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Electrophysiological impact of trazodone on the dopamine and norepinephrine systems in the rat brain

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Abstract

Previous study has documented the long-term effects of the antidepressant trazodone on the serotonin (5-HT) system. The present work examined the impact of sustained trazodone on ventral tegmental area (VTA) dopamine (DA) and locus ceruleus (LC) norepinephrine (NE) neurons firing activity, and characterized its effects at 5-HT_{2C}, 5-HT_{2A} receptors and α_1- and α_2-adrenoceptors. Electrophysiological recordings were carried out in anesthetized rats. Subcutaneously implanted minipumps delivered vehicle or trazodone (10 mg/kg/day) for 2 or 14 days. Administration of trazodone for 2 and 14 days did not alter the firing activity of DA neurons. Systemic injection of trazodone, however, reversed the inhibitory effect of the 5-HT_{2C} receptor agonist Ro 60,0175 on the DA neuronal firing, suggesting an antagonistic action of trazodone at this receptor. Administration of trazodone for 2 days significantly enhanced the NE neurons firing. Despite a return of the NE neurons firing rate to the baseline following 14-day trazodone, the percentage of neurons discharging in burst was increased by this regimen. Administration of trazodone for 14 days enhanced the tonic activation of postsynaptic α_2-adrenoceptors, as indicated by the disinhibitory effect of the α_2-adrenoceptor antagonist idazoxan on hippocampus pyramidal neurons firing. The inhibitory effect of acute trazodone on dorsal raphe (DR) 5-HT neurons firing was shown to be through the 5-HT_{1A} receptor. Systemic injection of trazodone reversed the inhibitory action of 5-HT_{2A} agonist DOI on the NE neurons firing rate, indicating its antagonistic action at 5-HT_{2A} receptors. The enhancement in α_2-adrenergic transmission by trazodone, and its 5-HT_{2A} and 5-HT_{2C} receptor antagonism may contribute to its therapeutic action in major depression.

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); NE, norepinephrine; DA, dopamine; SSRI, selective serotonin reuptake inhibitor; WAY100,635, N-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride; DOI, [1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane hydrochloride)].

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1. Introduction

Trazodone, a "second generation" antidepressant, was the first triazolopyridine derivative to be developed for the treatment of major depression. Pharmacologically, trazodone is considerably different from the "classical" antidepressants as it is devoid of effects on monoamine oxidase (MAO) activity (Hyslop et al., 1988), and produces minimal effects on norepinephrine (NE) reuptake (Richelson and Pfenning, 1984; Owens et al., 1997). The pharmacological profile of trazodone on serotonin (5-HT) system is, however, complex. Trazodone selectively blocks 5-HT transporters (5-HTT) *in vitro* (Richelson and Pfenning, 1984; Owens et al., 1997) and *in vivo* (Ghanbari et al., 2010), although its potency to block 5-HTT is markedly less than that of selective 5-HT reuptake inhibitors (SSRIs). The effects of sustained trazodone administration on the 5-HT system resemble that of SSRIs. Indeed, a previous electrophysiological study showed that short-term administration of trazodone suppressed the firing rate of dorsal raphe (DR) 5-HT neurons, in part, due to overactivation of somatodendritic 5-HT_{1A} autoreceptors induced by blockade of 5-HTT and a subsequent increase in 5-HT. The 5-HT neurons firing rate, however, recovered to baseline following a prolonged regimen. The recovery of 5-HT neurons firing was allowed by the desensitization of somatodendritic 5-HT_{1A} autoreceptors (Ghanbari et al., 2010), a phenomenon that has been repeatedly documented following long-term administration of SSRIs.

In addition to the blocking property at 5-HTT, trazodone displays moderate potency at 5-$HT_{2A/2C}$ receptors (Cusack et al., 1994; Owens et al., 1997; Millan, 2006) which could contribute to its mechanism of action. In line with this, direct application of trazodone into the frontal cortex of rats markedly enhanced extracellular 5-HT levels, an effect that was prevented by local perfusion of the 5-$HT_{2A/2C}$ receptor antagonist ketanserin (Pazzagli et al., 1999). Furthermore, it is noteworthy that microdialysis experiments have consistently shown that antagonistic property at 5-HT_{2C} receptors elicit enhancement of extracellular dopamine (DA) levels in the rat brain (Di Matteo et al., 1999; Gobert and Millan, 1999; Gobert et al., 2000), whereas that of 5-HT_{2A} receptors remains controversial (Schmidt and Fadayel, 1995; Gobert and Millan, 1999). From an electrophysiological point of view, the antagonistic property at 5-$HT_{2A/2C}$ receptors is of importance. In fact, pharmacological studies showed that selective blockade of 5-HT_{2A} and 5-HT_{2C} receptors restored the inhibited firing rate of locus coeruleus (LC) NE neurons (Dremencov et al., 2007a) and ventral tegmental area (VTA) DA neurons (Dremencov et al., 2009), respectively, induced by sustained administration of the SSRI escitalopram.

