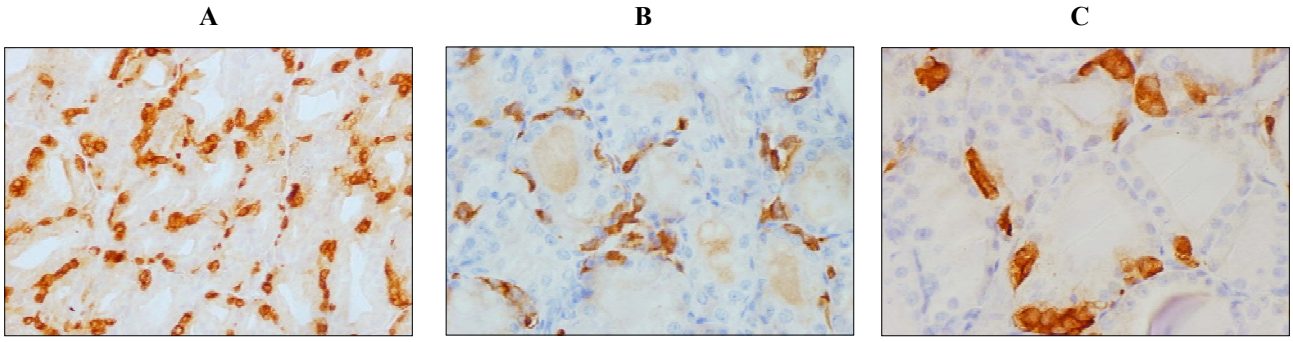


Figure 2. Somatostatin-immunoreactivity: A) Thyroid gland of control rats (x 400); B) Thyroid gland of rats treated with R-(+)-methanandamide (x 400); C) Thyroid gland of rats treated with CP 55,940 (x 400).

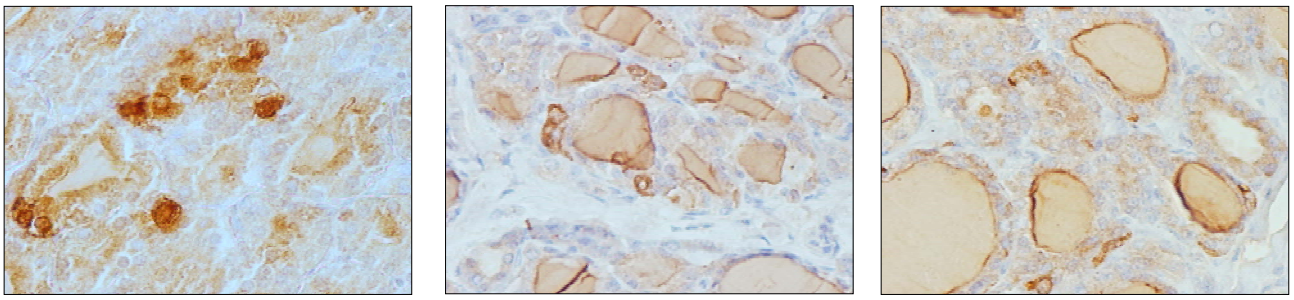
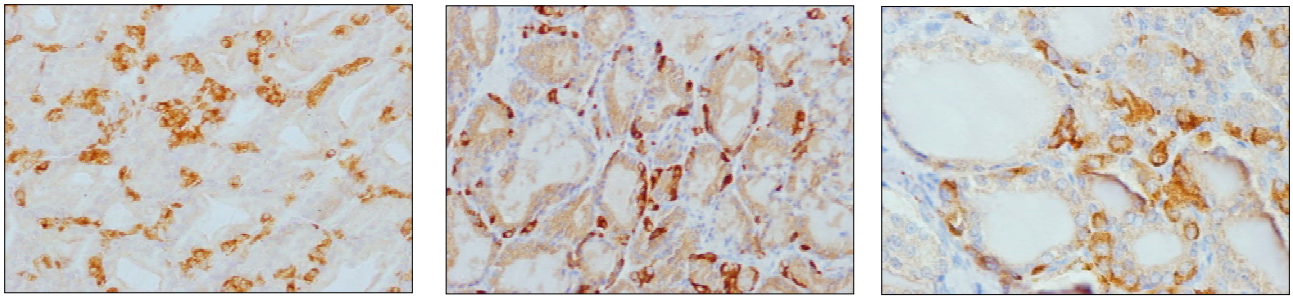


