PAC1 Receptor-Mediated Relaxation of Longitudinal Muscle of the Mouse Proximal Colon

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ABSTRACT—Since pituitary adenylate cyclase-activating polypeptide (PACAP) was shown to partially mediate nonadrenergic, noncholinergic (NANC) relaxation of longitudinal muscle of the proximal colon of ICR mice, we further studied the receptor subtype activated by PACAP by using a mutant mouse whose PAC1 receptors are markedly reduced. In wild-type mice, the PACAP-mediated component of NANC relaxation was 33%, but it was absent in the mutant mice. The potency of exogenous PACAP in inducing relaxation in the mutant mice was one hundredth of that in wild-type mice. VPAC1 and VPAC2 receptors were not suggested to have any role in the relaxation. These results suggest that PACAP mediates NANC relaxation of longitudinal muscle of mouse proximal colon via PAC1 receptors.

Keywords: PAC1 receptor, Pituitary adenylate cyclase-activating polypeptide, Nonadrenergic, noncholinergic relaxation

Exogenously added pituitary adenylate cyclase-activating polypeptide (PACAP) reduced basal contractions in all regions of the rat gastrointestinal tract (1) and induced relaxation in the guinea pig tenia caeci (2) and stomach (3), human colon (4), rat ileum (5) and rabbit pyloric muscle (6) in vitro. PACAP was also shown to be released by nerve stimulation in guinea pig tenia coli (7) and by oral stretch of segments to be responsible for descending relaxation in the rat mid colon (8). Involvement of PACAP in the non-adrenergic, noncholinergic (NANC) relaxation was further suggested by using selective PACAP receptor antagonists (8). We have first suggested that PACAP mediates NANC inhibitory responses of longitudinal muscle of rat distal colon via activation of the tyrosine kinase (9)-apamin-sensitive K⁺ channels (10) pathway.

PACAP receptor was initially classified into two types, Type I (specific to PACAP) and Type II (similar affinities to PACAP and vasoactive intestinal polypeptide (VIP)). The cDNAs encoding rat PACAP Type I receptors were cloned from the rat brain (11) and distribution of the mRNA for the receptors in the brain was studied by an in situ hybridization method (12). At present, Type I is called PAC1 and Type II is subclassified into VPAC1 and VPAC2. However, there is no selective antagonist for PAC1, VPAC1 and VPAC2 receptors. Although many reports strongly suggest the role of PACAP on NANC inhibitory response as noted above, the receptor subtype involved in PACAP-mediated relaxation in the gastrointestinal tract has not been studied in detail. Recently, a mouse line carrying targeted deletion of the signal peptide of the PAC1 receptor, which expresses the receptor with a markedly reduced level on the plasma membrane, was generated (13). In the present study, we studied the role of PAC1 receptors on PACAP-mediated relaxation by using the mutant mice.

Gene disrupted mice, PAC1 receptor exon 2−/− mice, were generated by the method described in the previous paper (13). PAC1 receptor exon 2−/− mice and wild-type mice (PAC1 receptor exon 2+/+ mice) (both sexes, 8- to 10-week-old, 25 – 35 g) were lightly anaesthetized with diethyl ether and then stunned by a blow on the head and bled via carotid arteries. Whole segment of the proximal colon (1.5 cm in length) was removed and placed in Tyrode
solution consisting of 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 0.42 mM NaH₂PO₄, 11.9 mM NaHCO₃ and 5.6 mM glucose. The whole segments were suspended in an organ bath filled with Tyrode solution aerated with 5% CO₂ in O₂ and maintained at 37°C. Atropine (1 μM) and guanethidine (5 μM) were present throughout the experiment to block cholinergic and adrenergic responses, respectively. The longitudinal muscle of each segment was subjected to a load of 1.0 g to obtain the most reproducible responses and stable resting tone. The preparations were equilibrated for at least 30 min before the experiments. Responses of the longitudinal muscle to electrical field stimulation (EFS) for 10 s with trains of 100 pulses of 0.5-ms width at 30 V were recorded isotonically with a 10-min interval between tests. The extent of relaxation was expressed as the area under the line of resting tone that was drawn on the bottom of resting spontaneous contractile activity (Fig. 1, dotted lines). VIP fragment VIP₁₀–₂₈ was purchased from Sigma Chemical Co., St. Louis, MO, USA. PACAP27 and PACAP fragment PACAP₆–₃₈ were purchased from The Peptide Institute, Osaka. Papaverine hydrochloride was from Wako Pure Chemical Industries, Ltd., Osaka.

