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An immunohistochemical study of the thyroid parafollicular (C) cells in rats treated with cannabinoids — preliminary investigations

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The purpose of the present study was to evaluate the effect of a single intraperitoneal injection of a stable analogue of endogenous cannabinoid anandamide — R-(+)-methanandamide (2.5 mg/kg) and CP 55,940 (0.25 mg/kg), an egzogenous CB1 receptor-agonist, on the calcitonin (CT) immunoreactivity of the thyroid parafollicular (C) cells. Four hours after injection with both cannabinoids CT immunoreactivity, evaluated with an avidin-biotin peroxidase complex method by means of rabbit antibodies against CT, was seen to be enhanced in the parafollicular cells in comparison to those of the control group. In thyroids taken from cannabinoid-treated rats the majority of follicles, particularly those located peripherally were large in size, and had low epithelium. Moreover, dilatation of the blood vessels was observed. These changes were accompanied by a significant decrease in CT plasma level, without changes in calcium concentrations. This is the first evidence that a single injection of the cannabinoids R-(+)-methanandamide and CP 55,940 significantly decreases the activity of thyroid C cells.

key words: thyroid C cells, cannabinoids, immunohistochemistry, rats

INTRODUCTION

In 1970 Lomax [4] reported the inhibitory effect of marijuana on thyroid function and indicated the hypothalamus as the putative site of its action. Further studies performed with Δ^9THC, the principal psychoactive constituent of marijuana, [2, 5] and WIN 55,212-2 [7], a selective CB1 receptor agonist, showing diminution of T_3 and T_4 plasma levels after a single injection of these compounds, confirmed the above observation. The discovery of cannabinoid receptors and the identification of their natural ligands, have contributed to the advancement of cannabinoid neurobiology and pharmacology. Mammalian tissues contain two types of cannabinoid receptors, CB1 and CB2, both of which have been cloned [6]. CB1 receptors are predominantly present in the central nervous system and also in certain peripheral tissues [6]. The high levels of CB1 mRNA during the late embryological stages of the rat thyroid [1], and the recently demonstrated presence of cannabinoid CB1 mRNA and protein in the adult rat thyroid, both in the follicular and parafollicular (C) cells [7], may point to the involvement of cannabinoid receptors in mediation of thyroid gland activity. Although, the levels of CB1 mRNA in the thyroid gland were much lower than those in the brain areas, the

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presence of FAAH mRNA in the thyroid supports the existence of an endocannabinoid system in this gland [7].

The purpose of the present study was the evaluation of a single intraperitoneal injection of a stable analogue of endogenous cannabinoid anandamide — R-(+)-methanandamide (2.5 mg/kg) and CP 55,940 (0.25 mg/kg), an egzogenous CB1 receptor-agonist, on calcitonin (CT) plasma level and the CT immunoreactivity of the thyroid parafollicular cells.

MATERIAL AND METHODS

Male Wistar rats weighing 180–185 g were used following 7 days of acclimatisation to the laboratory conditions. The animals were housed in plastic cages, 4 animals per cage, in a temperature-controlled room (20° C) and constant humidity, with a 12/12 light/dark cycle. Food and water were freely accessible. All procedures were performed in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Local Ethics Committee in Białystok.

R-(+)-methanandamide (Tocris) and CP 55,940 (Tocris), dissolved in a 19% solution of 2-hydroxypropyl-B-cyclodextrin (RBI) were injected once, at the intraperitoneal dose of 2.5 mg/kg and 0.25 mg/kg, respectively. The control rats were injected with the vehicle solution only. Each group consisted of 10 animals. Four hours after the cannabinoid injection, under pentobarbital sodium anaesthesia (50 mg/kg), the blood was taken from the abdominal aorta of each rat to determine plasma CT concentration by RIA. The rats were subsequently thyreoidectomised. Both thyroid lobes were placed in Bouin's fluid for 24 hours. An immunohistochemical reaction used for detecting calcitonin in C cells was conducted on 5 μm-thick paraffin sections of the thyroid glands. In this procedure specific rabbit antisera against CT, which can be found only in C cells, were used. In the above immunohistochemical study the ABC (avidin-biotin peroxidase complex) method according to Hsu et al. [3] was applied.

Statistical analysis: statistical comparison for 2 means were made by a Student's *t*-test.

RESULTS AND DISCUSSION

Four hours after a single intraperitoneal injection of both cannabinoids, a stable analogue of endogenous cannabinoid, anandamide — R-(+)-methanandamide and CP 55,940, an egzogenous CB1 receptor-agonist, an enhancement of CT immunoreactivity was observed in a majority of thyroid parafollicular cells (Fig. 1) in comparison to the control group (Fig. 2). Moreover, in thyroids taken from cannabinoid-treated rats the majority of follicles, particu-

