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Central tachykinin NK_3 receptors in the inhibitory action on the rat colonic propulsion of a new tachykinin, PG-KII

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Abstract

The inhibitory action of the natural selective tachykinin NK_3 receptor agonist, PG-KII, (pGlu-Pro-Asn-Pro-Asp-Glu-Phe-Val-Gly-Leu-Met-NH_2), on colonic propulsion was studied in rats after central administration. Intracerebroventricular injection of PG-KII (0.1, 1, 10 and 100 ng/rat) produced a dose-related inhibition of colonic propulsion, measured as the increase in the mean expulsion time of a 5-mm glass bead placed in the distal colon. At the same doses as PG-KII, the selective tachykinin NK_3 receptor agonist, senktide, (succ-[Asp^6-$MePhe^8$] substance P-(6–11)), induced a similar dose-related inhibition. Conversely, substance P (0.1, 1 and 10 μg/rat), a tachykinin NK_1-preferring receptor agonist, had weaker antipropulsive effects, neurokinin A (0.1, 1 and 10 μg/rat), a tachykinin NK_2-preferring receptor agonist, at the highest dose used only slightly inhibited colonic propulsion and neurokinin B (0.1, 1 and 10 μg/rat), a tachykinin NK_3-preferring receptor agonist, left propulsion unchanged. Pretreatment with the selective tachykinin NK_3 receptor antagonist, 3-indolycarbonyl-Hyp-Phg-*N*(me)-Bzl, referred as to R820 (6.2 μg/rat), prevented PG-KII-induced colonic antipropulsion, whereas the tachykinin NK_1 receptor antagonist, (*S*)-1-(2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl] ethyl)-4-phenyl-1-azoniabicyclo[2.2.2] octane chloride, referred to as SR 140,333 (1 μg/rat), and the tachykinin NK_2 receptor antagonist, ([Tyr^5,D-$Trp^{6,8,9}$, Arg^{10}] neurokinin A-(4–10)), referred to as Men 10,376 (5 μg/rat), left it unchanged. These findings show that of the tachykinins tested, PG-KII and senktide are the most potent central inhibitors of colonic propulsion in the rat, suggesting that the central tachykinin NK_3 receptor system plays an inhibitory role in modulating colonic transit. As well as confirming the selectivity of PG-KII for tachykinin NK_3 receptors, we show that PG-KII provides useful information about the physiological role of central tachykinin NK_3 receptors and that glass bead expulsion test is a reliable non-invasive in vivo method for evaluating the tachykinin NK_3 receptor selectivity of new synthetic or natural tachykinins.

Keywords: Tachykinin NK_3 receptor agonist; Colonic propulsion; (Rat)

1. Introduction

Tachykinins are widely distributed in the central nervous system (Saffroy et al., 1988) and in the gastrointestinal tract (Mussap et al., 1993). Although they participate in the control of many gastrointestinal functions, their central and peripheral physiological role in the control of peristalsis remains unclear.

The most investigated action of tachykinins in the gut relates to their role as neuromuscular excitatory transmitters through the activation of tachykinin NK_1 (substance P-preferring) or tachykinin NK_2 (neurokinin A-preferring) receptors (Barthó and Holzer, 1985; Holzer-Petsche, 1995; Shuttleworth and Keef, 1995; Maggi et al., 1997). Tachykinins can also affect intestinal motility by releasing inhibitory transmitters via tachykinin NK_1 and NK_3 receptors (Holzer and Holzer-Petsche, 1997). Evidence for tachykinin NK_1 and NK_3 receptor agonist inhibition of motor activity comes mainly from in vitro studies in the canine colon (Hou et al., 1989), the rat distal colon (Scheurer et al., 1994) and the guinea pig small and large intestine (Maggi et al., 1993, 1994a,b,c,d; Giuliani and Maggi, 1995). The role of tachykinin NK_3 receptors in intestinal motility under in vivo conditions remains less clear, because of the lack of potent and selective tachykinin NK_3 receptor agonists and antagonists. One study only has reported preliminary in vivo evidence that tonic activation of peripheral tachykinin NK_3 receptors by endogenous

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tachykinins stimulates inhibitory reflexes during prolonged localised distension of the rat colon (Lecci et al., 1996). Nothing is known about the role of central tachykinin NK_3 receptors in the regulation of colonic motility.

