Neuropeptide Y receptors differentially modulate G-protein-activated inwardly rectifying K^+ channels and high-voltage-activated Ca^{2+} channels in rat thalamic neurons

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(Received 11 May 2000; accepted after revision 4 October 2000)

1. Using whole-cell patch-clamp recordings, infrared videomicroscopy and fast focal solution exchange methods, the actions of neuropeptide Y (NPY) were examined in thalamic slices of postnatal (10–16 days) rats.

2. NPY activated a K^+-selective current in neurons of the thalamic reticular nucleus (RT; 20/29 neurons) and ventral basal complex (VB; 19/25 neurons). The currents in both nuclei had activation and deactivation kinetics that were very similar to those of $GABA_B$ receptor-induced currents, were totally blocked by 0·1 mM Ba^{2+} and showed voltage-dependent relaxation. These properties indicate that the NPY-sensitive K^+ current is mediated by G-protein-activated, inwardly rectifying K^+ (GIRK) channels.

3. In RT neurons, NPY application reversibly reduced high-voltage-activated (HVA) currents to $33 \pm 5\%$ ($n = 40$) of the control level but did not affect the T-type currents. Inhibition of Ca^{2+} currents was voltage independent and was largely mediated by effects on N- and P/Q-type channels.

4. NPY activation of GIRK channels was mediated via NPY_1 receptors, whereas inhibition of N- and P/Q-type Ca^{2+} channels was mediated by NPY_2 receptors.

5. These results show that neuropeptide Y activates K^+ channels and simultaneously inhibits HVA Ca^{2+} channels via different receptor subtypes.

The interaction of G-proteins with ion channels or associated regulatory proteins is well established as a major modulatory mechanism of many voltage-dependent ion channels. Some of the best-studied forms of modulation involve direct interaction between $G\beta\gamma$ and voltage-gated Ca^{2+} channels or G-protein-coupled inwardly rectifying K^+ channels (GIRK channels). Many metabotropic neurotransmitter receptors, including $5\text{-}HT_{1A}$ (Li & Bayliss, 1998), $GABA_B$ (Swartz & Bean, 1992), glutamate mGluR (Swartz & Bean, 1992; Sahara & Westbrook, 1993), α2-adrenergic (Li & Bayliss, 1998) and muscarinic acetylcholine m1, m2 and m4 (Fernandez-Fernandez *et al.* 1999), can be selectively coupled to either GIRK, high-voltage-activated (HVA) Ca^{2+} channels, or both (reviewed by Hille, 1994; Dolphin, 1998; Yamada *et al.* 1998). The physiological consequences of these interactions require further investigation.

Signalling specificity *in vivo* is likely to be complicated because multiple receptor subtypes may be segregated at different cellular locations such as synaptic terminals, soma and dendrites, and coupled to different ion channels. In the experiments reported here and in the accompanying paper (Sun *et al.* 2001), we investigated the pre- and postsynaptic modulatory actions of neuropeptide Y (NPY) in thalamic brain slices. The slice preparation offered a number of technical advantages, including: the preservation of reciprocal intrinsic connections between GABAergic neurons of the nucleus reticularis (RT) and thalamocortical relay neurons in the adjacent ventral basal complex (VB; see Huguenard & Prince, 1994); a dense expression of NPY receptors at developmental ages > postnatal day 5 (P5) in both nuclei (Gehlert *et al.* 1992; Dumont *et al.* 1993); the possibility of recording whole-cell voltage-gated Ca^{2+} current (Huguenard & Prince, 1994; Bayliss *et al.* 1997*a*,*b*; Destexhe *et al.* 1998); and a dense expression of GIRK and N-, P/Q- and T-type Ca^{2+} channels on neurons in these structures (Ponce *et al.* 1996; Murer *et al.* 1997; Talley *et al.* 1999). Our experiments revealed a robust coupling between NPY receptor subtypes and GIRK or Ca^{2+} channels. Results reported here, together with those of the accompanying paper (Sun *et al.* 2001), support the conclusion that there is functional segregation of NPY_1 receptors with GIRK channels at somatic and dendritic locations, and NPY_2 receptors with Ca^{2+} channels at inhibitory GABAergic presynaptic terminals.

METHODS

Preparations

All experiments were carried out using a protocol approved by the Stanford Institutional Animal Care and Use Committee. Young Sprague-Dawley rats (10–16 days old) were deeply anaesthetized with pentobarbital sodium (55 mg kg^{-1}) and decapitated. The brains were quickly removed and placed into cold, oxygenated slicing medium (~4 °C). The slicing medium contained (mM): 2·5 KCl, 1·25 NaH_2PO_4, 10 $MgCl_2$, 0·5 $CaCl_2$, 26 $NaHCO_3$, 11 glucose and 234 sucrose. Tissue slices (300–400 μm) were cut in the horizontal plane using a vibratome (Ted Pella, Inc., St Louis, MO, USA), transferred to a holding chamber and incubated (35 °C) for at least 1 h before recording. Individual slices were then transferred to a recording chamber fixed to a modified microscope stage and allowed to equilibrate for at least 30 min before recording. Slices were minimally submerged and continuously superfused with oxygenated physiological saline at a rate of 4·0 ml min^{-1}. Recordings of GIRK currents were made at a temperature of 35 ± 1 °C, whereas recordings of Ca^{2+} currents were performed at room temperature (23 °C). The baseline physiological perfusion solution contained (mM): 126 NaCl, 2·5 KCl, 1·25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose. These solutions were gassed with 95% O_2–5% CO_2 to a final pH of 7·4.

Whole-cell patch-clamp recording

Whole-cell recordings were obtained using visualized slice patch techniques (Edwards *et al.* 1989; Stuart *et al.* 1993) and a modified microscope (Zeiss Axioskop) with a fixed stage. A low-power objective (× 2·5) was used to identify the various thalamic nuclei, and a high-power water immersion objective (× 40) with Nomarski optics and infrared video was used to visualize individual neurons.

Recording pipettes were fabricated from capillary glass (WPF, M1B150F-4), using a Sutter Instrument P80 puller, and had tip resistances of 2–5 MΩ when filled with the intracellular solutions below. An Axopatch-1A amplifier (Axon Instruments, Foster City, CA, USA) was used for voltage-clamp recordings. Access resistance in whole-cell recordings ranged from 4 to 12 MΩ, and 50–75% of this was electronically compensated. Current and voltage protocols were generated using pCLAMP software (Axon Instruments), and data were stored on the hard disk of an IBM PC-compatible computer, while an optical disk was used for long-term storage of experimental records. The following software packages were used for data analysis: Clampfit, PStat (Axon Instruments), SCAN (courtesy of J. Dempster, Strathclyde, UK), Winplot (courtesy of N. Dale, St Andrews University, UK), Origin (Microcal) and locally written programs, Metatape and Detector (J. R. Huguenard).

