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Rapid communication

Proadrenomedullin N-terminal peptide and cortistatin activation of MrgX2 receptor is based on a common structural motif

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Abstract

The G protein-coupled receptor MrgX2 belongs to the large family of the Mas-related genes or sensory neuron-specific G protein-coupled receptors. The MrgX2 receptor has been shown to be activated by the peptides cortistatin and proadrenomedullin N-terminal peptides (PAMP). Here we investigated the structure activity relationship of PAMP and identified key structural features that are shared with cortistatin and might explain why two apparently unrelated peptides are able to activate a single G protein-coupled receptor.

Keywords: Nociception; Receptor; Surrogate ligand

MrgX2 receptor is a G protein-coupled receptor and a member of the Mas-related genes, a family of more than 50 G protein-coupled receptors that are sequentially similar but that differs in numbers among different species (Dong et al., 2001). Seven MrgX receptors exist that are specific to human (Choi and Lahn, 2003). The Mas-related genes are of particular interest because they share a unique anatomical expression in that they are almost restricted to small diameter primary sensory neurons, pointing at these receptors as important regulator of nociception (Lembo et al., 2002). The Mas-related genes share a second particularity, they exhibit unexpected pharmacological properties. They breach the dogma of receptor-ligand specificity by having shown to be activated by ligands that are diverse in structure and chemical nature. For instance the MrgA receptors have been shown to be activated by RFamide-like peptides (Dong et al., 2001) as well as the organic base adenine (Bender et al., 2002). MrgX2 receptor has been reported to be activated by the neuropeptide cortistatin (Robas et al., 2003) and by two other peptides PAMP-12 and PAMP-20 (Kamohara et al., 2005). These two sets of peptides are physiologically unrelated and indeed cortistatin is known to be a highly potent receptor agonist at all five somatostatin receptors (Spier and de Lecea, 2000), which led Kamohara et al. to propose MrgX2 receptor as the endogenous PAMP receptor. We independently discovered PAMP as an MrgX2 receptor selective agonist but considered PAMP as a surrogate ligand since PAMP is highly conserved between human and rodents while the MrgX2 receptor is human specific. To understand why MrgX2 receptor can bind different bioactive peptides we have carried out a structure activity relationship study. We co-transfected the MrgX2 receptor with chimeric $G\alpha_{q/i3}$ proteins in HEK-293 cells and monitored receptor activation through changes in intracellular calcium, using a Fluorescent Plate Reader System (Nothacker et al., 2000).

Systematic C-terminal deletion of PAMP [1–20] resulted in the total loss of activity highlighting the importance of the C-terminal end (Table 1). From the N-terminal end, removing the first three amino acids led to a 75% loss of

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Table 1
Structure activity relationship of proadrenomedullin and cortistatin related peptides at MrgX2 receptor

Peptide	Sequence	Relative[b] activity ± S.D. (%)
PAMP[1–17]	ARLDVASEFRKK**W**NK**W**A	3.3 ± 1.9
PAMP[1–14]	ARLDVASEFRKK**W**N	2.0 ± 0.5
PAMP[1–11]	ARLDVASEFRK	2.3 ± 0.4
PAMP[1–8]	ARLDVASE	3.0 ± 0.2
PAMP[1–4]	ARLDV	3.3 ± 1.7
PAMP[16–20]	**W**ALS**R**-amide	2.7 ± 0.8
PAMP[13–20]	**W**NK**W**ALS**R**-amide	97.2 ± 5.8
PAMP[10–20]	RKK**W**NK**W**ALS**R**-amide	85.0 ± 18.2
PAMP[9–20]	FRKK**W**NK**W**ALS**R**-amide	100.0 ± 12.6
PAMP[7–20]	SEFRKK**W**NK**W**ALS**R**-amide	53.2 ± 5.2
PAMP[4–20]	**D**VASEFRKK**W**NK**W**ALS**R**-amide	23.2 ± 5.7
hPAMP[1–20]	ARLDVASEFRKK**W**NK**W**ALS**R**-amide	45.9 ± 4.5
Cortistatin-14	PCKNFF**W**KT**F**SSC**K**	100.0 ± 12.0[a]
Cortistatin-17	**D**RMPCKNFF**W**KT**F**SSC**K**	25.3 ± 3.0[a]
Somatostatin[3–10]	CKNFF**W**KT	4.6 ± 1.1[a]
Somatostatin-14	AGCKNFF**W**KT**F**TSC	3.2 ± 0.2[a]
Somatostatin [7–14]	F**W**KT**F**TSC	1.4 ± 0.2[a]
Somatostatin [2–9]	GCKNFF**W**K	1.1 ± 0.2[a]
Minimal proposed	**W**xx**W**xxx**R**	
core structures	**W**xx**F**xxx**K**	

Data represent concentration response curves in triplicate over two independent experiments. [a]data were taken from Robas et al. (2003) and normalized to cortistatin-14 activity; [b]relative activities were calculated for responses at doses of 1 μM. Structurally important amino acids mentioned in the text are highlighted in bold.

activity. This loss might be attributed to the then N-terminally exposed acidic amino acid and the consequent generation of repulsive electrostatic forces. A similar phenomenon has been seen for cortistatin-17 where N-terminal addition of a negatively charged amino acid led to a marked decrease in efficacy (Table 1), supporting a common activation mechanism between cortistatin and PAMP.

Interestingly, further N-terminal deletions led to peptides that regained efficacy and were even more potent than the full length PAMP[1–20]. These data are in agreement with the report of Kamohara and colleagues, who found that the naturally occurring PAMP12 (equivalent to PAMP[9–20] of Table 1) is more potent than PAMP[1–20] at activating MrgX2 receptor. The N-terminus can be deleted by up to twelve amino acids without significant loss of activity. PAMP[13–20] was equipotent to PAMP[9–20] delineating the minimal active structure as an octapeptide. Any further deletions resulted in total loss of activity.

Comparison of the minimal active structure of PAMP with cortistatin-14 shows some surprising similarities that might explain similar efficacy of the apparently unrelated peptides. The active core of PAMP consists of two aromatic Trp residues and a C-terminally located basic arginine all arranged in a specifically spaced pattern (Table 1). A similar pattern of aromatic and basic amino acids is also found in cortistatin-14 but not in somatostatin-14 that lacks the C-terminal basic amino acid and is therefore inactive.

We are aware of the limitations of our study and neither assessed the potency of the peptides nor studied the role of individual amino acids within the core structure. It should also be considered that some of the non active peptides may be antagonists and thus still may bind with high affinity.

In conclusion, we suggest that both cortistatin and PAMP bind to and activate MrgX2 receptor at the same receptor site due to a common internal structural motif that is centered around an octapeptide that alternates aromatic and basic amino acids. This leads us to predict that PAMP and cortistatin will bind MrgX2 receptor in a competitive manner. We therefore propose to consider both ligands as MrgX2 receptor surrogates for as long as none have been shown to activate the receptor in vivo.

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