Recently, a novel kassinin-like peptide, PG-KII, (pGlu-Pro-Asn-Pro-Asp-Glu-Phe-Val-Gly-Leu-Met-NH_2), has been isolated from the skin of the Australian myobatrachid frog, *Pseudophryne güntheri* (Simmaco et al., 1990). Our previous reports (Improta et al., 1996; Polidori et al., 1997) indicated that in the rat, after central administration, PG-KII modulates inhibition of gastric acid secretion and alcohol intake, two functions thought to be mediated mainly by tachykinin NK_3 receptors. Furthermore, after peripheral administration, PG-KII stimulates saliva secretion through cholinergic pathways, as does neurokinin B (Broccardo et al., 1996). All these data provide evidence confirming PG-KII as a potent and selective tachykinin NK_3 receptor agonist.

This newly available highly selective tachykinin NK_3 receptor agonist seemed an important tool for investigating the role of central tachykinin NK_3 receptors in the regulation of in vivo colonic motility. Continuing research into the possible physiological role of the central tachykinin NK_3 receptor system in the mediation of gut motility in the rat, our main aim in this study was to characterize the actions of this novel natural tachykinin on colonic transit. Hence, we compared the effects induced on rat colonic propulsion by intracerebroventricular (i.c.v.) injection of PG-KII with those induced by various tachykinins including substance P (a tachykinin NK_1-preferring receptor agonist), neurokinin A (a tachykinin NK_2-preferring receptor agonist), neurokinin B (a tachykinin NK_3-preferring receptor agonist) and senktide (a synthetic and selective tachykinin NK_3 receptor agonist). We also evaluated the activity of PG-KII at central tachykinin receptors in rats pretreated with selective tachykinin NK_1, NK_2 and NK_3 receptor antagonists. The fundamental role of the central opioid receptor system in modulating colonic motility (Broccardo and Improta, 1992, 1998) prompted us also to investigate a possible central tachykinin–opioid receptor system interaction.

2. Materials and methods

2.1. Animals

All animal experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/EEC). Male Wistar rats (Morini, S. Polo D'Enza, Italy) weighing 200–250 g were used for the experiments. All rats were examined at 09:00 h. Rats were placed individually in plastic cages under standard temperature conditions (22°), with 12-h light/dark cycles and food and water ad libitum.

2.2. Surgery

At least 3 days before the experiments, rats were given light diethyl ether anesthesia and a permanent polyethylene cannula for i.c.v. injection was implanted into a skull hole drilled stereotaxically to the coordinates of the left lateral ventricle (Pellegrino et al., 1979).

2.3. Colonic propulsion test

Distal colonic propulsion was measured according to the method of Raffa et al. (1987) as previously described by Jacoby and Lopez (1984). Immediately after i.c.v administration of tachykinins, a single 5-mm diameter glass bead was inserted 3 cm into the distal colon of each rat. The time required for expulsion of the glass bead was determined (to the nearest 0.1 min) for each animal. Inhibition of colonic propulsion was measured as the increase in mean expulsion time of the glass bead compared with that of vehicle-treated rats (controls). The higher the mean expulsion time value, the stronger the inhibition of colonic propulsion.

2.4. Drugs

PG-KII (pGlu-Pro-Asn-Pro-Asp-Glu-Phe-Val-Gly-Leu-Met-NH_2) was purchased from Neosystem, Strasbourg, France. The selective tachykinin NK_3 receptor agonist (succ-[Asp^6-$MePhe^8$] substance P-(6–11)), referred to as senktide, substance P, neurokinin A and neurokinin B were purchased from Peninsula Laboratories, San Carlos, CA.

