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Research Report

Tachyphylaxis to 5-HT_3-receptor-mediated activation of vagal afferents is prevented by co-activation of 5-HT_2 receptors

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ABSTRACT

Functional studies have provided evidence that 5-HT_3 ion-channel receptors (5-HT_3Rs) on vagal cardiopulmonary afferents mediating the Bezold–Jarisch reflex (BJR) rapidly desensitize upon repeated exposure to selective 5-HT_3R agonists. G-protein-coupled 5-HT_2 receptors (5-HT_2Rs) also exist on vagal afferents, although activation of these receptors does not elicit the BJR. However, there is in vivo evidence that 5-HT_2Rs may regulate the activity of 5-HT_3Rs. The aim of this study was to determine whether co-activation of 5-HT_2Rs prevents desensitization of 5-HT_3Rs mediating the BJR in conscious rats. The principal findings were that (1) tachyphylaxis rapidly developed to the BJR-mediated hemodynamic responses elicited by successive injections of 5-HT_3R agonists and (2) co-injection of the selective 5-HT_2R agonist, α-methyl-5-HT, prevented tachyphylaxis to the BJR-mediated hemodynamic responses elicited by the 5-HT_3R agonists. Additional studies provided evidence that (1) tachyphylaxis to the 5-HT_3R agonists was not due to impairment of the central or efferent processing of the BJR, and (2) the pressor responses elicited by α-methyl-5-HT were not responsible for preventing tachyphylaxis to the BJR reflex responses elicited by 5-HT_3R agonists. These results suggest that the loss of response to 5-HT_3R agonists is due to desensitization of 5-HT_3Rs on vagal afferents mediating the BJR and that co-activation of 5-HT_2Rs prevents the desensitization of these 5-HT_3Rs.

1. Introduction

Systemic injections of 5-hydroxytryptamine (5-HT) elicit the Bezold–Jarisch reflex (BJR) via activation of 5-HT_3 receptors (5-HT_3Rs) on vagal cardiopulmonary afferents (Fozard, 1984; Richardson and Engel, 1986; Thoren, 1979). The BJR responses include pronounced reductions in mean arterial blood pressure (MAP), which are mediated predominantly by vagal-efferent-induced falls in heart rate (HR) and cardiac output (CO), although transient reductions in sympathetic nerve activity (SNA) also contribute to the responses (Fozard, 1984; Richardson and Engel, 1986; Thoren, 1979). 5-$HT_{3A,3B}$Rs

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are ligand-gated ion-channels which conduct Na^+ and K^+ ions (Derkach and Suprenant, 1989; Julius et al., 1990; Julius, 1991; Maricq et al., 1991; Zifa and Fillion, 1992). 5-HT_3R-mediated changes in the electrical activity of cultured neurons are subject to rapid tachyphylaxis (Andrade and Chaput, 1991; Lambert et al., 1989; Neijt et al., 1988; Peters and Lambert, 1989). Moreover, successive systemic injections of the selective 5-HT_3R agonists phenylbiguanide (PBG) and 2-methyl-5-HT produce progressively and markedly smaller BJR responses in conscious rats (Pires and Ramage, 1990; Whalen et al., 2000). Development of tachyphylaxis to PBG and 2-methyl-5-HT was unlikely to be due to a loss of central or efferent processing of the BJR since the ability of the S-nitrosothiol, L-S-nitrosocysteine (L-SNC) (Ignarro, 1990; Myers et al., 1990; Rosenblum, 1992) to activate the BJR was not affected in rats which received successive injections of the 5-HT_3R agonists (Whalen et al., 2000). Taken together, these findings suggest that tachyphylaxis to PBG and 2-methyl-5-HT is due to the down-regulation of 5-HT_3Rs on vagal cardiopulmonary afferents mediating the BJR.