Since the largest participation of PACAP in NANC relaxation was shown in the proximal colon of ICR mouse in the previous study (14), the role of PAC1 receptors in NANC relaxation was studied in the proximal colon of wild-type and the mutant mice. We first studied the resting tone of the proximal colonic segments and extent of EFS-induced NANC relaxation in both mice. The maximal relaxation induced by 30 μM papaverine (9.2 ± 2.9% of length of the segments, mean ± S.E.M., n = 7) in wild-type mice was shown in the proximal colon of ICR mouse in the previous study (14), the role of PAC1 receptors in NANC relaxation was studied in the proximal colon of wild-type and the mutant mice. We first studied the resting tone of the proximal colonic segments and extent of EFS-induced NANC relaxation in both mice. The maximal relaxation induced by 30 μM papaverine (9.2 ± 2.9% of length of the segments, mean ± S.E.M., n = 7) in wild-type mice was shown in the proximal colon of ICR mouse.

**Fig. 1.** Effects of PACAP₆–₃₈ on EFS-induced relaxation in the proximal colon of wild-type and the mutant mice. Relaxation was induced by EFS before and after treatment of the segments with 1 μM PACAP₆–₃₈ for 20 min. The continuous lines indicate the presence of PACAP₆–₃₈ or papaverine. Bold black lines indicate duration of EFS for 10 s. After recording normal spontaneous movements, the chart was run at a fast speed immediately before the stimulation so that the relaxant response could be recorded clearly. The maximal relaxation was induced by treatment of the segments with 30 μM papaverine. Inset: relaxations induced by EFS in the presence of PACAP₆–₃₈ are expressed as percentages of the control response before addition of the drug. Columns and bars represent means ± S.E.M. in 4 (wild) and 6 (mutant) experiments.
mice was similar in extent to that in the mutant mice (12.5 ± 3.7%, n = 7), suggesting that resting tones of the segments from wild-type and the mutant mice are similar levels. The extents of EFS-induced relaxations, expressed as percent of the maximal relaxation induced by 30 μM papaverine (15), were also similar in the wild-type and mutant mice, 24.7 ± 3.7% (n = 8) and 28.7 ± 4.5% (n = 10), respectively. These results suggest that the resting tone and extent of EFS-induced relaxation are not changed in the mutant mice. Next, participation of PACAP in NANC relaxation was studied in wild-type mice by using a PACAP receptor antagonist, PACAP6–38, at 1 μM, a concentration at which it exhibited its maximum inhibitory effect on NANC relaxation in the distal colon of Wistar-ST rats (10). PACAP6–38 at 1 μM inhibited the relaxation by 33.3 ± 4.6% (n = 4). However, it did not inhibit the relaxation in the mutant mice (2.5 ± 2.4% inhibition, n = 6) (Fig. 1). The results suggest that the PACAP-mediated component of NANC relaxation is absent in the mutant mice. Then, the effect of exogenously added PACAP on the longitudinal muscle of the proximal colonic segments was studied. Exogenous PACAP induced gradual relaxation of longitudinal muscle segments obtained from both wild-type and the mutant mice. However, the potency of PACAP in the mutant mice was significantly lower than that in wild-type mice (Fig. 2): IC_{50} values are 0.3 and 30 nM for wild-type and mutant mice, respectively, suggesting involvement of PAC1 receptors in PACAP-induced relaxation. Finally, we tried to examine the possibility of compensation for lack of PAC1 receptors by VPAC1 and VPAC2 receptors in the mutant mice, since the extent of EFS-induced NANC relaxation in mutant mice did not decrease in spite of the lack of PACAP-mediated component as noted above. Since a VIP fragment, VIP_{10–28}, antagonizes the effect of VIP, the fragment must predominantly block VPAC1 and VPAC2 receptors. VIP_{10–28} at 3 μM, which exerted its maximal inhibitory effect on NANC relaxation in the distal colon of Wistar-ST rats (16), had no significant effect on NANC relaxation (data not shown, n = 2) or exogenous PACAP-induced relaxation (n = 3) in the mutant mice. The result indicates that VPAC1 and VPAC2 receptors do not compensate for lack of PAC1 receptors in the mutant mice. The question why PACAP at a high concentration can induce the maximal response in the mutant mice (Fig. 2) remained unsolved. In the mutant mice with a targeted deletion of the signal peptide of PAC1 receptor, PAC1 receptors expressed were largely retained on intracellular membranes as an immature glycoprotein (13). However, it seems likely that PAC1 receptors, which are markedly reduced in number but present in a sufficient amount to induce the maximal relaxation in response to a high concentration of exogenous PACAP, are also expressed on the plasma membrane of smooth muscle cells in the mutant mice. Namely, a large amount of spare PAC1 receptors are present in the wild-type mouse proximal colon.

In summary, it was suggested that PACAP partly mediates NANC relaxation of longitudinal muscle of the proximal colon of mouse via activation of PAC1 receptors.

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