PG-KII (0.1, 1, 10 and 100 ng/rat), senktide (1, 10 and 100 ng/rat), substance P, neurokinin A and neurokinin B (0.1, 1 and 10 μg/rat) were dissolved in 0.9% NaCl and administered by i.c.v. injection in a volume of 5 μl/rat.

The selective tachykinin NK_1 receptor antagonist, (*S*)-1-(2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl] ethyl)-4-phenyl-1-azoniabicyclo-[2.2.2] octane chloride, referred to as SR 140,333 (Edmonds-Alt et al., 1993a,b), and the selective tachykinin NK_2 receptor antagonist, ([Tyr^5, D-$Trp^{6,8,9}$, Arg^{10}] neurokinin A-(4–10)), referred to as Men 10,376 (Maggi et al., 1991), were a gift from Dr. Maggi Menarini Laboratories, Florence, Italy. The selective tachykinin NK_3 receptor antagonist, 3-indolycarbonyl-Hyp-Phg-*N*(me)-Bzl, referred as to R820 (Regoli et al., 1994; Nguyen-Le et al., 1995), was a gift from Dr. Regoli, Pharmacology, Sherbrooke, Quebec, Canada. SR 140,333 (1 μg/rat), Men 10,376 (5 μg/rat) and R820 (6.2 μg/rat) were dissolved in 10% dimethyl sulphoxide (DMSO), further diluted to the final concentration with distilled water and administered by i.c.v. injection. Naloxone (1 mg/kg, s.c.), a

non-selective opioid receptor antagonist, was purchased from Endo Laboratories and dissolved in distilled water. All the antagonists were administered 4 min before tachykinins.

2.5. Statistical analysis

All results are expressed as means ± S.E. Data were evaluated with a one-way analysis of variance (ANOVA) and Duncan's multiple range test on an Apple II Computer. P values equal to or less than 0.01 were considered to indicate statistical significance.

3. Results

The mean colonic bead expulsion time for vehicle-treated rats was 2.26 ± 0.34 min. All the tachykinins tested inhibited colonic propulsion: the rank order of potency was PG-KII = senktide ≫ substance P ⋙ neurokinin A ≥ neurokinin B. PG-KII (0.1, 1, 10 and 100 ng/rat) increased the mean expulsion time significantly and in a dose-related manner; the highest dose tested (100 ng/rat) increased the colonic propulsion time to 37.32 ± 6.4 min and even a very small dose (100 pg/rat) significantly inhibited colonic propulsion (Fig. 1). At the same doses as PG-KII, the synthetic tachykinin NK_3 receptor agonist, senktide, induced a similar dose-related inhibition of colonic propulsion. Conversely, substance P (0.1, 1 and 10 μg/rat) inhibited colonic propulsion only at doses about 1000 times higher than those of PG-KII and senktide. Neurokinin A had an even weaker inhibitory action on colonic propulsion, and neurokinin B left colonic propulsion unchanged (Fig. 1).

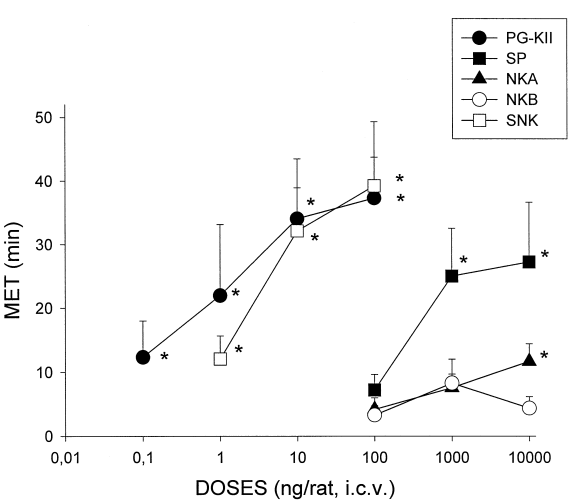

Fig. 1. The dose–response curves for the colonic antipropulsive effects of various tachykinins administered i.c.v. Each point represents the mean ± S.E of values for eight or more rats, expressed as mean expulsion time (MET) of the glass bead inserted into the distal colon. The mean expulsion time for vehicle-treated rats was 2.26 ± 0.34 min. Difference from vehicle-treated rats $^*P > 0.01$.

