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Agmatine and a cannabinoid agonist, WIN 55212-2, interact to produce a hypothermic synergy

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Abstract

Agmatine blocks morphine withdrawal symptoms and enhances morphine analgesia in rats. Yet, the role of agmatine in the pharmacological effects of other abused drugs has not been investigated. The present study investigates the effect of agmatine administration on the hypothermic response to cannabinoids. Hypothermia is an effective endpoint because cannabinoid agonists produce a rapid, reproducible, and significant decrease in body temperature that is abolished by cannabinoid CB_1 receptor antagonists. WIN 55212-2, a cannabinoid agonist, was administered to rats by itself and with agmatine. WIN 55212-2 (1, 2.5, 5 and 10 mg/kg, i.m.) caused a significant hypothermia. Agmatine (10, 25 and 50 mg/kg, i.p.) was ineffective. For combined administration, agmatine (50 mg/kg, i.p.) enhanced the hypothermic effect of WIN 55212-2 (1, 2.5, 5 and 10 mg/kg, i.m.). The enhancement was strongly synergistic, indicated by a 2.7-fold increase in the relative potency of WIN 55212-2. The central administration of agmatine (25 and 50 μg/rat, i.c.v.) significantly increased the hypothermic effect of WIN 55212-2 (2.5 mg/kg, i.m.). This indicates that agmatine acts through a central mechanism to augment cannabinoid-evoked hypothermia. Idazoxan (2 mg/kg, i.p.), an imidazoline antagonist, blocked the enhancement by agmatine, thus suggesting that imidazoline receptor activation is required for agmatine to enhance cannabinoid-evoked hypothermia. The present data reveal that agmatine and a cannabinoid agonist interact to produce a hypothermic synergy in rats. These results show that agmatine acts in the brain and via imidazoline receptors to enhance cannabinoid-evoked hypothermia.

Keywords: Agmatine; WIN 55212-2; Cannabinoid; Hypothermia; Imidazoline; CB_1

1. Introduction

Agmatine is an endogenous biogenic amine that has been identified in nearly all of the organs of rats, including brain and plasma (Raasch et al., 1995). It is formed in the mammalian brain from arginine by the enzyme arginine decarboxylase and hydrolyzed by the enzyme agmatinase (Regunathan et al., 1995; Sastre et al., 1996). The brains regions with the greatest levels of agmatine are the hypothalamus, periaqueductal gray, locus coeruleus, cerebral cortex, hippocampus, amygdala, thalamus and striatum (Reis and Regunathan, 2000). The majority of agmatine immunoreactivity is detected in neurons whereas glial cells contain little agmatine immunoreactivity but do contain arginine decarboxylase (Regunathan et al., 1995). Agmatine exerts a wide range of biological activities on several organ systems, including the central nervous system, where it has been proposed to act as a neurotransmitter (Nguyen et al., 2003; Li et al., 2003). In fact, agmatine satisfies at least five criteria for neurotransmitter classification as it is: (1) synthesized in the brain (Li et al., 1994); (2) stored in synaptic vesicles (Kuzirian et al., 1986); (3) released by depolarization (Reis and Regunathan, 2000); (4) accumulated by uptake (Sastre et al., 1997); and (5) inactivated by agmatinase (Sastre et al., 1996). Brain concentrations of agmatine correlate well with other established transmitters, and the presence of agmatine in the extracellular fluid suggests that it may be involved in intercellular signaling (Reis and Regunathan, 2000). The site of action of agmatine remains unclear. Prior work demonstrates that agmatine interacts with imidazoline receptors, α_2-adrenoceptors, nicotinic receptors and 5-HT_3 receptors (Loring, 1990; Piletz et al., 1995; Reis and Regunathan, 2000). Agmatine also has neuroprotectant properties, presumably through the modulation of the NMDA subclass of glutamate receptors and inhibition of nitric oxide

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synthase (NOS) (Feng et al., 2005; Fairbanks et al., 2000; Gilad et al., 1996; Reis et al., 1998).

