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SPECIAL REPORT

Involvement of G-protein αi1 subunits in activation of G-protein gated inward rectifying K^+ channels (GIRK1) by human NPY_1 receptors

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This study investigated the type of G-protein α subunit(s) that human neuropeptide Y $(NPY)_1$ receptors preferentially utilize when activating G-protein gated K^+ currents. Two electrode voltage-clamp recordings were made from *Xenopus* oocytes that had been injected with cDNAs encoding either human NPY_1 or $D_{2(short)}$ dopamine receptors, and GIRK1 a cloned rat brain K^+ channel. These receptors were also co-injected with G-protein αi1, αi2, αi3 and αo1 subunits to determine which subunit(s) modulate the efficiency of signal transduction. In NPY_1 receptor injected cells neuropeptide Y (100 nM) caused a 53 ± 10 nA inward current ($n = 14$; $EC_{50} = 3$ nM) and this effect was blocked by pertussis toxin (500 ng ml^{-1} 24 h). Activation of GIRK1 currents by neuropeptide Y was selectively potentiated by αi1 subunit cDNA whereas coupling dopamine of D_2 receptors to this channel was not.

Keywords: NPY_1 receptors; D_2 dopamine receptors; G-proteins; G-protein activated K channels (GIRK1/KGB); pertussis toxin

Introduction In the hippocampus neuropeptide Y (NPY) decreases neuronal excitability by activation of NPY_1 receptors (Colmers *et al.*, 1987). Cloned NPY_1 receptors also inhibit adenylate cyclase activity by activation of pertussis toxin-sensitive G-proteins (e.g. Herzog *et al.*, 1992). D_2 dopamine receptors have similarly been shown to regulate pertussis toxin-sensitive G-proteins, and in rat pituitary lactotrophs this specifically involves αi2 subunits (Albert & Morris, 1994). Whether cloned human NPY_1 receptors regulate K^+ channel activity by specific G-protein αi subunits remains to be determined.

The cDNA encoding a G-protein gated potassium channel (GIRK1) has recently been cloned from rat heart tissue (Dascal *et al.*, 1993; Kubo *et al.*, 1993). This channel is 501 amino acids long with two putative transmembrane spanning helices, and can form inwardly rectifying K^+ channels with a single channel conductance of 40 pS. Furthermore when expressed in *Xenopus* oocytes this channel can be activated by co-expressed $5\text{-}HT_{1A}$, muscarinic m_2 and opiate receptors. Thus GIRK1 channels belong to a structurally and functionally unique K^+ channel family. In heart tissue it is likely that GIRK1 channels mediate the bradycardia associated with muscarinic receptor activation following acetylcholine release from parasympathetic nerve terminals (Kubo *et al.*, 1993). G-protein gated K^+ channels are also involved in modulation of neuronal activity in the central nervous system. To further understand the signal transduction mechanisms associated with human NPY_1 receptors (Herzog *et al.*, 1992) we have investigated whether these receptors can activate GIRK1 channels when expressed in *Xenopus* oocytes. The ability of transfected G-protein α subunits to enhance the efficiency of receptor-effector coupling was also examined, and compared to that of human D_2 dopamine receptors.

Methods Two electrode voltage-clamp recordings were made from *Xenopus* oocytes that had been transiently transfected with GIRK1 channel cDNA (e.g. Dascal *et al.*, 1993) by nuclear injection, and with human NPY_1 or $D_{2(short)}$ dopamine receptors. Using the published coding region of the GIRK1 channel a homolog was cloned from a rat brain cDNA library by PCR. The identity of the sequence was confirmed by ^{32}P-oligonucleotides probes and restriction analysis. Injected cells were perfused with an extracellular solution that contained (in mM); NaCl 96, HEPES 5, KCl 2, $CaCl_2$ 1, $MgCl_2$ 1, pH 7.5, at 22°C. Cells were voltage-clamped at −80 mV and after equilibration in high potassium containing saline (96 mM KCl and 2 mM NaCl instead of 2 mM KCl and 96 mM NaCl) to reverse the K^+ ion gradient, the ability of NPY and quinpirole to activate inward currents was examined. Responses to D_2 and NPY_1 receptor activation were maximal 2-3 days following cDNA injections. The effects of co-expressing G-protein αi1, αi2, αi3 and αo1 subunits were studied in parallel. Quinpirole and S(−)-sulpiride were purchased from Research Biochemicals Inc., and human neuropeptide Y from Sigma. Data are expressed as the mean ± s.e.mean.