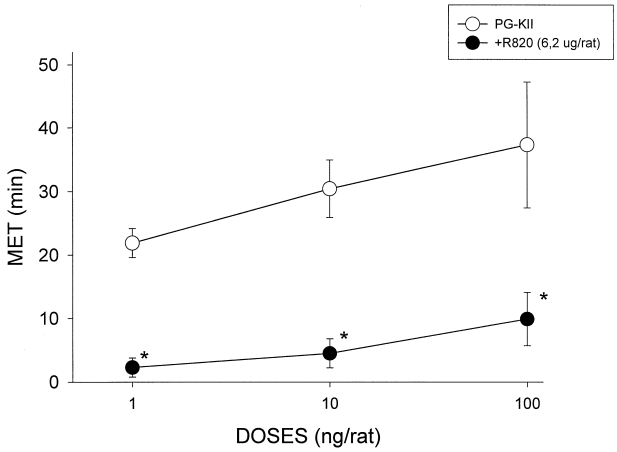

Fig. 2. Mean expulsion time (MET) of a glass bead after injection of PG-KII alone (1, 10 and 100 ng/rat, i.c.v.) or after i.c.v. pretreatment with the tachykinin NK_3 receptor antagonist, R820 (6.2 μg/rat) in rats. Each point represents the mean ± S.E. for at least eight rats. Difference from PG-KII-treated rats $^*P < 0.01$.

No significant difference was observed between the mean colonic bead expulsion time in vehicle-treated and tachykinin NK_3 receptor antagonist-treated rats. Pretreatment with the highly selective tachykinin NK_3 receptor antagonist, R820 (6.2 μg/rat), invariably prevented the colonic antipropulsive activity of PG-KII (Fig. 2).

The tachykinin NK_1 receptor antagonist, SR 140,333 (1 μg/rat), and the tachykinin NK_2 receptor antagonist, Men 10,376 (5 μg/rat), left the mean expulsion time in vehicle-treated rats unchanged. They also failed to antagonize PG-KII-induced inhibition of distal colonic propulsion (Table 1).

Pretreatment with the opioid receptor antagonist, naloxone (1 mg/kg, s.c.), left colonic propulsion in vehicle-treated rats unchanged. It also failed to block the inhibitory effect of PG-KII (100 ng/rat) on distal colonic propulsion in the rat (Table 1).

Table 1
Colonic antipropulsive effects induced by a single dose of PG-KII (100 ng/rat, i.c.v.) in vehicle-pretreated or tachykinin NK_1 receptor antagonist (SR 140,333)-, tachykinin NK_2 receptor antagonist (Men 10,376)- and opioid receptor antagonist (naloxone)-pretreated rats. Each point represents the mean expulsion time (MET) ± S.E. of a glass bead for at least eight rats

Pretreatment	Treatment	Mean expulsion time (min)
Vehicle	Vehicle	2.26 ± 0.34
SR 140,333 (1 μg/rat, i.c.v.)	Vehicle	3.12 ± 1.32
Men 10,376 (5 μg/rat, i.c.v.)	Vehicle	5.02 ± 1.87
Naloxone (1 mg/kg, s.c.)	Vehicle	2.01 ± 0.58
Vehicle	PG-KII	37.32 ± 6.44[a]
SR 140,333 (1 μg/rat, i.c.v.)	PG-KII	26.44 ± 2.25[a]
Men 10,376 (5 μg/rat, i.c.v.)	PG-KII	30.94 ± 4.50[a]
Naloxone (1 mg/kg, s.c.)	PG-KII	34.00 ± 5.83[a]

[a] $P < 0.01$ difference from vehicle-treated rats.

