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Short communication

Co-distribution of the cannabinoid CB_1 receptor and the 5-HT transporter in the rat amygdale

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Abstract

Cannabis sometimes causes dysphoria rather than euphoria; however, evidence relating to the interaction between the cannabinoid and 5-hydroxtryptamine (5-HT) systems is limited, especially in areas of the brain such as the amygdale. Here we report that cannabinoid CB_1 receptors and 5-HT transporter proteins are co-distributed in the amygdale, suggesting the possibility that activation of cannabinoid CB_1 receptors might cause a reduction in 5-HT release, similar to its effect on other neurotransmitters, thereby resulting in dysphoria.

Keywords: Cannabinoid CB_1 receptor; 5-hydroxtryptamine; Amygdale

Although cannabis use usually results in euphoric experiences, clinical trials of medicinal cannabinoids suggest that some people experience dysphoria rather than euphoria (e.g., Campbell et al., 2001). Some studies also demonstrate that cannabis users can even experience panic and phobic attacks, which anecdotal reports suggest can be particularly severe in those concurrently using selective serotonin reuptake inhibitors (SSRIs) (e.g., Deas et al., 2000).

Pre-synaptic cannabinoid CB_1 receptors have been demonstrated to inhibit the release of many neurotransmitters, including γ-aminobutyric acid (GABA), glutamate, dopamine, noradrenaline, acetylcholine and 5-hydroxytryptamine (5-HT) (e.g., Nakazi et al., 2000), and this effect is thought to play a major role in the effects that cannabinoids such as delta-9-tetrahydrocannabinol (delta-9-THC) have on brain function. A reduction in 5-HT release caused by the activation of cannabinoid CB_1 receptors might be expected to have a significant dysphoric effect. Functional electrophysiological studies in the neocortex have shown that activation of cannabinoid CB_1 receptors results in decreased 5-HT release (Nakazi et al., 2000). However, to the best of our knowledge, the only co-distribution studies investigating the cannabinoid CB_1 receptor and 5-HT systems, have been restricted to 5-HT receptors. Therefore, the aim of this study was to determine whether the cannabinoid CB_1 receptor is co-distributed with the pre-synaptic 5-HT transporter protein, which is responsible for the reuptake of this neurotransmitter from the synaptic cleft. Since the amygdale are an area of the brain particularly involved in fear responses (e.g., Adolphs et al., 1995), we chose to look for co-distribution in the different sub-regions of the amygdale.

Using methods approved by the Animal Ethics Committee at the University of Otago, cannabinoid CB_1 receptor and 5-HT transporter immunoreactivity was investigated in coronal brain sections. Following decapitation without anaesthesia, brains from five male Wistar rats (150–250 g) were rapidly removed and snap frozen in O.C.T compound. Ten-micrometer serial cryosections were then cut at 0.5 mm intervals, thaw mounted on poly-L-lysine (Sigma, AU) coated slides, post-fixed in 4% paraformaldahyde and processed for immunochemical assay. Using an affinity-purified polyclonal goat anti-human antibody raised against the N-terminus of human cannabinoid CB_1 receptor protein (Santa Cruz Biotechnology, USA), sections were incubated in cannabinoid CB_1 receptor–antibody solution overnight at 4 °C, before incubation with an fluorescein isothiocyanate (FITC)-conjugated donkey anti-goat secondary antibody (Jackson Laboratories, USA). Sections were then incubated in a primary antibody solution of a guinea pig anti-rat antibody raised against the 5-HT transporter protein (Chemicon, USA) overnight at 4 °C, before incubation with a tetramethylrhodamine

