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Role of tachykinin NK_1 and NK_2 receptors in colonic sensitivity and stress-induced defecation in gerbils

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Abstract

The pharmacology of tachykinin NK receptors varies greatly among species. The aim of the present study was to assess the role of NK_1 and NK_2 receptors in mediating colorectal distension-evoked nociception and psychological stress-induced defecation in gerbils, a species with human-like NK receptor pharmacology. The effects of the selective NK_1 and NK_2 receptor antagonists, aprepitant and saredutant, on acute (1 h) restraint stress-evoked defecation and plasma adenocorticotropin (ACTH) levels in gerbils were assessed. The effects of antagonists alone or in combination on colorectal distension-evoked visceral pain in conscious gerbils were evaluated using the visceromotor response as a surrogate marker of pain. Restraint stress increased fecal pellet output 2–3-fold and plasma ACTH levels 9-fold. Aprepitant inhibited the defecatory and endocrine responses to stress by 50%, while saredutant completely normalized the same parameters. Visceral pain responses during colorectal distension were attenuated by both compounds, but aprepitant (19±6% inhibition, $P<0.01$) was slightly more effective than saredutant (10±9% inhibition, $P<0.05$). A combination of both compounds resulted in an additive effect (30±10% inhibition, $P<0.01$). The results demonstrate that NK_1 and NK_2 receptors are involved in stress-related colonic motor alterations and visceral pain responses in gerbils and that combined antagonism provides enhanced inhibition of visceral pain responses. This suggests that for therapeutic use in for instance functional gastrointestinal disorders, dual NK_1/NK_2 receptor antagonists may provide better clinical outcome than selective compounds.

Keywords: Aprepitant; Saredutant; Colorectal distension; Stress; Visceral pain; Tachykinin; NK receptor; Gerbil; Colonic motility

1. Introduction

The tachykinins substance P, neurokinin A and neurokinin B are peptides that share the C-terminal sequence Phe-X-Gly-Leu-MetNH_2. They exert their biological effects by binding to G-protein coupled tachykinin NK receptors. Substance P is the preferred ligand for the tachykinin NK_1 receptor while neurokinin A and neurokinin B are the preferred ligands for NK_2 and NK_3 receptors, respectively (Maggi, 1995). However, considering the structural similarities of the C-terminal part of the peptides, it is not surprising that all three ligands act as full agonists at all three NK receptors.

The irritable bowel syndrome is a functional disorder characterized by discomfort or pain from the gastrointestinal tract (for a review see Drossman, 2006). Disturbances in gastrointestinal motility manifested as diarrhea or constipation are also present. Antagonists for NK receptors are considered as potentially useful in treating the irritable bowel syndrome and have been reviewed elsewhere (Lecci et al., 2002, 2004; Sanger, 2004). In the gastrointestinal tract, the tachykinins, especially substance P and neurokinin A, are present throughout the enteric nervous system in both myenteric and submucosal ganglia (Sternini et al., 1989). Tachykinin NK_1 receptors are present in myenteric and submucosal neurons, circular and longitudinal smooth muscle cells and epithelial cells (Portbury et al., 1996;

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Vannucchi et al., 1997; Southwell and Furness, 2001). Tachykinin NK_2 receptors are predominantly expressed in smooth muscle cells, but might also be present in neuronal cells (Lecci et al., 2006). Substance P and neurokinin A evoke excitatory motor responses and are believed to be the main non-cholinergic component of excitatory transmission to the muscle cells (Improta and Broccardo, 2006). However, NK_1 and NK_2 receptors do not appear to play major roles in intestinal motility under normal conditions, when cholinergic transmission is intact. On the other hand, NK_1 and NK_2 receptors appear to contribute to increased intestinal motility and secretion under pathophysiological conditions, for example during infection or inflammation (Croci et al., 1997; Carini et al., 2001; Moriarty et al., 2001; Koon and Pothoulakis, 2006).

Psychological stress stimulates colon motility in several species including man (Almy et al., 1950; Martinez and Taché, 2006; Taché and Bonaz, 2007). A few studies have investigated the effects of selective NK_1 and NK_2 receptor antagonists on stress-induced colonic motility in animals. The selective NK_1 receptor antagonists RP67580 and TAK637 reduced stress-induced defecation in rats and gerbils respectively (Ikeda et al., 1995; Okano et al., 2001, 2005). In contrast, studies using selective NK_2 receptor antagonists have generated conflicting results. The selective tachykinin NK_2 receptor antagonist SR48968 (saredutant) did not affect stress-induced defecation in rats (Ikeda et al., 1995) while MEN10627, another selective tachykinin NK_2 receptor antagonist, inhibited defecation in rats under similar stress conditions (Evangelista, 2001).