4. Discussion

First of all, in this study, i.c.v. injection of the natural tachykinin PG-KII potently inhibited distal colonic propulsion increasing, in a significant and dose-dependent way, the mean expulsion time of a glass bead placed in the distal colon of rats. Because R820, the most suitable tachykinin NK_3 receptor antagonist currently available for studies of the rat central nervous system (Cellier et al., 1997), prevented the PG-KII-induced inhibitory effect, whereas the tachykinin NK_1 and NK_2 receptor antagonists did not, we confirm our previous in vitro and in vivo data (Improta et al., 1996; Polidori et al., 1997) and show that the natural peptide PG-KII is a selective agonist for central tachykinin NK_3 receptors.

Of the studied tachykinins, the most potent inhibitors of colonic propulsion were PG-KII and senktide, which is known as a highly selective tachykinin NK_3 receptor agonist. This finding suggests an important role of the central tachykinin NK_3 receptor system in the inhibitory control of colonic propulsion. However, a central role fits in poorly with our observation that even at the highest doses, the natural tachykinin NK_3-preferring receptor agonist, neurokinin B, had no significant inhibitory effect on colonic propulsion. In other studies, centrally injected neurokinin B also proved less potent than senktide, for example, in inhibiting gastric secretion and in certain cardiovascular effects (Improta and Broccardo, 1990; Culman et al., 1995). The most likely explanation is the fast degradation of centrally injected neurokinin B in the ventricular system or its low selectivity for central tachykinin NK_3 receptors.

Overall, these data fit in well with recently reported results describing the in vivo and in vitro inhibitory effects of tachykinin NK_3 receptor agonists in the colon (Giuliani and Maggi, 1995; Lecci et al., 1996; Holzer and Holzer-Petsche, 1997) and lend support to a central site of action. Accordingly, many reports describe the presence of neurokinin B, the tachykinin NK_3 receptors and the mRNA for both in the central nervous system (Dam et al., 1990; Lucas et al., 1992; Merchenthaler et al., 1992; Ding et al., 1996; Shughrue et al., 1996). Furthermore, evidence that colorectal distension activates various brain regions, including the caudal intermediate reticular nucleus, comes from a study showing increased expression of proto-oncogenes (Lantekaari-Minet et al., 1993).

In our distal colonic propulsion test, substance P was far less ($\sim$1000 times) potent than the tachykinin NK_3 receptor agonists, and neurokinin A inhibited colonic propulsion slightly and only at the highest dose used. At these high concentrations, substance P and neurokinin A may lose their already poor selectivity, and activate tachykinin NK_3 receptors, thus confirming the inhibitory role of central tachykinin NK_3 receptors on colonic propulsion. Nonetheless, we cannot altogether exclude a partial inhibitory role of central tachykinin NK_1 receptors in this function. Previous research (Julia et al., 1994) on the possible physiological role of the central tachykinin receptor system in the regulation of colonic motility in the rat has addressed the involvement of central tachykinin NK_1 and NK_2 receptors in colonic motor disturbances induced by rectal distension. Central administration of the tachykinin NK_1 receptor antagonist, CP96,345, reduced the rectal distension-induced colonic inhibitory reflex, thus suggesting that central tachykinin NK_1 receptors mediate this reflex while central tachykinin NK_2 receptors are involved only in the transmission of visceral pain.

The known fundamental role of the central opioid receptor system in the modulation of colonic motility (Broccardo and Improta, 1992, 1998) prompted us to investigate whether pretreatment of rats with naloxone, a peripherally-administered opioid receptor antagonist that generally crosses the blood–brain barrier and may therefore, modify central actions, would change the antipropulsive action of PG-KII. Because it failed to do so, we conclude that PG-KII-induced antipropulsion is not mediated by a central tachykinin–opioid receptor system interaction.

In conclusion, we confirm the selectivity of PG-KII for the tachykinin NK_3 receptors, showing that this novel natural tachykinin NK_3 receptor agonist provides useful information about the consequences of central tachykinin NK_3 receptor stimulation. We also suggest that the central tachykinin NK_3 receptor system has a key physiological role in the inhibitory modulation of colonic propulsion in the rat. Our experience indicates that the glass bead expulsion test is a reliable non-invasive, in vivo method for evaluating the tachykinin NK_3 receptor selectivity of new synthetic or natural tachykinins.

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