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Research Report

Transient receptor potential vanilloid 1 channels modulate the anxiolytic effect of diazepam

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ABSTRACT

The present study investigated the interaction between the vanilloid and GABAergic systems on anxiety. Swiss mice were subjected to social interaction test, an animal model for assessing anxiety-related behavior, after intracerebroventricular administration of capsaicin, (TRPV1 agonist) or capsazepine, (TRPV1 antagonist) either alone or in combination with traditional anxiolytic drug, diazepam. Results showed that capsaicin (1, 10, and 100 μg/mouse) decreased the interaction time exhibiting an anxiogenic-like response, while capsazepine (10, and 100 μg/mouse) produced anxiolytic-like response similar to that of diazepam (0.25–4 mg/kg, i.p). Prior administration of capsaicin at a dose, inactive per se (0.1 μg/mouse) attenuated the anxiolytic effect of diazepam, whereas, co-administration of capsazepine and diazepam both in their sub-effective as well as effective doses exhibited significant anxiolytic-like effect. Interestingly, the combined treatment of diazepam (2 mg/kg) and capsazepine (100 μg/mouse) produced no sedative or locomotor deficit effects. On the contrary, a higher dose of diazepam (>2 mg/kg) alone was found to be a sedative or locomotor depressant, indicating that the anxiolytic effect of diazepam, at least in part involve TRPV1 receptor. Morever, capsazepine pretreatment blocked the anxiogenic effect of capsaicin (1, and 100 μg/mouse). Taken together, these findings suggest that blockade of TRPV1 might be a functional tool to prevent the risks associated with the long-term use of benzodiazepines.

1. Introduction

The transient receptor potential (TRP) vanilloid type 1 (TRPV1) are nonselective cation channels that belong to the TRP family of proteins. Activators of TRPV1 channels are either painful chemical stimulus, or physical stimulus such as heat (>43 °C) or low pH. The endogenous ligands for this receptor include anandamide, N-arachidonoyldopamine, or oleoylethanolamide (Starowicz et al., 2007b), which are also known as endovanilloids. After exposure to these stimuli (heat, low pH, endovanilloids) TRPV1 becomes permeable to Na^+ and Ca^{2+} ions, causing the neurons to depolarize and fire action potentials (Starowicz et al., 2007b). Studies demonstrate the presence of these receptors in a wide number of organs and tissues; and are the novel therapeutic

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Abbreviations: AEA, anandamide; ANOVA, analysis of variance; AM404, N-(4-hydroxyphenyl)-arachidonylethanolamide; CAP, capsaicin; CB_1, cannabinoid receptor type 1; CZP, capsazepine; DMSO, dimethylsulfoxide; DZP, diazepam; FAAH, fatty acid amide hydrolase; GABARAP, GABA receptor associated proteins; SIT, social interaction test; TRPV1, transient receptor potential vanilloid 1 cation channels; URB597, cyclohexylcarbamic acid-3′-carbamoyl-biphenyl-3-yl ester

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targets for the treatment of inflammatory and chronic neuropathic pain in the peripheral nervous system (Starowicz et al., 2007b).

Immunocytochemical studies have shown the presence of TRPV1 receptors in different brain areas such as hippocampus, hypothalamus, substantia nigra, cortex, amygdala and periaqueductal gray (Cristino et al., 2006; Mezey et al., 2000; Tóth et al., 2005) indicating their involvement in complex physiologic responses within the central nervous system. For instance,stimulation of TRPV1 by capsaicin, a TRPV1 agonist has shown to evoke a concentration-dependent release of calcitonin gene-related peptide (Wardle et al., 1997); and has shown to promote calcium dependent glutamate release from the hypothalamic slices and lumbar dorsal horn (Sasamura et al., 1998). They have shown to play an important role in various physiological functions such as pain, anxiety, thermoregulation, and movement (Starowicz et al., 2007b). TRPV1 knockout mice have shown to reduce anxiety-like behavior and impaired fear conditioning (Marsch et al., 2007). Systemic or local injection of capsazepine, a TRPV1 antagonist in dorsolateral periaqueductal gray has shown to induce anxiolytic-like effect in various behavioral paradigms (Kasckow et al., 2004; Terzian et al., 2009). Lee et al. (2006) showed that pre-treatment of capsazepine attenuated the capsaicin induced hypolocomotion, further suggesting their role in locomotion.