For the recording and isolation of GIRK currents, the physiological perfusion solution contained (mM): 98·5 NaCl, 30 KCl, 1·25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose. This solution was gassed with 95% O_2–5% CO_2 to a final pH of 7·4. The pipette saline was modified according to Sodickson & Bean (1996) and was composed of (mM): 100 potassium gluconate, 13 KCl, 9 $MgCl_2$, 0·07 $CaCl_2$, 10 EGTA, 10·0 Hepes, 2 Na_2-ATP and 0·4 Na-GTP. The pH was adjusted to 7·4 and osmolarity to 280 mosmol l^{-1}. For the recording and isolation of Ca^{2+} currents in thalamic slices, the external solution was composed of (mM): 120 NaCl, 20 tetraethylammonium chloride (TEACl), 3 KCl, 2·5 $CaCl_2$, 2 $MgCl_2$, 10 Hepes, 5 CsCl, 1 4-aminopyridine (4-AP); and 1 μM tetrodotoxin (TTX); pH 7·3, osmolarity adjusted to 292 mosmol l^{-1}. This solution was equilibrated with 100% O_2. The pipette solution contained (mM): 117 caesium gluconate, 13 KCl, 1 $MgCl_2$, 0·07 $CaCl_2$, 0·1 EGTA, 10 Hepes, 10 TEACl, 2·0 Na_2-ATP and 0·4 Na-GTP; pH 7·4, osmolarity adjusted to 280 mosmol l^{-1}. The efficacy of voltage clamp and space clamp was verified by studying the kinetics of transient inward T-type Ca^{2+} currents. The criterion for effective voltage- and space clamp was a smoothly activating current, whose *I–V* relationship could be well fitted with the Hodgkin & Huxley model (Dale, 1995*a*, *b*). In the case of T-type currents, the efficacy of voltage control was checked by measuring and fitting instantaneous *I–V* curves, steady-state inactivation and time-dependent recovery from inactivation (Huguenard, 1996, 1998). We obtained relatively reliable voltage-clamp recordings from ~40 RT neurons and ~10 VB neurons, particularly those near the surface of the slice. When the decay phase of the T-type current (elicited at different depolarizing voltages between −70 and −40 mV) was fitted with a single exponential, the time constant of inactivation (τ) of T-type currents in RT and VB neurons was 70 ± 8 ms ($n = 18$, test potential (V_{test}) = −50 mV, holding potential (V_h) = −90 mV) and 26 ± 3 ms ($n = 7$, $P < 0{\cdot}01$), respectively. These values are similar to those of T-type currents recorded in acutely isolated thalamic neurons (see Coulter *et al.* 1989; Huguenard & Prince, 1992; Huguenard, 1998).

Drugs

Drugs were applied focally through a multi-barrel microperfusion pipette that was positioned within 1 mm of the cell. NPY analogues were obtained from Peninsula Laboratories (Belmont, CA, USA). Concentrated NPY stock solutions were dissolved in ultra-pure water to a final concentration of 0·1 M and stored at −70 °C. Stock NPY solutions were diluted in physiological perfusion solution to final concentrations of 100 nM to 1 μM 1 h before use. Concentrated NPY(18–36) and BIBP3226 solutions were also stored at −70 °C, diluted to the final concentration in physiological perfusion solution and applied via multi-barrel focal perfusion. Baclofen was obtained from RBI (Natick, MA, USA), and GTP-γ-S was from Sigma (St Louis, MO, USA). Ion channel blockers ω-agatoxin TK, ω-conotoxin GVIA and ω-conotoxin MVIIC were all obtained from Sigma, CsCl and TTX from Sigma, and TEACl from GFS Chemicals (Columbus, OH, USA).

Statistics

All data are presented as means ± S.E.M. Analysis by Student's *t* test was performed for paired and unpaired observations. *P* values of less than 0·05 were considered statistically significant.

RESULTS

Activation of GIRK currents by NPY

The effects of NPY on K^+ currents were examined in 70 RT and VB neurons (Figs 1 and 4). Voltage-ramp commands (1000 ms), from −150 to +10 mV, delivered to neurons in slices bathed in perfusate containing high-K^+ saline (30 mM) were used to elicit K^+ currents. Inward voltage-gated Na^+ and Ca^{2+} currents and outward Ca^{2+}-activated K^+ currents were eliminated by simultaneous bath perfusion of TTX (1 μM) and Cd^{2+} (200 μM). NPY application resulted in K^+ current activation in both RT (20/29) and VB (19/25) neurons (Figs 1*A* and 4). The reversal potential of NPY-sensitive current was −44 ± 3 mV ($n = 8$), which approximated the potassium equilibrium potential under our recording conditions. The amplitude of NPY-sensitive current at −150 mV was 490 ± 70 pA ($n = 20$), or about 20% of the total current elicited by voltage ramps (2900 ± 160 pA). The washin and washout time courses of NPY-induced current were fitted with single exponential

functions that had time constants of 5·4 ± 2·4 and 8·4 ± 3·6 s, respectively ($n = 10$; e.g. Fig. 1*A*3). These were very similar to the time courses of baclofen-induced current (5·2 ± 2·3 and 6·1 ± 2·5 s, $n = 6$; e.g. Fig. 1*B*3) and about twofold slower than the time course for direct block of GIRK currents by Ba^{2+} in these slices ($n = 6$; not shown). The *I–V* plot of NPY-sensitive current in 20/39 neurons demonstrated strong inward rectification (Fig. 1*A*2). This property was almost identical to that of baclofen-sensitive current in these neurons (Fig. 1*A*2 *vs.* *B*2). In the remaining cells, NPY-sensitive currents showed either less inward rectification or were linear (e.g. Fig. 4*A*). In these neurons, baclofen-sensitive current showed similar voltage dependence (e.g. Fig. 4*A*1, inset). In three cells, we found that the NPY and baclofen effects on K^+ currents were partially occlusive (not shown). The similar kinetic properties of NPY- and baclofen-induced currents suggest that they are produced by a homologous class of channels, probably GIRK channels (see Yamada *et al.* 1998).

The K^+ selectivity of the NPY-sensitive currents was studied by examining their reversal potential and amplitude in perfusate containing different K^+ concentrations ($[K^+]_o$). Reversal potentials examined in 2·5 (−97 ± 3 mV), 10

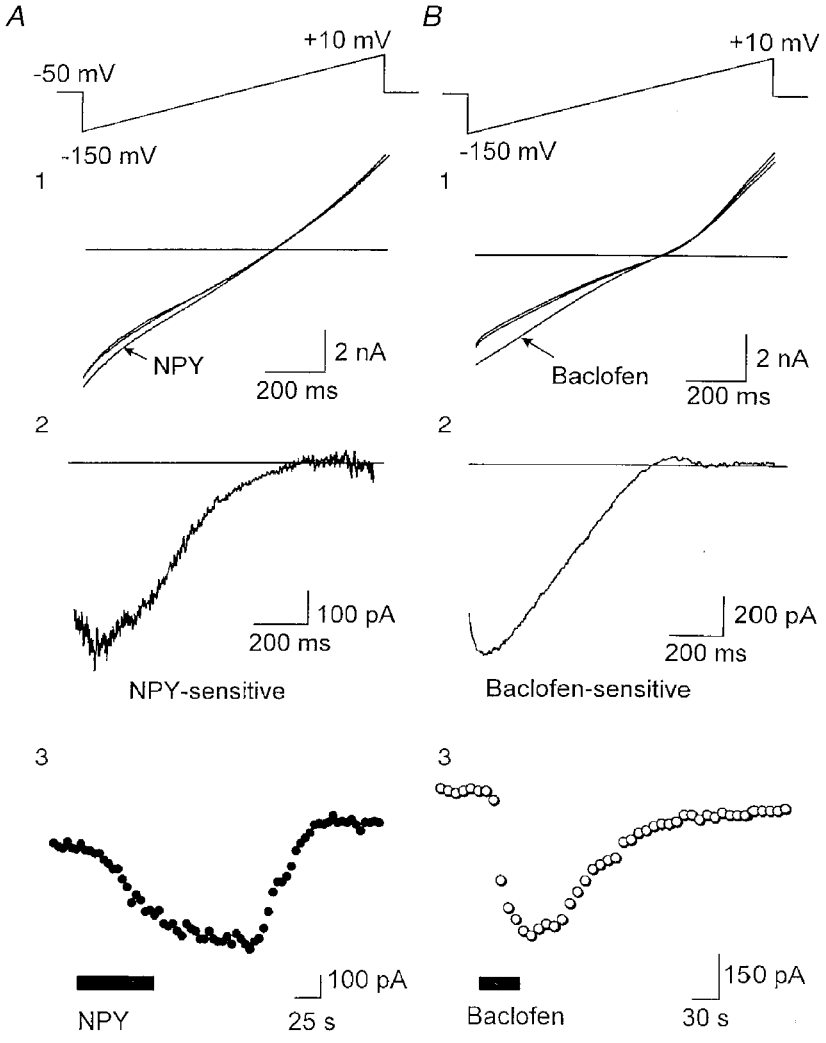

Figure 1. Activation of GIRK currents by NPY and baclofen in thalamic slices from young rats (P15)

A, NPY-activated currents recorded in 30 mM external K^+ in a VB neuron. 1, currents elicited by voltage ramps from −150 to +10 mV in the absence (before and after NPY application) and presence of 500 nM NPY. Each current trace was averaged from 10 consecutive responses. 2, NPY-activated current obtained by subtraction of the traces in NPY from the mean of those obtained before and after NPY application. 3, time-dependent kinetics of activation and deactivation of NPY-sensitive current measured at −120 mV. *B*, baclofen-activated currents recorded in 30 mM external K^+ in a VB neuron. 1, currents elicited by voltage ramps in the absence (before and after baclofen application) and presence of 20 μM baclofen. Each current trace was averaged from 10 consecutive responses. 2, baclofen-activated current obtained as in *A*2. 3, time-dependent kinetics of activation and deactivation of baclofen-sensitive current measured at −120 mV. The horizontal lines in *A*1 and 2, and *B*1 and 2 indicate the zero current level.