5-HT_2 receptor (5-HT_2R) subtypes are G-protein-coupled receptors (Fozard, 1990; Glaum et al., 1988; Goudie and Lathley, 1990; Hartig, 1992; Pritchett et al.; Probst et al., 1992). The sequence of events following agonist-induced occupation of 5-HT_2Rs includes (1) G-protein-mediated activation of phospholipases C and A_2, (2) phosphoinositol-mediated mobilization of intracellular pools of Ca^{2+}, (3) activation of protein kinase C (PKC), (4) Ca^{2+}-mediated activation of Ca^{2+}-activated chloride channels (Cl_{Ca}-channels) resulting in cell depolarization and (5) depolarization-induced activation of voltage-sensitive Ca^{2+}-channels (Ca^{2+} vs. -channels) (Abdel-Latif, 1986; Dabire et al., 1990; Felder et al., 1990; Fenuik and Humphrey, 1989; Hollenberg, 1988; Saxena et al., 1989; Hartig et al., 1990; Hartig, 1992). There is considerable evidence that 5-HT_2Rs exist on vagal afferents (Christian et al., 1989; Dabire et al., 1990; Lacolley et al., 1990a,b; Meller et al., 1991, 1992; Pires and Ramage, 1990; Yoshioka et al., 1992). For example, activation of methysergide-sensitive (most-likely to be) 5-HT_2Rs and/or 5-HT_1Rs (see Zifa and Fillion, 1992) has pronounced effects on the changes in electrical activity of vagal afferent perikarya elicited by 5-HT (Christian et al., 1989). Systemic injections of 5-HT_2R agonists facilitate 5-HT_3R-mediated activation of nociceptive vagal cardiopulmonary afferents (Meller et al., 1991, 1992). However, systemic injections of 5-HT_2R agonists do not directly elicit the BJR (Meller et al., 1991). Thus, 5-HT_2Rs on vagal afferents mediating the BJR may modulate 5-HT_3R activity. Specifically, the signal transduction mechanisms activated by 5-HT_2Rs (Abdel-Latif, 1986; Dabire et al., 1990; Felder et al., 1990; Fenuik and Humphrey, 1989; Hollenberg, 1988; Saxena et al., 1989) may regulate the desensitization of 5-HT_3Rs on vagal afferents mediating the BJR.

The main aim of this study was to determine whether co-activation of 5-HT_2Rs modulates the development of tachyphylaxis to 5-HT_3R-mediated activation of vagal afferents mediating the BJR in conscious rats. In these studies, we determined whether co-injection of the selective 5-HT_2R agonist α-methyl-5-HT (Fozard, 1990; Richardson and Engel, 1986; Zifa and Fillion, 1992) would prevent tachyphylaxis to the BJR-induced hemodynamic responses which occurs after successive injections of PBG or 2-methyl-5-HT. The pressor responses elicited by each injection of α-methyl-5-HT, which are due to direct and centrally mediated constriction of resistance vessels (see Muntzel et al., 1996), may be involved in the ability of α-methyl-5-HT to prevent tachyphylaxis to PBG and 2-methyl-5-HT. More specifically, it is possible that the baroreflex-mediated reductions in HR and CO in response to the α-methyl-5-HT pressor response in some way prevent the development of tachyphylaxis to the 5-HT_3R agonists. Accordingly, we also determined whether co-administration of the selective α_1-adrenoceptor agonist phenylephrine modulates the development of tachyphylaxis to 5-HT_3R agonists. Phenylephrine exerts pressor responses via constriction of resistance arteries (see Kooy and Lewis, 1996) and activates similar signal transduction pathways to 5-HT_2Rs (Hartig et al., 1990; Lamb and Barna, 1988, 1998; Satake et al., 1992). The use of phenylephrine has an important additional benefit in that this α_1-adrenoceptor agonist will not directly activate vagal afferents. More specifically, although vagal afferents express β-adrenoceptors (Watkins et al., 1996), they do not appear to express α-adrenoceptors (see Meller et al., 1991, 1992).

2. Results

2.1. *Resting hemodynamic parameters*

Resting hemodynamic values remained constant in each experiment. More specifically, the development of tachyphylaxis to the 5-HT_3R agonists PBG and 2-methyl-5-HT had no affect on resting hemodynamic values ($P > 0.05$, for all comparisons, data not shown).

2.2. *Tachyphylaxis to 5-HT_3R agonists*

The BJR changes in HR, BP_D and CO and in HR, BP_D and RSNA elicited by 5 injections of PBG are summarized in Figs. 1 and 2, respectively. The first injection of PBG elicited pronounced falls in HR, BP_D, CO and RSNA, but not TPR (data not shown). Subsequent injections of PBG elicited progressively and markedly smaller responses. The BJR responses elicited by 5-HT or L-SNC before and after the injections of PBG are also summarized in Figs. 1 and 2. The 5-HT responses were markedly attenuated after the injections of PBG, whereas the L-SNC responses were not affected. The BJR responses elicited by 5 injections of 2-methyl-5-HT are summarized in Table 1. The BJR responses elicited by 5-HT and L-SNC before and after the injections of 2-methyl-5-HT are also shown. The 2-methyl-5-HT-induced responses were subject to pronounced tachyphylaxis. The 5-HT-induced responses were markedly diminished after development of tachyphylaxis to 2-methyl-5-HT, whereas the L-SNC responses were not affected.

2.3. *Tachyphylaxis to 5-HT_3R agonists does not involve a loss of vagal-efferent function*

The BJR responses elicited by 5 injections of PBG or 2-methyl-5-HT in pentobarbital-anesthetized rats were subject to substantial tachyphylaxis (see Table 2). The hemodynamic responses elicited by ES of the peripheral end of the transected