Results Neuropeptide Y (100 nM) activated an inward current of 53 ± 10 nA in 14/27 cells injected with NPY_1 + GIRK1 cDNA. No effects of neuropeptide Y were observed in cells injected with GIRK1 cDNA alone or in combination with D_2 dopamine receptors. The maximal inward current following D_2 receptor activation with quinpirole (1 μM) was comparable to that of NPY_1 receptor activation (106 ± 20 nA, 34/45 cells). The EC_{50} for neuropeptide Y in NPY_1 receptor transfected cells was 3 nM ($pEC_{50} = 8.61 \pm 0.34$, $n = 4$; Figure 1) whereas for quinpirole in D_2 receptor injected oocytes was 6 nM ($pEC_{50} = 8.22 \pm 0.44$, $n = 3$). These effects of quinpirole were reversibly inhibited by the selective D_2 receptor antagonist S(−)-sulpiride (to $3 \pm 2\%$ of control at 1 μM, $n = 3$) and were not observed in oocytes injected with GIRK1 + NPY_1 receptors instead of the D_2 subtype.

Activation of GIRK1 currents by NPY_1 or D_2 dopamine receptors was significantly reduced in oocytes pretreated with pertussis toxin (500 ng ml^{-1}; Figure 1c). Consistent with the involvement of PTX-sensitive G-proteins co-injection of cells with NPY_1 + GIRK1 + αi1 cDNA increased the efficiency of

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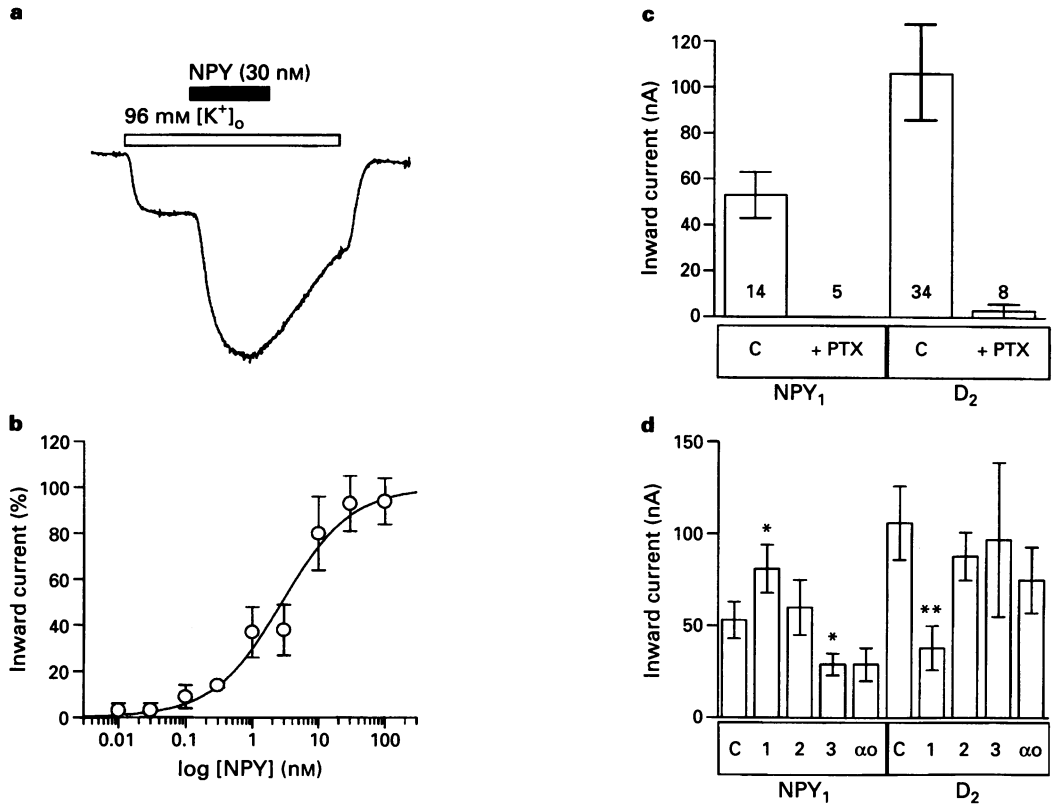