(−75 ± 2 mV), 20 (−57 ± 2 mV), 30 (−44 ± 2 mV) and 40 mM $[K^+]_o$ (32 ± 2 mV; $n = 16–25$) agreed very well with those calculated from the theoretical Nernst equation for potassium: $(RT/F)\ln([K^+]_o/[K^+]_i)$, where R is the universal gas constant, T is absolute temperature and F is Faraday's constant. The amplitude of NPY-sensitive current measured at a fixed membrane potential (−120 mV) also increased linearly with $[K^+]_o$ ($n = 16–25$; not shown). Thus the NPY-induced current was highly K^+ selective.

We next tested the effects of intracellular dialysis with the non-hydrolysable GTP analogue GTP-γ-S (1 μM) on the NPY-induced currents. In four cells recorded under these conditions, NPY irreversibly activated a larger current (~400 pA at −110 mV) than that obtained at the same voltage using standard pipette solution (~250 pA, $P < 0.01$; not shown), suggesting G-protein involvement.

Low concentrations of Ba^{2+} can block GIRK channel currents in many CNS neurons (Sodickson & Bean, 1996, 1998; Bayliss *et al.* 1997*a*; Fernandez-Fernandez *et al.* 1999; Takigawa & Alzheimer, 1999; reviewed by Yamada *et al.* 1998). We therefore tested the effects of Ba^{2+} on the NPY-sensitive K^+ current. Ba^{2+} (0·1 mM) totally blocked the NPY-sensitive current (Fig. 2*C* and *D*; $n = 6$) in a voltage-independent manner (Fig. 2*A–C*). In 5/8 neurons, the Ba^{2+}-sensitive currents were virtually identical to the NPY-sensitive current (Fig. 2*A* *vs.* *B*, and *C*), while in 3/8 neurons, they were larger (not shown), perhaps due to some Ba^{2+}-sensitive tonically activated inward rectification conductance (see Fernandez-Fernandez *et al.* 1999; Takigawa & Alzheimer, 1999). Focal addition of 0·1 mM Ba^{2+} alone blocked currents with inward rectification kinetics in 4/6 neurons (~150 pA at −110 mV; not shown). In neurons pre-perfused with solutions containing 0·1 mM Ba^{2+}, NPY had virtually no further effects ($n = 5$; not shown), suggesting that NPY-sensitive currents are completely Ba^{2+} sensitive. The possibility that the effects of 0·1 mM Ba^{2+} might have been due to block of Ca^{2+}-activated K^+ conductances (see Smart, 1987) was eliminated as we used 200 μM Cd^{2+} to block Ca^{2+} influx via voltage-gated Ca^{2+} channels. Thus

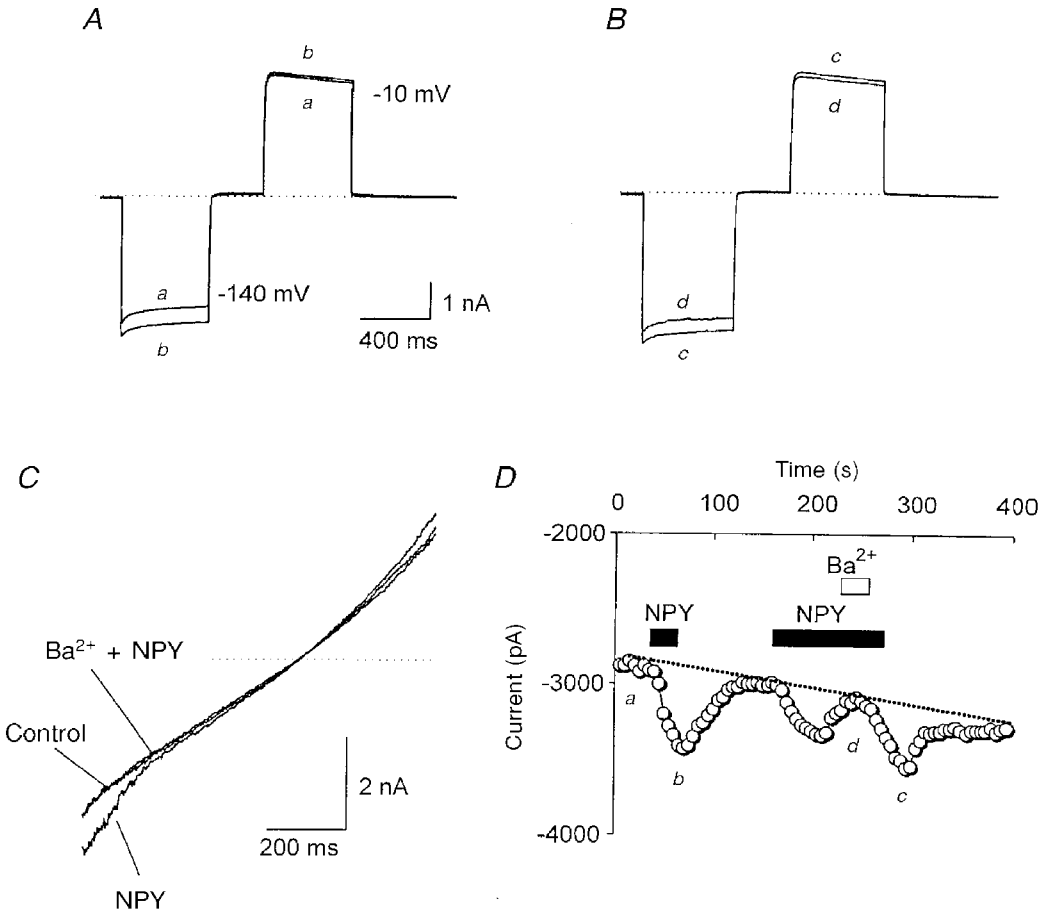

Figure 2. Block of NPY-activated K^+ currents by 0·1 mM Ba^{2+}

A and *B*, currents elicited by steps to −140 and −10 mV from a holding potential of −55 mV in the presence (*Ab* and *Bc*) and absence (*Aa*) of NPY (200 nM), and with both NPY and 0·1 mM Ba^{2+} (*Bd*) in an RT neuron. *C*, voltage-ramp responses in another cell showing Ba^{2+} block of NPY-sensitive current. *D*, time series measurements of currents elicited at −140 mV in the same neuron as in *A* and *B*, showing that 0·1 mM Ba^{2+} blocked NPY-activated K^+ currents. Filled horizontal bars, 200 nM NPY; open bar, 0·1 mM Ba^{2+}. *a–d* correspond to current traces in *A* and *B*. The horizontal dotted lines in *A–C* indicate the zero current level.

GIRK channels appear to be a principal target for modulation by NPY.