A number of pharmacological actions of agmatine have been well documented. Through a peripheral mechanism of action, agmatine modulates insulin and glucose metabolism, elevates renal sodium and water excretion, and stimulates epinephrine and norepinephrine release from adrenal cells (Reis and Regunathan, 2000). Central actions of agmatine include a weak analgesic action, anti-depressant like effects, release of luteinizing hormone releasing hormone from the hypothalamus, reduction of seizure-evoked glutamate levels in the frontal cortex, attenuation of neuropathic pain, anti-convulsant effects, and improvement of locomotor function following spinal cord injury (Reis and Regunathan, 2000; Schwartz et al., 1997; Zomkowski et al., 2005; Kalra et al., 1995; Su et al., 2004; Onal et al., 2003; Feng et al., 2005). Perhaps the best characterized effect of agmatine is its biphasic action on morphine withdrawal and analgesia (Su et al., 2003). Agmatine, when given with morphine: (1) blocks all symptoms of morphine withdrawal; (2) enhances acute morphine analgesia; and (3) prevents the development of tolerance to morphine analgesia (Kolesnikov et al., 1996; Aricioglu-Kartal and Uzbay, 1997; Reis and Regunathan, 2000). Agmatine also interacts with non-opioid systems to modulate endpoints other than analgesia and withdrawal. One endpoint is body temperature. Agmatine administration to rats blocks the hyperthermia caused by restraint stress and the bacterial endotoxin, lipopolysaccharide (LPS) (Aricioglu and Regunathan, 2005), and reduces pain induced by inflammation in animal models (Bradley, 1997; Fairbanks et al., 2000).

Despite the wide range of biological activities exerted by agmatine, its effects on addictive substances other than morphine have not been investigated. One of the most widely abused recreational drugs is marijuana (SAMSHA, 2000). Cannabis and its derivative compounds, collectively known as cannabinoids, produce an array of pharmacological effects in animals and humans (Martin, 2005). These effects include sedation, cognitive dysfunction, short-term memory impairment, time assessment alteration, perceptual changes, motor incoordination, poor executive function, analgesia, and immunosuppression (Howlett et al., 2004). In animal models, hypothermia is an effective endpoint for evaluating cannabinoid activity and interactions of cannabinoids with other endogenous systems (Ovadia et al., 1995; Compton et al., 1992; Rawls et al., 2002, 2004). The endpoint is useful because cannabinoid agonists produce a reproducible hypothermia that is rapid in onset, persistent in duration, and abolished by cannabinoid CB_1 receptor antagonists (Compton et al., 1992; Rawls et al., 2002).

One of the most important sites of agmatine action is imidazoline receptors (Piletz et al., 1995). Three main imidazoline receptor classes are recognized: the I_1 imidazoline receptor, which mediates the sympatho-inhibitory actions to lower blood pressure; the I_2 receptor, which is an allosteric binding site of monoamine oxidase; and the I_3 receptor, which regulates insulin secretion from pancreatic beta cells (Head and Mayorov, 2006). Evidence indicates that imidazoline receptors are important in a number of pathophysiological conditions, including hypertension, depression, left ventricular hypertrophy, diabetes, hyperlipidemia, cell proliferation, inflammation, ischemia, and addiction (Halaris and Piletz, 2003; Mukaddam-Daher and Gutkowska, 2004; Lumb et al., 2004). Because of the therapeutic relevance of imidazoline receptors, it is important to identify endogenous receptor systems with which imidazoline receptors interact and to develop and characterize new drugs that act at these receptors.

In the present study, we first examined the effects of peripheral and central agmatine administration on cannabinoid-evoked hypothermia in rats. We then determined the involvement of imidazoline receptors in the effects of agmatine on cannabinoid-induced hypothermia. Experiments revealed that agmatine and a cannabinoid agonist interact to produce a hypothermic synergy which depends on imidazoline receptor activation.

2. Methods

2.1. Animals

All animal use procedures were conducted in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the Temple University Animal Care and Use Committee. Male Sprague-Dawley rats (Zivic-Miller, Pittsburgh, PA, USA) weighing 175–200 g were housed 2 per cage for a minimum of 5 days before experimental use. Rats were maintained on a 12-hr light/dark cycle and fed rat chow and water ad libitum.

2.2. Drug preparation and administration

WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6H-pyrrolo[3,2,1ij]quinolin-6-one], WIN 55212-3 [*S*-(-)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-napthanlenyl) methanone mesylate], yohimbine, and agmatine sulfate were purchased from Tocris-Cookson (St. Louis, MO, USA). Idazoxan hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). WIN 55212-2 and WIN 55212-3 were dissolved in a 10% cremophor/saline solution and injected intramuscularly (i.m.) into the right thigh (Rawls et al., 2002, 2004). Agmatine, idazoxan and yohimbine were dissolved in pyrogen-free distilled water and injected intraperitoneally (i.p.).

2.3. Cannula implantation

Rats were anesthetized with an i.p. injection of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg). A polyethylene cannula was implanted stereotaxically into the right lateral ventricle (Rawls et al., 2002). Dental acrylic was used to secure the cannula to the cranium.

2.4. Experimental protocol

Experiments were always started between 8:00 and 9:00 a.m. to minimize the effects of circadian variation. One week after surgery, rats were placed individually into an environmental room maintained at a constant temperature of 21±0.3 °C and