Substance P and neurokinin A are also expressed in intestinal extrinsic primary afferent nerve endings emanating from dorsal root ganglia (Sternini et al., 1989). A number of studies have implicated NK_1, NK_2 and NK_3 receptors in mediating visceral nociception during colorectal distension or after chemical stimulation of the gut (Julia et al., 1994; Julia and Bueno, 1997; McLean et al., 1997; 1998; Laird et al., 2000; 2001a; Toulouse et al., 2000; Kamp et al., 2001; Okano et al., 2002; Birder et al., 2003; Gaudreau and Plourde, 2003; Greenwood-Van Meerveld et al., 2003; Bradesi et al., 2006). In most cases, the efficacy of NK receptor antagonists is most prominent during hypersensitive conditions evoked by either colonic inflammation (acute and/or chronic) or by psychological stress.

The pharmacology of small molecule tachykinin NK_1 receptor antagonists varies greatly among species, while species-related differences for NK_2 receptor antagonists are not as prominent (unpublished observations). Antagonists that bind to human NK_1 receptors often have lower affinity (in some cases 1,000-fold lower) for mouse and rat NK_1 receptors. This has hindered the evaluation of NK_1 receptor antagonists in models of colorectal distension, mainly developed in rats (Ness and Gebhart, 1988; Tammpere et al., 2005) and mice (Larsson et al., 2003; Arvidsson et al., 2006). Alternatively, when assessing the efficacy of NK_1 receptor antagonists intended for future clinical use, colorectal distension has been performed in rabbits (Okano et al., 2002) and guinea pigs (Greenwood-Van Meerveld et al., 2003). Gerbils represent a rodent species with NK_1 receptor pharmacology comparable to humans (Beresford et al., 1991; Engberg et al., 2007) and have been used in models of depression (Varty et al., 2003) and anxiety (Cheeta et al., 2001) when evaluating the efficacy of NK_1 receptor antagonists *in vivo*.

The current study evaluates the role of NK_1 and NK_2 receptors in stress-induced defecation and adenocorticotropin (ACTH) hormone release in gerbils. Also, for the first time, the role of NK_1 and NK_2 receptors in visceral hypersensitivity produced by repeated noxious colorectal distensions in gerbils was investigated. This was done using the selective NK_1 and NK_2 receptor antagonists aprepitant (Hale et al., 1998) and saredutant (Advenier et al., 1992). The doses of antagonist chosen were based on their effects in the NK_1 receptor mediated gerbil foot tapping assay (Bristow and Young, 1994; Lindström et al., 2007; Sundqvist et al., 2007) and NK_2 receptor agonist-evoked fecal pellet output.

2. Materials and methods

2.1. Chemicals

5-[(2(*R*)-[1(*R*)-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3(*S*)-(4-fluorophenyl)-4-morpholinyl)methyl]-2,4-dihydro-1,2,4-triazol-3-one] (MK869 or aprepitant, Hale et al., 1998) and (S)-N-methyl-N(4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl)butyl)benzamide (SR48968 or saredutant, Advenier et al., 1992) were synthesized at AstraZeneca. Lys-Asp-Ser-Phe-Val-Gly-R-lactam-Leu-Met-NH_2 (GR64349), a selective tachykinin NK_2 receptor agonist, was purchased from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany). A vehicle containing ethanol/solutol/saline (5/5/90) was used to dissolve the compounds, which were administered i.p. at a volume of 5 ml/kg.

2.2. Animals

Male Mongolian gerbils, aged about seven weeks and weighing 55–70 g upon arrival, were purchased from Charles River (Sulzfeld, Germany). The gerbils were group housed in cages containing an enriched environment including hay, plastic tubes, nesting material and sand. Food and water were available *ad libitum* and the cages were placed in temperature and humidity-controlled holding rooms. The animals were allowed at least 7 days to acclimatize to the housing conditions before handling. All experiments were approved by the local animal ethical committee of Göteborg, Sweden.

2.3. NK_2 receptor agonist-induced pellet output

Gerbils were accustomed to individual grid-wire cages five times for 30–60 min on separate days before experiments. The effect of selective NK_2 receptor stimulation on fecal pellet output was studied in initial experiments by i.p. administration of the selective NK_2 receptor agonist GR64349 at various doses. The gerbils were placed in the cages and the number of fecal pellets excreted was counted for 60 min after treatment, at 15 min intervals. From these experiments, a maximally effective dose of GR64349 (100 μg/kg i.p., equivalent to 109 nmol/kg)