Some GIRK channels, particularly those composed of $GIRK_1$ subunits, demonstrate robust time-dependent slow relaxation, whereas in others these properties are much less prominent (Yamada *et al.* 1998). We tested the hypothesis that a particular subtype of GIRK channels is targeted by NPY by using a series of hyperpolarizing steps from −55 to −160 mV and depolarizing steps from −55 to −10 mV to test NPY actions. In six RT and VB neurons, the NPY-sensitive currents consisted of an instantaneous component followed by a time-dependent relaxation (Fig. 3*B* and *D*), similar to acetylcholine-activated K^+ current in cardiac cells (Yamada *et al.* 1998). The time constant of slow relaxation obtained by single exponential fitting (Fig. 3*B*) showed a voltage-dependent change consistent with the kinetics of

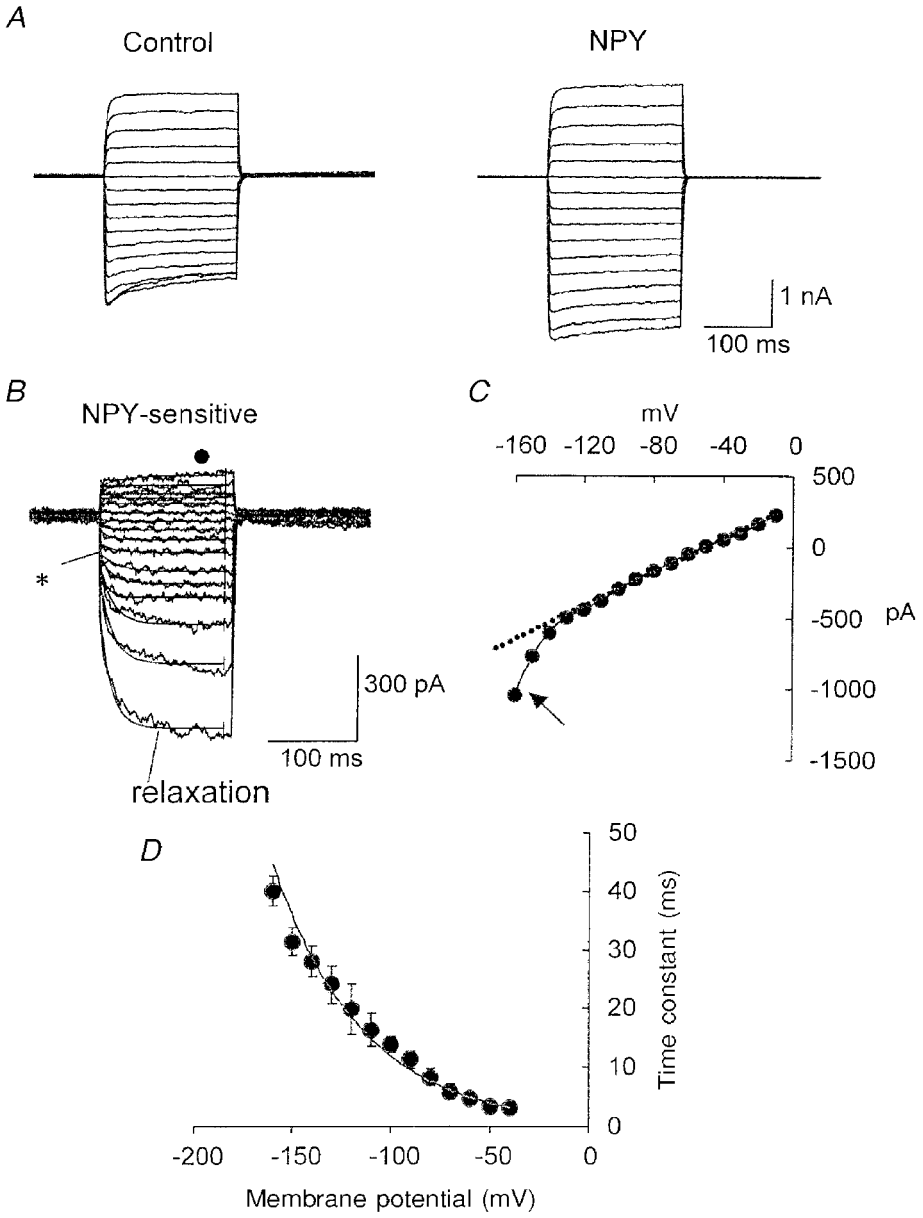

Figure 3. Inward rectification and voltage-dependent relaxation of NPY-activated K^+ currents
A, currents elicited by voltage steps ranging from −160 to 0 mV in the absence (left; Control) and presence (right) of 500 nM NPY in an RT neuron. *B*, NPY-sensitive currents obtained by subtraction of the two sets of currents in *A*. * Instantaneous current jumps were followed by time-dependent slow relaxations, which could be fitted with a single exponential curve (fitting errors become prominent at voltages negative to −120 mV). *C*, steady-state *I–V* relationship of NPY-sensitive current showing that the current flowed more readily in the inward direction (−160 mV, arrow) than in the outward direction (0 mV). The conductance was nearly linear at test voltages between −110 and −50 mV. *D*, voltage-dependent relaxation measured by fitting the decaying phase of the NPY-sensitive currents with a single exponential. The continuous line is the best fit of the single exponential equation: $y = -184 + 157e^{(-V/22)}$.

$GIRK_1$ channels. These findings suggest that NPY-activated GIRK currents are carried via $GIRK_1$-containing channels in these neurons. Other GIRK channels (non-$GIRK_1$-containing channels) could be responsible for the NPY-sensitive currents in the remaining neurons ($n = 4$), where much less relaxation was observed (not shown).

GABAergic RT neurons differ from glutamatergic VB neurons in many of their active and passive membrane properties (Huguenard & McCormick, 1992; McCormick & Huguenard, 1992; Huguenard & Prince, 1994; Huguenard, 1998; Destexhe *et al.* 1998; Huntsman *et al.* 1999). We therefore compared the NPY-sensitive currents in these two nuclei. The amplitude of NPY-sensitive currents was very similar in RT ($n = 11$) *vs.* VB ($n = 11$, $P > 0{\cdot}5$; Fig. 4). Since VB neurons have much larger somatic-dendritic membrane areas than RT neurons (see McCormick & Huguenard, 1992), this suggests that the density of NPY-induced currents may vary in the two nuclei. The density of NPY-induced currents was in fact much larger in neurons from RT than from VB (Fig. 4*B*2, $P < 0{\cdot}01$, $n = 11$ for both RT and VB). $GABA_B$ receptor-mediated slow inhibitory postsynaptic potentials play a role in regulating the firing mode of RT neurons (Ulrich & Huguenard, 1996). To assess whether a similar function could also be subserved by the NPY receptor, we compared the density of NPY- and baclofen-induced currents in these cells, and found that the density of NPY-sensitive currents was ~75% of that of baclofen-sensitive currents (not shown).