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isothiocyanate (TRITC)-conjugated donkey anti-guinea pig secondary antibody (Jackson Laboratories, USA) solution for 2 h at 4 °C. Nuclei were counterstained with 100 ng/ml Hoescht 33342 (Sigma, AU). Individual photographs of the FITC labeled cannabinoid CB_1 receptor, TRITC labeled 5-HT transporter, and Hoescht stain were then taken. The cannabinoid CB_1 receptor protein was labeled green and the 5-HT transporter labelled red with blue nuclei. Morphological analysis and photography employed a Zeiss Axioplan MC80 BX microscope, Zeiss AxioCam HRc digital camera and Zeiss Axiovision 3.1 software (Carl Zeiss Vision GmBH, Germany). Five separate sets of images were taken at 0.5 mm intervals in the amygdale. All analysis was carried out using Adobe Photoshop 5.0 (Adobe Software, USA). Images were first converted to grayscale and inverted. Matching images for both the 5-HT transporter and cannabinoid CB_1 receptor labeling were systematically sampled using 50 μm sided squares. Sampling consisted of overlaying computer images with a grid and sampling every 10th square from a randomly chosen square from between 1 and 10 squares from the top left corner. Mean luminosity was recorded for each sampled square in matching images. In this way, paired measurements for luminosity at corresponding areas in the 5-HT transporter and cannabinoid CB_1 receptor-labeled images were obtained. Paired measurements were then used to calculate a correlation coefficient (r). These calculations were made for each of five paired images in each amygdale sub-region in each rat, and the r estimates were averaged. The mean and standard errors of these averages were then calculated for all the rats, and the means used as an index of co-distribution. A one way analysis of variance (ANOVA) was also performed to compare co-distribution across the amygdale sub-regions.

Cannabinoid CB_1 receptor–immunoreactivity was strongly co-distributed with 5-HT transporter–immunoreactivity throughout the amygdale (Fig. 1), with the degree of co-distribution being greatest in the rostral terminus of the anterior amygdaloid area. In all areas of the amygdale except one, the correlation coefficient was greater than 0.75 (Fig. 1). ANOVA showed that the degree of co-distribution differed significantly across the amygdale sub-regions ($F=9.6$, $P<0.0001$).

In order to test for spurious correlations between cannabinoid CB_1 receptor and 5-HT transporter–immunoreactivity, we also labeled sections with cannabinoid CB_1 receptor antibody together with an antibody specific for smooth muscle actin (Sigma, AU), a protein that is found exclusively in the walls of blood vessels, and is therefore not co-distributed with the cannabinoid CB_1 receptor and can be used as a negative control. Calculations for the index of co-distribution returned a very low value for these sections ($r<0.3$). We therefore concluded that the high r values we calculated for cannabinoid CB_1 receptor and 5-HT transporter labeling are reliable.

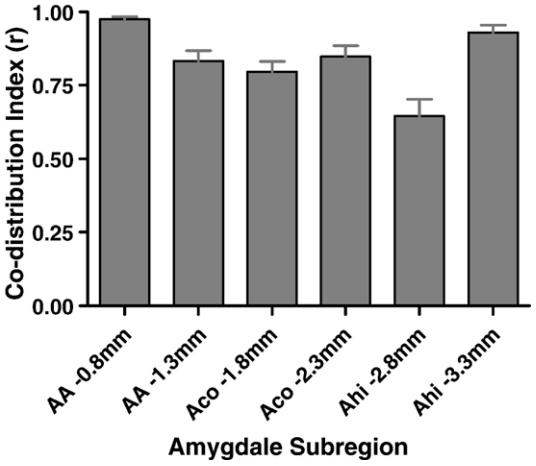

Fig. 1. Correlations between intensity of cannabinoid CB_1 receptor and 5-HT transporter labeling throughout the amygdale. (AA) Anterior amygdaloid area; (ACo) anterior cortical amygdaloid nucleus; (Ahi) amygdaloid–hippocampal area. Numbers in brackets indicate distances from bregma. Columns indicate means of 5 rats and error bars indicate standard errors.

Here we show, for the first time, that cannabinoid CB_1 receptors are co-distributed with the 5-HT transporter protein and therefore are likely to be localized to pre-synaptic 5-HT terminals, and that this is the case in an area of the brain – the amygdale – known to be specifically involved in mediating fear and panic reactions (Adolphs et al., 1995). We therefore speculate that the activation of cannabinoid CB_1 receptors can potentially reduce 5-HT release in the amygdale, thereby initiating panic behaviour.

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