Stimulation of cannabinoid (CB1) and prostanoid (EP2) receptors opens BKCa channels and relaxes ocular trabecular meshwork[☆]

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Abstract

Prostanoids and cannabinoids have ocular hypotensive and neuroprotective properties. The effect of the prostanoid AH13205 (EP2), the thromboxane-mimetic U46619, the cannabinoid (CB) agonists WIN55212-2 and CP 55,940, endothelin-1 (ET-1) and 8-bromo-cAMP on the membrane currents of trabecular meshwork (TM) cells were measured using the patch-clamp technique and compared to their effects on TM contractility. Previous studies show relaxation of TM to AH 13205 and other substances that elevate cAMP, while U46619 and endothelin-1 contract TM. This study shows that after contraction (100%) with carbachol (10^{-6} M), the CB agonist CP 55,940 dose-dependently reduced contractility to $83\pm4\%$ ($n=9$) (10^{-6} M) and $61\pm10\%$, ($n=7$) (10^{-5} M). In the presence of both the CB1 antagonist AM251 (10^{-6} M) and CP 55,940 (10^{-5} M), the contractile response to carbachol reached $84\pm3\%$ ($n=6$) of the original level. In patch-clamp experiments, membrane permeable 8-bromo-cAMP (10^{-4} M) had no effect on currents of TM cells. In contrast, AH 13205 and two cannabinoids reversibly enhanced outward current through high-conductance Ca^{2+}-activated K^+ channels (BKCa, BK, maxi-K) to the following values (in % of the initial value at 100 mV): AH 13205 (10^{-5} M): $200\pm28\%$ ($n=6$), CP 55,940 (10^{-6} M): $196\pm33\%$ ($n=7$), CP 55,940 (10^{-5} M): $484\pm113\%$ ($n=7$), WIN55212-2 (10^{-5} M): $205\pm41\%$ ($n=10$). Iberiotoxin (10^{-7} M) completely blocked these responses. The current response to CP 55,940 (10^{-5} M) could be partially blocked by the CB1 antagonist AM251 (10^{-6} M). Conversely, the contractile agents in this study either caused a transient reduction in outward current (ET-1(5×10^{-8} M)) or had no effect (U46619 (10^{-6} M)). We conclude that stimulation of EP2 and CB1 receptors in TM is coupled to the activation of BKCa channels via a non-diffusible second messenger cascade. This effect may contribute to the relaxant activity of EP2 and CB1 agonists in isolated TM strips, modulating ocular outflow.
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1. Introduction

Glaucoma continues to be a leading cause of blindness worldwide. In many cases, conventional therapeutic intervention fails to stop the progression of the disease, either because intraocular pressure (IOP) levels fail to fall sufficiently, or because degeneration of retinal ganglion cells and the optical nerve continue despite low values of IOP (Sugrue, 1997). The therapeutic potential of both cannabinoids and prostanoids has long been known (Green and Kim, 1976) and interest in them is enhanced by mounting evidence of their neuroprotective properties (Sugrue, 1997; Woodward et al., 1997; Mechoulam et al., 2002). By measuring the contractile response of trabecular meshwork strips (TM) and studying membrane currents of isolated human TM cells using the patch-clamp technique, this study attempted to investigate a possible mechanism by

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which two cannabinoids, WIN55212-2 and CP 55,940 and the prostanoid AH13205 (Woodward et al., 1995; Krauss et al., 1997a) reduce intraocular pressure. The effects were compared to the responses obtained by application of endothelin-1 (Lepple-Wienhues et al., 1991; Noske et al., 1997) and the thromboxane-mimetic U-46619 (Krauss et al., 1997a,b). Endothelin-like immunoreactivity has been implicated in the pathogenesis of glaucoma (Noske et al., 1997).

After formation by the eye's bilayered ciliary epithelium (Civan, 2003), aqueous humour is either drained through the sclera after passing through ciliary muscle bundles (uveoscleral route), or through the trabecular meshwork into Schlemm's canal (conventional route). Species and age-related differences exist in the relative importance of the two pathways. Outflow through the trabecular route appears to be predominant in the healthy human eye (Johnson and Johnson, 2001; Brubaker, 2003), and can be enhanced by contraction of the ciliary muscle via its insertion at the scleral spur, distending the trabecular meshwork (Wiederholt and Stumpff, 1998; Brubaker, 2003; Llobet et al., 2003). Alternately, it has been shown that smooth muscle relaxing substances decrease trabecular meshwork tone (Wiederholt and Stumpff, 1998; Brubaker, 2003; Llobet et al., 2003), leading to an increase in intertrabecular spaces through which outflow can occur (Wiederholt et al., 1995; Gilabert et al., 1997; Llobet et al., 1999).