Voltage-independent inhibition of N- and P/Q-type, but not T-type, Ca^{2+} channels by NPY in thalamus

Whole-cell Ca^{2+} currents recorded from thalamic neurons *in vitro* contain both T-type and HVA components. These can be distinguished by their voltage dependence of activation and inactivation. T-type currents were elicited at test potentials more positive to −70 mV and were inactivated during a 100 ms test pulse (Fig. 5*A*1), while the HVA currents were evoked at potentials of −40 to +10 mV (Fig. 5*A*). Using a triple pulse protocol from an initial

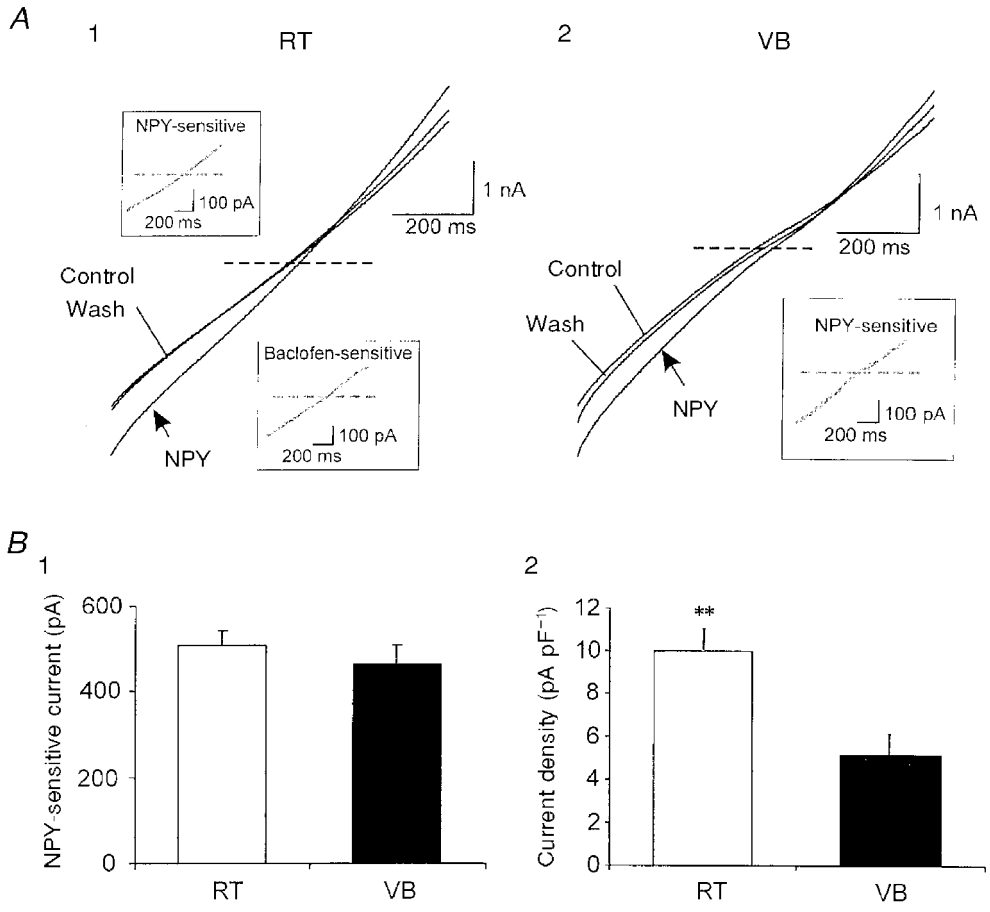

Figure 4. Comparison of NPY-sensitive currents in RT *vs.* VB neurons

A, currents elicited by voltage ramps from −150 to +10 mV in the absence (before (Control) and after (Wash) NPY application) and presence of 500 nM NPY in RT (1) and VB (2) neurons. Each current trace was averaged from 10 consecutive responses. Insets, NPY- or baclofen-sensitive currents, obtained by subtraction. The horizontal dashed lines indicate the zero current level. *B*1, NPY-sensitive current, measured at −140 mV in RT ($n = 11$) and VB ($n = 11$) neurons. 2, density of NPY-sensitive currents in RT ($n = 11$) and VB ($n = 11$) neurons. Current density was normalized to membrane capacitance, which was estimated from compensation of capacity transients immediately after membrane rupture. $^{**}P < 0{\cdot}01$.

holding potential of −90 mV, T-type and HVA currents were elicited separately, and the effects of NPY on each were examined (Fig. 5*B*1). In this experiment, we focused on the modulation of Ca^{2+} currents in RT neurons because these cells have much smaller somatic and dendritic membrane areas, resulting in better voltage control. In 40 RT neurons, NPY reversibly reduced the HVA but not the T-type currents (see e.g. Fig. 5*B*). The mean inhibition of HVA currents by 100 nM to 1 μM NPY was 33 ± 5% (Fig. 8*B*3). The percentage inhibition measured from current amplitudes during activation voltage steps was similar to that measured from tail currents (e.g. Fig. 5*B*1).

The biophysical properties of neuropeptidergic modulation of the HVA currents were examined. The reduction of HVA Ca^{2+} current was not accompanied by the slowing of activation (e.g. Fig. 5*B*1) that usually occurs during direct G-protein modulation (for review see Hille, 1994; Dolphin, 1998), and did not show any sensitivity to membrane potential (Figs 5*A* and 6*A*; see Sun & Dale, 1997). Furthermore, the inhibition of HVA currents by NPY was not even partially relieved by a long duration positive prepulse to +100 mV (Fig. 6*B*; $n = 5$), suggesting that the inhibition of HVA currents by NPY was voltage independent (see Sun & Dale, 1997, 1998; Dolphin, 1998). In control solutions, these prepulses had very little effect on the HVA Ca^{2+} currents ($n = 4$; not shown), indicating that changes in leak induced by such pulses do not alter evoked Ca^{2+} currents.

To further identify the HVA channel types modulated by NPY in RT neurons, we used ω-conotoxin fraction GVIA, a selective N-type Ca^{2+} channel blocker (Feldman *et al.* 1987; Randall & Tsien, 1995), ω-agatoxin TK, a P/Q-type channel blocker (Sun & Dale, 1997), and ω-conotoxin fraction MVIIC, a P/Q-type channel blocker (Randall & Tsien, 1995) (Fig. 7). ω-Conotoxin MVIIC (1 μM) and ω-agatoxin TK (500 nM) blocked the HVA current to a similar extent (by 68 ± 10%, $n = 7$ and 65 ± 18%, $n = 9$, respectively; Fig. 7*B*1 and *C*1) while block by ω-conotoxin GVIA (1 μM)

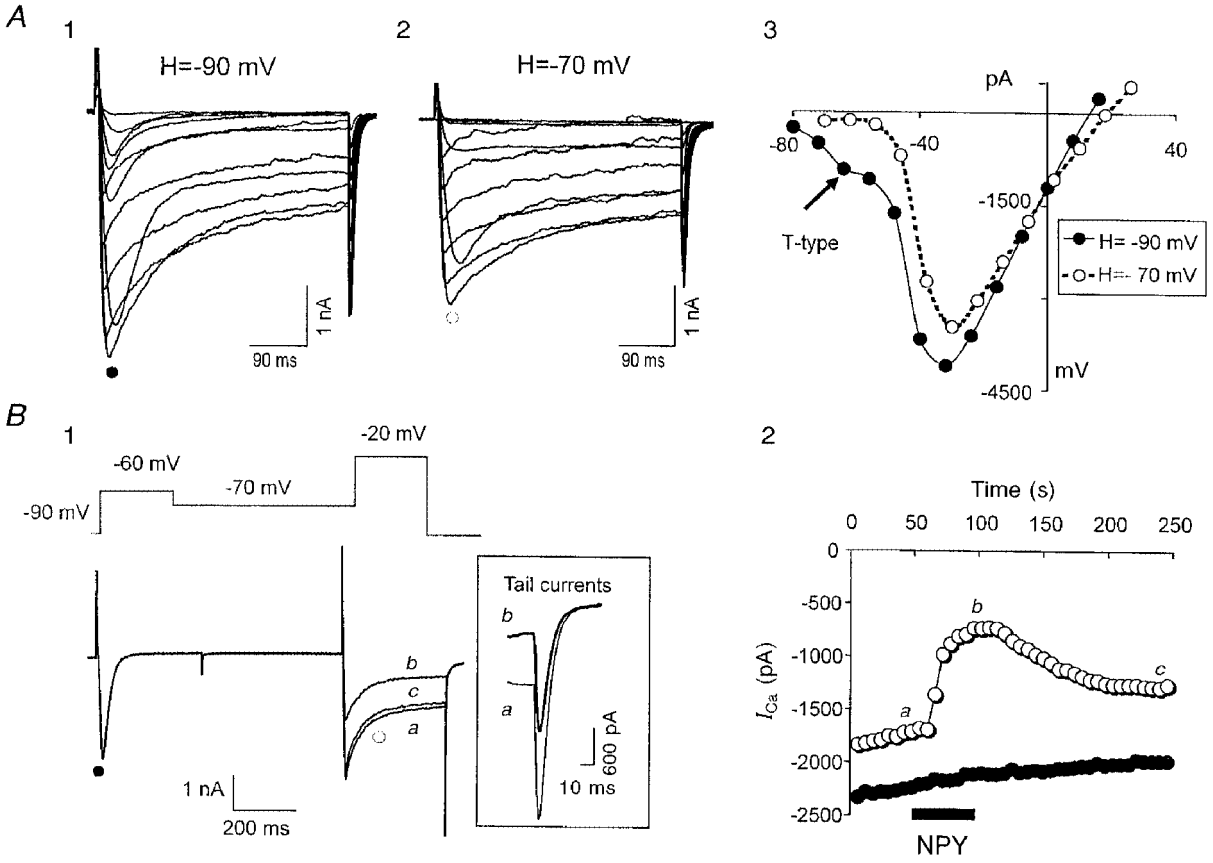

Figure 5. Inhibition by NPY of the high-voltage-activated (HVA) but not the low-voltage-activated (LVA or T-type) Ca^{2+} currents in an RT neuron