Figure 3. Synaptophisin-immunoreactivity: A) Thyroid gland of control rats (x 200); B) Thyroid gland of rats treated with R-(+)-methanandamide (x 200); C) Thyroid gland of rats treated with CP 55,940 (x 400).



were injected only with the vehicle solution. Four hours after the injections, under pentobarbital sodium anaesthesia (50 mg/kg), both thyroid lobes were extracted and fixed in Bouin's fluid. Paraffin 5-µm sections were made. Immunocytochemical reactions were performed, using the ABC technique. Specific antibodies were used against: calcitonin, synaptophysin (SY), somatostatin (DAKO) and CGRP (Sigma, Aldrich). Control reactions yielded negative results.

Four hours after a single injection of both cannabinoids, the majority of thyroid follicles, particularly those, peripherally located, were of large size with a low epithelium, and blood-vessels were dilated. In addition, the application of CP 55,940 and, in a lesser degree, of R-(+)-methanandamide, caused an enhancement of the immunoreactivity of CGRP (Fig. 1 A, B, C) and synaptophysin (Fig. 3 A, B, C), while both cannabinoids attenuated the immunoreactivity of somatostatin (Fig. 2 A, B, C), as compared to respective values in the control group. Moreover, the enhancement of CT-immunoreactivity was similar to those, observed in our previous study [6].

Porcella et al. [5] have demonstrated the presence of CB1 receptors in the thyroid gland, which are, probably, tonically activated by endogenous cannabinoids, similarly as those in the central nervous system. Since cannabinoids exert an inhibitory action on the peptides release, the attenuation of SS-immunoreactivity is probably connected with their influence on SS synthesis. In our previous study, the enhancement of CT-immunoreactivity in parafollicular cells, accompanied by a significant diminution of CT plasma concentration, was observed after a single injection of both cannabinoids [6]. That observation points to an inhibiting role of cannabinoids on the secretion activity of C cells. Therefore, the activation of CB1 receptors, located on parafollicular cells [7], by R-(+)-Methanandamide and CP 55,940 probably leads to decreased CT plasma concentration, owing to the inhibition of calcium-dependent CT release. Since CT and CGRP are produced by the same gene and cannabinoids enhanced their immunoreactivity in C cells, the same mechanism of this action may be considered. Recently, we have demonstrated a mutual relation between parafollicular and

follicular cells *in vivo* [7]. The hypoactivity of follicular cells, evoked by an i.p. application of L-thyroxine, brought about an inhibition of C cells activity, expressed by attenuation of CT plasma levels and an enhancement of CT-immunoreactivity in C cells. Also Hillard et al. [3] observed a significant reduction of TSH, T_3 and T_4 serum levels after a single injection of Δ^9THC, with the maximal TSH decrease occurring one hour after the administration, followed by a significant diminution of T_3 and T_4 serum concentrations, observed 3 and 6 hours later, respectively. Also Nazar et al. [8] have demonstrated a meaningful attenuation of thyroxine plasma concentration 6 hours after Δ^9THC administration, either single or repeated for 3 days. These observations provide a presumption that cannabinoids may regulate the activity of C cells directly, via CB1 receptor, and also indirectly, by an influence on the activity of follicular cells. Another possibility of cannabinoid action may be the inhibition of noradrenaline release from sympathetic nerve terminals. The autonomic nervous system takes place in the regulation of the follicular and parafollicular cell activity. Current data indicate increased basal calcitonin plasma concentrations after an application of the ß-adrenergic receptor agonist, reversed by an administration of this receptor antagonist [9].

Synaptophysin, as evaluated in our study, is an integral membrane glycoprotein of the vesicles of synapses and neuroendocrine cells, which play some role in the neurotransmitter and hormone release from synaptic vesicles and neuroendocrine cells by making an exocytolitic fusion pore [10]. The increase of SY-immunoreactivity of C cells, caused by CP 55,940, and, in a lesser degree, by R-(+)-Methanandamide administration, may indicate an important role of synaptophysin in the regulation of peptide secretion from C cells, e.g., CT and CGRP.

This study indicates that a single injection of cannabinoids: R-(+)-methanandamide and CP 55,940, alters the immunoreactivity of regulatory peptides in the thyroid parafollicular cells. Therefore, the significant impact of cannabinoids on the secretory activity of C cells should be taken into consideration.

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