Interestingly, anandamide, the first proposed TRPV1 endogenous ligand is also an agonist at the type 1 cannabinoid (CB_1) receptor (Ross et al., 2001; Smart et al., 2000; Zygmunt et al., 1999). It has shown to activate TRPV1 at a concentration higher than required for CB_1. Moreover, studies demonstrated that anandamide-mediated biphasic effects are due to its interaction with these two receptors (Micale et al., 2009; Rubino et al., 2008). Rubino et al. (2008) showed that CB1-mediated anxiolytic effect of anandamide are at a low dose while at higher doses, anxiogenic effect is evident through TRPV1 activation. Evidence suggests that stimulation of CB_1 leads to a decrease in intracellular calcium influx in presynaptic terminals reducing the neurotransmitter release (Freund et al., 2003), whereas, activation of TRPV1 promotes Ca^{2+} influx in postsynaptic sites (Starowicz et al., 2007a,b). Probably, due to these contrasting effects, TRPV1 and CB_1 were found to relate with opposite effects on certain brain functions, such as emotionality, cognition, and synaptic plasticity (Cristino et al., 2006; Micale et al., 2009; Rubino et al., 2008). This supports the fact that CB_1 knockout mice exhibit anxiogenic behavior, whereas, TRPV1 knockout mice have shown reduced anxiety-like behavior and impaired fear conditioning (Marsch et al., 2007). Moreover, there is evidence that TRPV1 activation by anandamide is enhanced in the presence of agents, which are capable of prolonging the life span of anandamide (De Petrocellis et al., 2000; Rawls et al., 2006; Ross et al., 2001; Rubino et al., 2008). Inhibitors of anandamide degradation such as URB97, a FAAH (fatty acid amide hydrolase) inhibitor; or AM404, an anandamide transport inhibitor has been shown to increase synaptic concentrations of anandamide (Giuffrida et al., 2000, 2001; Piomelli et al., 2006). Further, these agents have shown to modulate anxiety-related behavior similar to that of anandamide (Bortolato et al., 2006; Kathuria et al., 2003; Rubino et al., 2008).

The GABAergic system, in particular, $GABA_A$, has a pivotal role in the regulation of anxiety. Benzodiazepines, the positive modulators of $GABA_A$ are still the most widely used anxiolytic compounds (Roy-Byrne, 2005). Interactions between the GABAergic and endocannabinoid systems in feeding behavior, anxiety and epilepsy have been extensively studied (García-Gutiérrez and Manzanares, 2010; Naderi et al., 2008; Rahminiwati and Nishimura, 1999). For instance, a recent study showed the decrease in functional activity of $GABA_A$ and $GABA_B$ receptor in cannabinoid CB_1 receptor knockout mice (Urigüen et al., 2011) indicating an interplay between the GABAergic and endocannabinoid systems. However, little attention has been paid to the interaction between TRPV1 receptor and GABAergic system in brain. In fact, recent studies now indicate an interaction between GABAergic system and TRPV1. Laínez et al. (2010), showed that GABA receptor associated proteins (GABARAP) is an important component of the TRPV1 signaling complex that contributes to increase in the channel expression on the plasma membrane, and modulate its functional activity at the level of channel gating and desensitization. In addition, another study showed that TRPV1 agonists require GABA input to pyramidal cells to modulate hippocampal synaptic plasticity (Bennion et al., 2011).

Based on the above evidence, the present study was designed to investigate the interaction between vanilloid and GABAergic systems on anxiety-like behavior in the mice model of social interaction test (SIT) (File, 1997; File and Hyde, 1978), a widely used model for assessing anxiety-like behavior, having good predictive, face and constructive validity. In order to test this hypothesis, Swiss mice were intracerebroventricularly (i.c.v.) administered with the TRPV1 agonist (capsaicin), the TRPV1 antagonist (capsazepine) and diazepam either alone or in combinations.

2. Results

2.1. *Experiment 1: influence of diazepam, capsaicin and capsazepine in SIT and locomotor activity*

One-way ANOVA followed by Dunnett's post hoc test showed that all treatments had a significant {DZP: [$F(5,36)=151.0$; $P<0.0001$] (Fig. 1A); CAP: [$F(4,30)=6.844$; $P=0.0005$] (Fig. 1B); CZP: [$F(4,30)=8.468$; $P=0.0001$] (Fig. 1C)} effect on the interaction time. Capsaicin (1, 10, and 100 μg/mouse) significantly ($P<0.05$, 0.01) decreased interaction time in SIT. In contrast, capsazepine (1, 10 and 100 μg/mouse) produced significant ($P<0.05$, 0.01) dose-dependent increase in the interaction time similar to that of diazepam (0.5, 1 and 2 mg/kg). However, capsaicin (0.1 μg/mouse), capsazepine (0.1 and 1 μg/mouse) and diazepam (0.25 and 4 mg/kg) did not show any effect.

Diazepam at a dose of 4 mg/kg showed significant ($P<0.01$) [$F(5,36)=76.75$, $P<0.0001$; (Fig. 2A)] decrease in locomotor counts whereas, no such effect was evident at the dose lower than 4 mg. Capsaicin and capsazepine did not have any effect on locomotor activity at any dose level {CAP: [$F(4,30)=1.765$; $P=1.765$] (Fig. 2B); CZP: [$F(4,30)=0.3749$; $P=0.82477$] (Fig. 2C)}.

2.2. *Experiment 2: influence of pretreatment of capsazepine on the effect of capsaicin in SIT and locomotor activity*

Pretreatment of capsazepine, at a dose (1 μg/mouse), inactive *per se* significantly {1st injection: [$F(1,36)=8.255$; $P<0.01$]; 2nd injection: [$F(2,36)=1.418$; $P=$n.s.]; interaction: [$F(2,36)=4.969$, $P<0.05$]} (Fig. 3A)} abolished the anxiogenic-like effect of capsaicin (1, and