A, whole-cell Ca^{2+} currents recorded using steps from a holding potential (H) of −90 (1) or −70 mV (2) to test potentials between −90 (or −70 mV) and +20 mV in an RT neuron. *A*3, *I–V* curves measured from the peak of the Ca^{2+} currents. *B*1, T-type (●) and HVA (○) currents, which were elicited by test potentials of −60 and −20 mV, respectively, from a holding potential of −90 mV. *a*, control; *b*, in the presence of 100 nM NPY; *c*, wash. Only HVA currents were reduced by NPY (*b*). Inset, HVA Ca^{2+} tail currents of traces in control (*a*) and NPY (*b*). *B*2, time series measurements showing that the effects of NPY were specific to HVA currents and were totally reversible in the same neuron (● and ○, and *a–c* correspond to the symbols and traces, respectively, in *B*1).

was less ($48 \pm 4\%$, $n = 7$; Fig. 7*A*1 and *C*1). Thus, in RT neurons, the HVA Ca^{2+} currents were predominantly carried through ω-conotoxin MVIIC- and ω-agatoxin TK-sensitive channels, presumably P/Q-type channels, and to a lesser extent by ω-conotoxin GVIA-sensitive channels, presumably N-type. The effects of peptide Ca^{2+} channel inhibitors ω-agatoxin TK, ω-conotoxin GVIA and ω-conotoxin MVIIC were partially reversible, which is consistent with results obtained in acutely isolated thalamic neurons (Kammermeier & Jones, 1997). Cd^{2+} (200 μM) blocked the rest of the remaining current (Fig. 7*A*1 and *C*1). We did not further characterize channels that were insensitive to ω-toxins.

We next examined possible NPY modulation of specific HVA Ca^{2+} channels. The effects of NPY alone and those in the presence of ω-conotoxin GVIA (1 μM), ω-agatoxin TK (200 nM) and ω-conotoxin MVIIC (1 μM) were studied to determine whether the blocking actions of NPY and each toxin were additive or occlusive. Our results showed that all these toxins occluded ~50% of the NPY effect (see Fig. 7*A*1 for an example of the effect and *C*2 for a summary). These results suggests that P-, Q- and N-type HVA channels are the predominant target of inhibition by NPY.

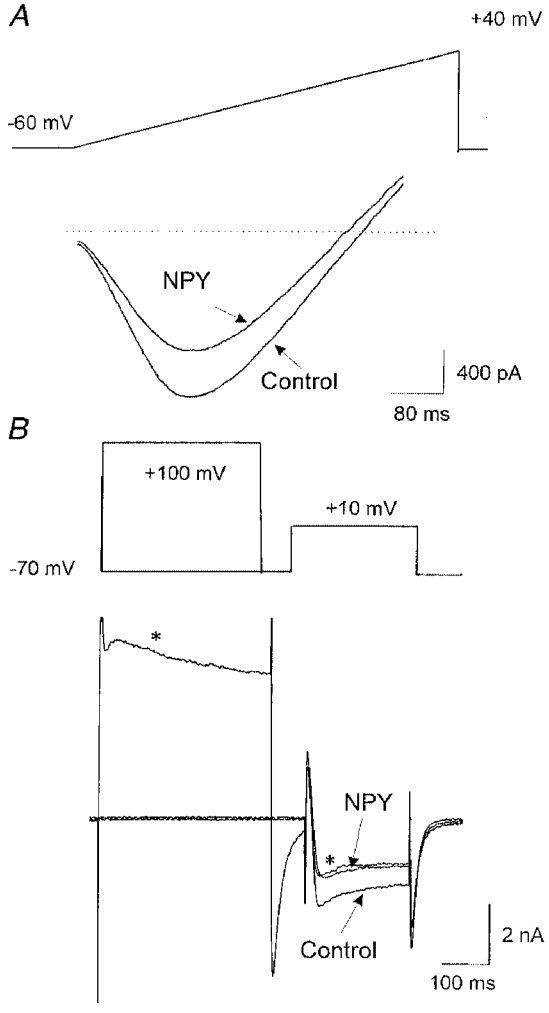

Figure 6. Voltage-independent inhibition of HVA Ca^{2+} currents in an RT neuron from rat (P12) thalamic slice
A, HVA Ca^{2+} currents elicited by 500 ms voltage ramps from −60 to + 40 mV, in the absence and presence of 200 nM NPY. The horizontal dotted line indicates the zero current level. *B*, inhibition of HVA currents in an RT neuron was not changed by applying a prepulse to +100 mV. *Trace elicited by test pulse with +100 mV prepulse in 200 nM NPY.

Selective coupling to GIRK channels and HVA Ca^{2+} channels by separate NPY receptor subtypes

To identify the NPY receptors involved in the modulation of HVA Ca^{2+} currents and activation of GIRK channels, selective agonists and antagonists were applied. The effects of NPY on GIRK currents were fully blocked by the selective NPY_1 receptor antagonist BIBP3226 (Doods *et al.* 1996; Fig. 8*A*1 and *A*3), suggesting that NPY_1 receptors are responsible for the activation of GIRK channels by NPY. However, BIBP3226 did not block the effects of NPY on Ca^{2+} currents (Fig. 8*B*1 and *B*3), suggesting that inhibition of HVA Ca^{2+} currents by NPY was not mediated by NPY_1 receptors. Likewise, the selective NPY_2 receptor agonist NPY(18–36) (Michel, 1991) fully mimicked the actions of NPY on HVA Ca^{2+} currents (Fig. 8*B*2 and *B*3) but had no effect on GIRK channels (Fig. 8*A*2 and *A*3), suggesting that NPY_2 receptors are responsible for the inhibition of HVA Ca^{2+} currents but are not involved in the activation of GIRK channels. These results suggest that NPY_1 and NPY_2 receptors are selectively coupled to GIRK channels and HVA Ca^{2+} channels, respectively.

DISCUSSION

Very little is known about the mechanisms underlying the selective coupling between a single neurotransmitter and different ion channels. Two possible explanations for this selectivity have been proposed: (1) receptor and ion channel colocalization and compartmentation, and (2) different levels of receptor expression, for example, a higher density of $5\text{-}HT_{1A}$ receptors in raphe neurons could allow 5-HT to modulate both GIRK and Ca^{2+} channels (Li & Bayliss, 1998). However, the latter explanation (2) cannot explain why activation of a receptor (e.g. $5\text{-}HT_{1A}$, $GABA_B$) will only modulate one of the several Ca^{2+} channels (e.g. N, P, Q, R), even though all are susceptible to modulation by neurotransmitters (see Lambert & Wilson, 1996; Sun & Dale, 1998; reviewed by Hille, 1994; Dolphin, 1998).