The other potential features of trazodone that may play a factor in its mechanism of action are its moderate and potent affinity for 5-HT_{1A} and α_1-adrenoceptors, respectively (Cusack et al., 1994; Owens et al., 1997; Millan, 2006). Indeed, trazodone functions as an agonist at Chinese hamster ovary cells expressing human 5-HT_{1A} receptors (Odagaki et al., 2005) as well as at 5-HT_{1A} receptors in the rat hippocampus (Ghanbari et al., 2010). In addition to 5-HTT blockade, the agonistic and/or antagonistic action of trazodone at 5-HT_{1A} and α_1-adrenoceptors, respectively, may in part contribute to the inhibition of DR 5-HT neurons firing rate induced by both systemic (Scuvée-Moreau and Dresse, 1982) and 2-day administration of this agent (Ghanbari et al., 2010). In support of this, systemic injection of the selective α_1-adrenoreceptor antagonist prazosin inhibits the firing rate of DR 5-HT neurons (Marwaha and Aghajanian, 1982).

Considering the functional interactions among the monoaminergic systems *in vivo*, the present experiments were thus undertaken to assess the impact of trazodone on DA and NE systems. Moreover, the study aimed at putting into evidence the antagonistic action of trazodone at 5-$HT_{2A/2C}$ receptors *in vivo* and examining the nature of impact of the antagonistic action of trazodone at α_1-adrenoceptor on DR 5-HT neurons.

2. Experimental procedures

2.1. Animals

The *in vivo* electrophysiological experiments were carried out in male Sprague–Dawley rats (Charles River, St. Constant, QC, Canada) weighing between 280 and 320 g at the time of recordings. The animals were kept, 2 per cage, under standard laboratory conditions (12:12 light–dark cycle with access to food and water *ad libitum*). The rats were allowed to acclimatize to their new environment for 1 week prior to start of any new treatments or experiments. All the experiments were approved by the local Animal Care Committee and conducted in accordance with the Canadian Council on Animal Care, for the care and use of laboratory animals.

2.2. Experimental preparations

The rats were anesthetized with isoflurane to implant, subcutaneously, the osmotic Alzet minipumps (Durect, Palo Alto, CA, USA), that ensure slow and steady release of trazodone (10 mg/kg/day) or the vehicle (hydroxy propyl-β-cyclodextrin 20%, used to dissolve the drug) for 2 or 14 days. Such durations were chosen because trazodone requires over 1 week to achieve steady state in humans (Stahl, 2009), but only days in rats (DeVane et al., 1999), due to its much shorter half-life in rats. The electrophysiological experiments were carried out with the minipumps in place. Prior to the electrophysiological recordings, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Supplemental doses of the anesthetic (100 mg/kg, i.p.) were given to maintain constant anesthesia and to prevent any nociceptive reaction to pinching of the hind paws. Body temperature was maintained at 37 °C throughout the experiment utilizing a thermistor-controlled heating pad. Prior to the electrophysiological experiments, a catheter was inserted in a lateral tail vein for systemic intravenous (i.v.) injection of pharmacological agents.

2.3. Electrophysiological recording of VTA DA neurons

The recording of VTA DA neurons was obtained with single-barreled glass micropipette lowered at 3.0–3.6 mm anterior to lambda and 0.6–1.0 to the midline suture. These neurons were encountered at the depth of 6.0 to 8.5 mm from the surface of brain. The presumed DA neurons were identified by well established electrophysiological criteria (Freeman et al., 1985) including: 1) spontaneous firing rate between 5 and 90 spikes/10 s, exhibiting bursting activity or irregular firing; 2) biphasic or triphasic waveforms, with an initial positive deflection (usually notched) followed by a prominent negative