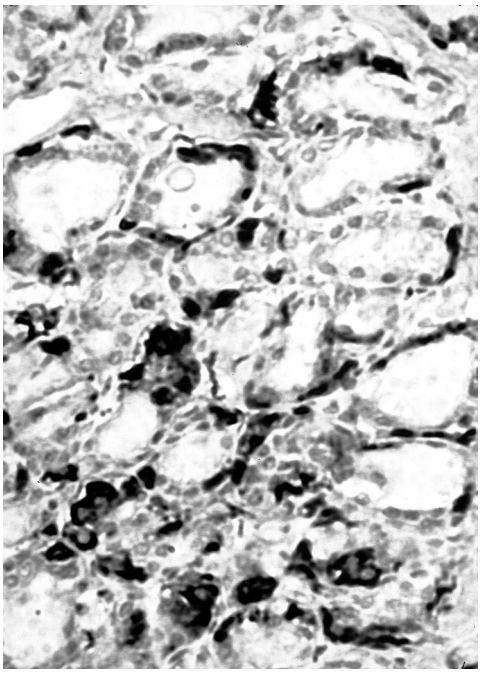

Figure 1. Light micrograph of the thyroid gland of a rat 4 hours after a single ip. injection of CP 55,940. The micrograph presents a piece of the gland with concentrations of C cells showing a strong immunohistochemical reaction for calcitonin; × 300.

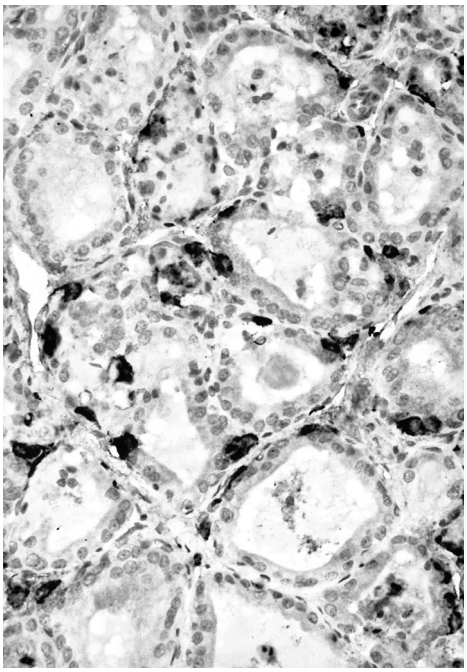

Figure 2. Light micrograph of the thyroid gland of a control rat. A differentiated intensity of immunohistochemical reaction for calcitonin in C cells is observed; × 300.

larly those located peripherally, were large in size and had low epithelium and the blood vessels were dilated. This picture is in agreement with an observation made by Hillard et al. [2] presenting significant reductions in TSH, T_3 and T_4 serum levels after a single injection of Δ^9THC, the principal psychoactive constituent of marijuana. The significant reductions in TSH serum levels were seen at 0.25, 0.5, 1.0 and 3.0 hours, with the maximal decrease occurring 1 hour after Δ^9THC administration, while a significant diminution in T_3 and T_4 serum concentrations was observed at 3 and 6 hours, respectively. Nazar et al. [5] have also demonstrated a significant attenuation of thyroxine plasma concentration 6 hours after a single Δ^9THC administration or after repeating the administration over three days. The enhancement of CT immunoreactivity in the parafollicular cells, accompanied by significant diminution of CT plasma concentration (Fig. 3), observed after a single injection of both cannabinoids, indicates that they also inhibit the secretion activity of C cells. This finding is consistent with the recent study performed by Porcella et al. [7] demonstrating the presence of an endocannabinoid system in the thyroid gland, which is probably tonically activated by endogenous cannabinoids, as in the central nervous system [8, 9]. Therefore, the activation of CB1 receptors, transducing signals through a pertussis toxin-sensitive G_i/G_o inhibitory pathway, located on parafollicular cells [7] by R-(+)-methanandamide and CP 55,940, probably leads to the inhibition of calcium dependent CT release, followed by a diminution in CT plasma concentration.

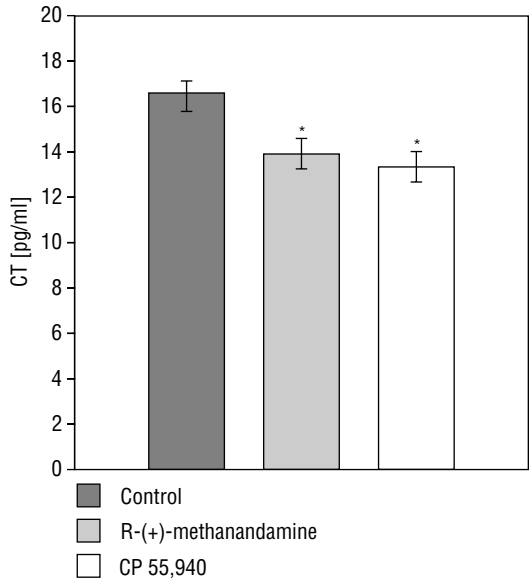

Figure 3. Effect of a single ip. injection of R-(+)-methanandamide (2.5 mg/kg) and CP 55,940 (0.25 mg/kg) on plasma calcitonin (CT) concentration. The columns represent means ± SEM of the values obtained from 10 rats; *$p < 0.05$ vs. those of a control group of rats, injected with a vehicle (Student's *t* test).

This is the first evidence that a single injection of the cannabinoids R-(+)-methanandamide and CP 55,940 significantly decreases the activity of the thyroid parafollicular (C) cells.

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