Alternative approaches have been used recently to study the effects of neurotransmitter receptor activation on heterologously expressed GIRK channels. In rat sympathetic neurons (Ruiz-Velasco & Ikeda, 1998), GIRK channel overexpression decreased basal Ca^{2+} channel facilitation and attenuated noradrenergic inhibition of Ca^{2+} channels. This might be due to competition of binding with G-proteins between two effectors: Ca^{2+} channels and GIRK channels. This explanation appears to contradict results indicating selective coupling in native neurons (see Li & Bayliss, 1998). Interestingly, another recent study, also using heterologously overexpressed GIRK channels in rat sympathetic neurons,

has shown that endogenous m2 and m4 receptors can selectively couple to GIRK and Ca^{2+} channels, respectively, with negligible cross-talk (Fernandez-Fernandez *et al.* 1999). The authors proposed that there might be a selective compartmentation of receptor and ion channels, so that the effects of m4 on Ca^{2+} channels and m2 on GIRK channels might result from a relatively tightly coupled receptor–G-protein–channel complex. This hypothesis has received some experimental support (see Neubig, 1994; Stanley & Mirotznik, 1997).

NPY can activate a family of homologous receptors (Y_1–Y_6) that are coupled to pertussis toxin (PTX)-sensitive G-proteins (Vezzani *et al.* 1999; Sun & Miller, 1999). Most of these receptors, including Y_1, Y_2 and Y_4, have the ability to regulate K^+ and Ca^{2+} channels expressed in *Xenopus* oocytes and in human embryonic kidney (HEK293) cells (Sun *et al.* 1998). Similarly, in acutely isolated rat arcuate nucleus neurons, NPY_1–Y_5 receptors can regulate both K^+ and Ca^{2+} channels simultaneously in the same cell (Sun & Miller, 1999). This does not seem to be the case in the

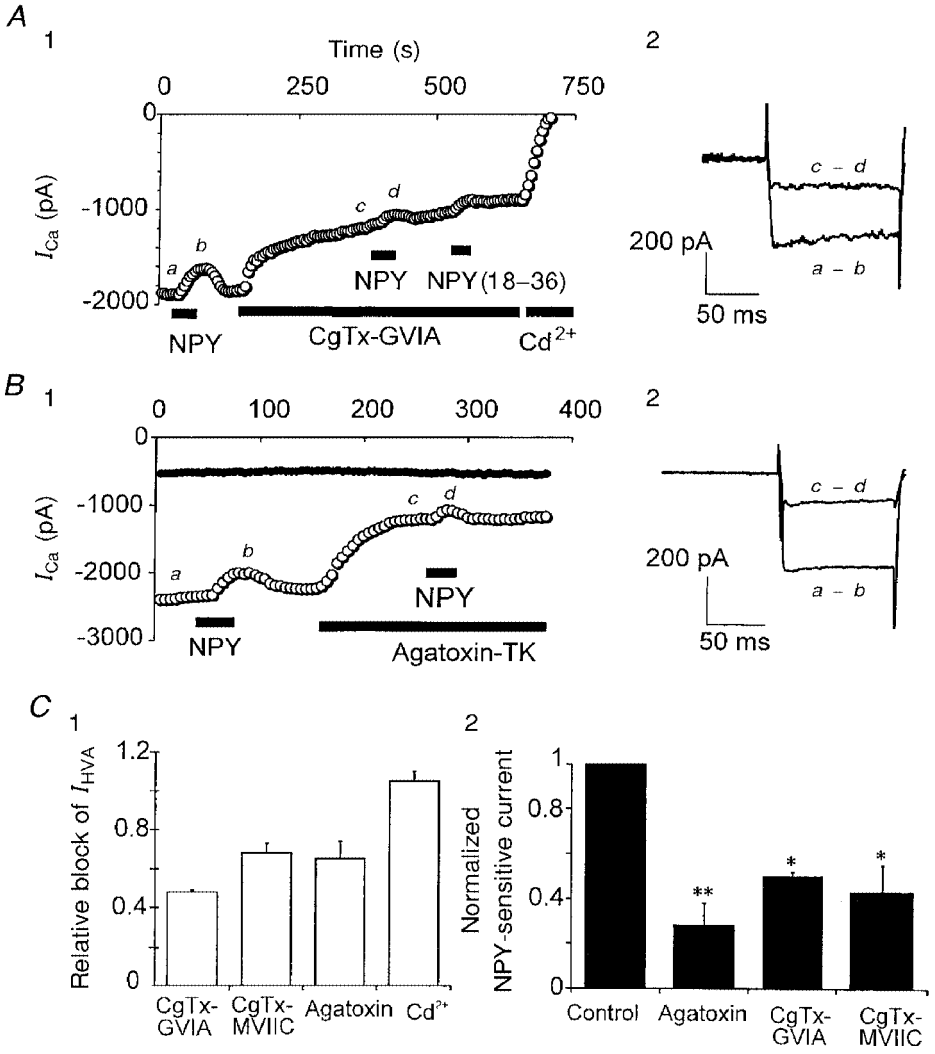

Figure 7. N-, P- and Q-type HVA Ca^{2+} channels are the target of inhibition by NPY

*A*1, time series measurements showing the effects of Ca^{2+} channel blockers ω-conotoxin GVIA (CgTx-GVIA; 1 μM) and Cd^{2+} (200 μM) on the HVA currents. The recording also shows that the inhibition of HVA currents by NPY (200 nM) and the responses to the specific Y_2 receptor agonist NPY(18–36) (500 nM) were partially occluded by ω-conotoxin GVIA (1 μM). *A*2, NPY-sensitive difference currents corresponding to measurements in *A*1. *B*1, time series showing the effects of Ca^{2+} channel blockers ω-agatoxin TK (Agatoxin-TK; 500 nM) on the HVA (○) and LVA (●) currents. The recording also shows that the inhibition of HVA currents by NPY (200 nM) was partially occluded by ω-agatoxin TK. *B*2, NPY-sensitive difference currents corresponding to measurements in *B*1. *C*1, summary of the effects of Ca^{2+} channel blockers on the HVA currents in RT neurons. Bars represent normalized mean fractional block by each toxin ($n = 7$–9). CgTx-MVIIC, ω-conotoxin MVIIC (1 μM). *C*2, summary of normalized NPY-sensitive current in the absence (Control) and presence of Ca^{2+} channel blockers. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$ *vs.* control ($n = 7$–9).

thalamus, where NPY_1 and NPY_2 receptors are differentially coupled to GIRK channels and Ca^{2+} channels, respectively. Our results support the 'compartmentation' hypothesis that suggests that receptor, ion channels, and G-proteins can be 'tied' together in certain forms of G-protein–channel complex.

We have demonstrated that NPY_1 receptor activation produces robust activation of GIRK currents in both RT and VB neurons. The inward rectification properties of GIRK channels were probably due to Mg^{2+} and polyamine block at negatively charged residues of $GIRK_1$ channels (see Yamada *et al.* 1998). Whereas homomultimeric $GIRK_1$ channels demonstrated strong inward rectification, other homomultimeric GIRK channel subtypes (such as $GIRK_2$ and $GIRK_4$) showed less inward rectification (see Yamada *et al.* 1998). In CNS neurons, multiple GIRK channel subunits usually form heteromultimeric channels and demonstrate a variety of differences in their voltage dependence of rectification. For example, in hippocampal neurons, $GABA_B$,