Figure 1 Activation of GIRK1 potassium currents by stimulation of human NPY_1 or D_2 dopamine receptors. (a) Membrane current from an oocyte injected with GIRK1 + NPY_1 receptor cDNA. The cell was voltage-clamped at −80 mV, and the current was monitored before and after application of a high potassium containing solution (open bar) during which time neuropeptide Y (NPY 30 nM) was also applied (solid bar). (b) Concentration-effect curve to neuropeptide Y. Each point represents the mean ± s.e.mean from 4 cells. The EC_{50} for neuropeptide Y was 3 nM. (c) Block of inward currents activated by neuropeptide Y (100 nM) and quinpirole (1 μM) in NPY_1 and D_2 injected cells respectively by pertussis toxin (500 ng ml^{-1} for 24 h). C = control cells injected with the cDNA for each receptor plus GIRK1. (d) Effect of co-expressing G-protein αi1 (1), αi2 (2), αi3 (3) and αo subunits on the maximum current activated by either neuropeptide Y (100 nM) or quinpirole (1 μM) in NPY_1 and D_2 injected oocytes. $*P<0.05$ and $**P<0.01$ significantly different from control (C; Students t test on Log transformed data). Data are from 6 to 41 cells for each condition.

receptor effector coupling by 51%. Conversely, in cells injected with D_2 + GIRK1 + αi1 the receptor activated current was significantly reduced by 64%. This effect was not mimicked by co-injection with αi2 subunit cDNA which had no effect on currents induced by either receptor. Co-expression of αi3 and αo1 G-protein subunits attenuated the NPY-mediated responses but not those by D_2 receptors (Figure 1D).

Discussion Therefore human NPY_1 receptors can activate inwardly rectifying K^+ channels when expressed in *Xenopus* oocytes by pertussis toxin sensitive G-proteins, and this can be potentiated by G-protein αi1 subunits. Co-injection of αi3 or αo1 G-protein subunits decreased the efficiency by which NPY_1 receptors activated GIRK1 currents whereas no effect of αi2 co-expression was observed. This indicates that the enhancement with αi1 subunits, which was presumably associated with endogenous βγ subunits, was due to a direct increase in the efficiency of signal transduction. Co-expression of G-protein α subunits has also been shown to decrease the efficiency by which muscarinic m_2 receptors gate GIRK1 channels and this is thought to be due to transfected α subunits sequestering endogenous βγ subunits that may also directly gate these channels (Takao *et al.*, 1994). However, free α-subunits may also directly associate with the receptor and thus reduce the efficiency of coupling via heteromeric αβγ complexes.

Like NPY_1 receptors, stimulation of human D_2 receptors activated GIRK1 channels via pertussis toxin sensitive G-proteins. However, in contrast to NPY_1 receptors the effect of D_2 receptor activation was not potentiated by co-expression with αi1 cDNA but instead was decreased. This suggests that expression of αi1 subunits, like the effect of αi3 and αo1 on NPY_1 receptors, may be sequestering βγ subunits required for efficient receptor-effector coupling. Furthermore, because co-expression of αi1 subunits had opposing effects on D_2 and NPY_1 receptors these data indicate that more than one subtype of βγ subunit may be involved in the regulation of GIRK1 channel activity. However, confirmation of this hypothesis requires a direct comparison between the effects of co-expressing these receptors with the various subtypes of G-protein β and γ subunits. GIRK1 channels can form heteromeric complexes with other inward rectifier K^+ channel subtypes (Krapivinsky *et al.*, 1995) and this may also influence the selectivity of G-protein subunits for these channels. Nevertheless this study demonstrates that human NPY_1 and D_2 receptors can modulate GIRK1 K^+ channel activity and that this is likely to be mediated by different pertussis toxin-sensitive G-proteins subunits.

Note added in proof
Since submitting this work it has been discovered that the pcDNA3 αi2 construct did not result in the expression of protein.

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