The exact nature of the contractile apparatus of trabecular meshwork has yet to be determined. Likewise, it is not known what tone the trabecular meshwork has in vivo. However, the sphincter pupillae and the ciliary muscle are innervated by the autonomic nervous system, which also projects endings into the area of the trabecular meshwork (Selbach et al., 2000). The dramatic effects of anticholinergic agents such as atropine on the ciliary muscle and the iris show that in vivo, the tissues of the anterior chamber are exposed to a baseline level of acetylcholine. The atropine-sensitive contractile response of trabecular meshwork to depolarization with high potassium solution demonstrates the presence of functional cholinergic nerve terminals in the excised tissue (Wiederholt and Stumpff, 1998). Physiologically, trabecular tone may also be regulated by the interaction of endogenically produced substances such as endothelin (Noske et al., 1997) and prostaglandins (Gilabert et al., 1997).

Therapeutically, approaches that relax trabecular meshwork should not only maintain the physiological rate of aqueous humour flow through the eye, but circumvent the potential threat that contractile agents pose to retinal circulation and neuronal health (Barone et al., 1995; Noske et al., 1997; Bengzon et al., 2002). In this line, both prostanoids and cannabinoids are promising candidates (Kaufman, 1998, 2003).

Significant progress has been made in identifying different prostanoid receptors in the eye (Woodward et al.,

1997). There is increasing evidence for the production of endogenic prostaglandins (Gilabert et al., 1997) in the eye, and PGE2 (Kaplan-Messas et al., 2003), in particular, may mediate the effects of many ocular hypotensive agents. Relaxation of precontracted strips of trabecular meshwork with the EP2 agonist AH13205 has been demonstrated (Krauss et al., 1997a).

Stimulation of EP2 receptors in trabecular meshwork (Zhao et al., 1995; Crider and Sharif, 2001; Schneemann et al., 2002) may explain the increase in outflow through the trabecular meshwork in in vitro models (Dijkstra et al., 1999) and monkeys (Woodward et al., 1995), in connection with a rise in cytosolic cAMP. In the intact eye, AH13205 enhanced outflow (Woodward et al., 1995), reducing IOP. Conversely, the thromboxane-mimetic U46619 has no impact on IOP (Krauss et al., 1997b), suggesting that the small contractile impact on ciliary muscle is not sufficient to overcome the strong contraction observed in the trabecular meshwork (Krauss et al., 1997a).

Only two types of cannabinoid receptors have been identified so far (Howlett et al., 2002) with mounting evidence indicating that further receptors may exist. CB1 receptors are mainly found in the central and peripheral nervous system (Batkai et al., 2001) while CB2 receptors are located primarily on immune cells (Howlett et al., 2002), with additional cannabinoid effects mediated by non-CB1 non-CB2 receptors (Howlett et al., 2002).

In the eye, CB1 receptors in tissues of both the inflow and outflow pathways (Straiker et al., 1999) reduce IOP by species and substance dependent effects on both inflow (Chien et al., 2003) and outflow (Beilin et al., 2000), with possible involvement of non-CB1 non-CB2 receptors (Pate et al., 1998; Beilin et al., 2000; Song and Slowey, 2000). The ability of the CB1 antagonist SR 141716 A to elevate IOP (Pate et al., 1998) suggests that endogenic cannabinoids (Freund et al., 2003) are involved in the physiological regulation of intraocular pressure. In addition, there is evidence for the biosynthesis of an endogenic cannabinoid, anandamide, in the trabecular meshwork (Stamer et al., 2001) and for its biodegradation to prostamides (Woodward et al., 2001) with hypotensive properties.

CB1 receptors appear to be expressed more highly in trabecular meshwork than in the ciliary muscle (Straiker et al., 1999; Stamer et al., 2001) where they elevate the production of cytosolic cAMP (Stamer et al., 2001). Both inhibition and elevation of cAMP has been reported for other tissues involving G-protein coupled cascades (Hampson et al., 2000; Howlett et al., 2002).

CB2 receptors are absent in TM (Porcella et al., 1998, 2000). The failure to lower IOP by stimulation of CB2 receptors (Mikawa et al., 1997; Laine et al., 2003) led to the false assumption that the therapeutic potential of cannabinoids could not be separated from their adverse effects on the central nervous system, but in recent years, new synthetic cannabinoids have been developed that allow cannabinoids to be applied topically (Pate et al., 1995;