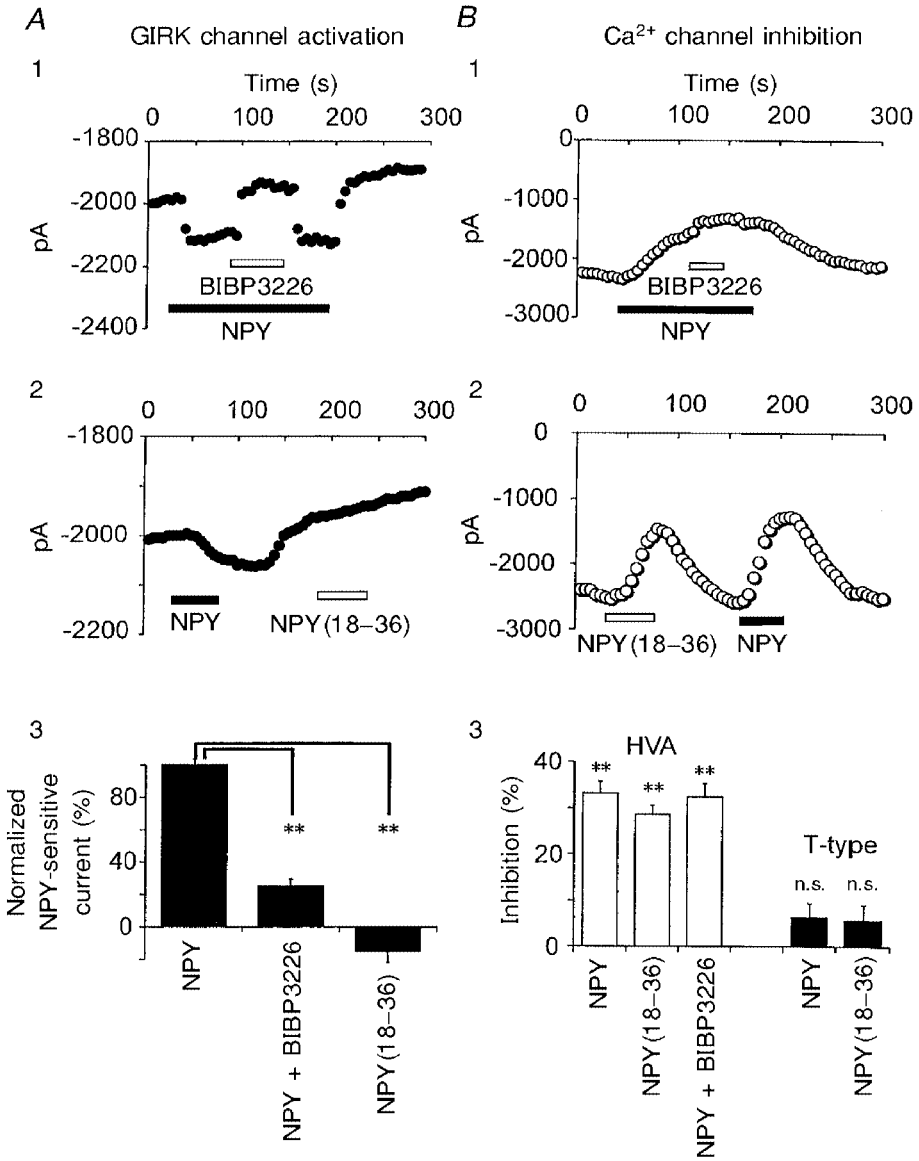

Figure 8. Pharmacological characterization of neuropeptidergic modulation of HVA Ca^{2+} currents and GIRK currents in RT neurons

*A*1, the activation of GIRK currents by 200 nM NPY was blocked by the NPY_1 receptor antagonist BIBP3226 (500 nM). *A*2, 200 nM NPY activated GIRK currents but 500 nM of the NPY_2 receptor agonist NPY(18–36) did not. *A*3, summary showing that activation of GIRK currents by NPY was blocked by BIBP3226 and was not mimicked by NPY(18–36). $^{**}P < 0{\cdot}01$, $n = 8$. *B*1, inhibition of HVA Ca^{2+} currents by NPY was not blocked by BIBP3226. *B*2, inhibition of HVA Ca^{2+} currents by NPY was mimicked by the NPY_2 agonist NPY(18–36). *B*3, summary showing the inhibition of HVA currents by NPY and NPY(18–36). $^{**}P < 0{\cdot}01$ *vs.* control (no NPY; $n = 21$). BIBP3226 did not block the effects of NPY. $^{**}P < 0{\cdot}01$ *vs.* control, $P > 0{\cdot}1$ *vs.* NPY alone ($n = 5$). Neither NPY nor NPY(18–36) inhibited T-type currents. $P > 0{\cdot}1$ *vs.* control ($n = 6$).

5-HT, and somatostatin receptor-mediated GIRK currents showed very little rectification across a wide range of voltages (see Sodickson & Bean, 1996, 1998; Lüscher *et al.* 1997). $GIRK_{1-4}$ channels are densely expressed in most rat thalamic nuclei. Therefore the discrepancies in the voltage dependence of NPY- and baclofen-induced currents (Figs 1*A* *vs.* 4*A*1 and *A*2; and 3) suggest that these different channels may form different types of functional heteromultimeric channels. In addition, if GIRK channels and neurotransmitters also interact at less electrically accessible dendrites (see Takigawa & Alzheimer, 1999), the inward rectification properties of these interactions may not be fully reflected in the somatic recordings.

In contrast to the voltage-gated K^+ channels, the open probability of GIRK channels is greatly increased by neurotransmitter binding to their cognate G-protein-coupled receptors. This offers a great advantage by allowing significant modulation of membrane conductance. In particular, GIRK channels could be preferentially located at dendrites (Drake *et al.* 1997; Takigawa & Alzheimer, 1999; Q.-Q. Sun, J. R. Huguenard & D. A. Prince, unpublished observations) where they might mediate dendritic hyperpolarization. We therefore propose that activation of these channels by neurotransmitter could significantly alter the excitability of postsynaptic neurons, place these neurons in a different firing mode, and thus could be critical in the fine tuning of thalamocortical functions.

In this study of thalamic neurons, we found that NPY_2, but not NPY_1, receptor activation inhibits N- and P/Q-type, but not T-type, channels via voltage-independent mechanisms in RT neurons. In previous studies, NPY_{1-5} receptors have all been found to regulate Ca^{2+} channel activity. NPY_1, NPY_2 and NPY_4 receptor activation can inhibit N-type and R-type Ca^{2+} channels expressed in HEK293 cells and *Xenopus* oocytes (Sun *et al.* 1998). NPY_{1-5} receptors also similarly inhibit HVA Ca^{2+} currents in rat arcuate nucleus (Sun & Miller, 1999). The actions of NPY in these reports appear to be at least partially voltage dependent. In rat nodose neurons, NPY_1 and NPY_2 receptors have opposite actions on Ca^{2+} currents (Wiley *et al.* 1993). However, only NPY_2 receptors have been found to inhibit Ca^{2+} channels and synaptic transmission in hippocampal CA1 neurons (Qian *et al.* 1997). In contrast, inhibition of N-type Ca^{2+} channels in rat dentate granule cells is mainly by NPY_1 receptors (McQuiston *et al.* 1996). Thus it appears that both Y_1 and Y_2 receptors regulate N- and P/Q-type Ca^{2+} channels through various mechanisms. This might occur at the G-protein level, e.g. NPY_2 receptors simultaneously activate both PTX-sensitive and -insensitive G-proteins in the same cell (see Lynch *et al.* 1994), or downstream from G-protein activation, e.g. NPY receptors activate cGMP-dependent protein kinase (see Lemos *et al.* 1997) or protein kinase A (PKA) pathways (Bryant & Hart, 1996). In cells that express native GIRK channels, only PTX-sensitive G-proteins have been reported (see Yamada *et al.* 1998); however, voltage-independent inhibition of HVA channels may be induced by both PTX-sensitive and -insensitive G-proteins (see Shapiro & Hille, 1993; Diversé-Pierluissi *et al.* 1995; Sun & Dale, 1999; reviewed by Dolphin, 1998). Thus the differential effect of NPY_1 and NPY_2 receptors on GIRK and Ca^{2+} channels, respectively, could also be explained by a differential mechanism of action following receptor activation rather than (or besides) a functional segregation of those receptors.

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Acknowledgements

This work was supported by NIH grant NS12151 from the National Institute of Neurological Disorders and Stroke, and the Pimley Research and Training Funds.

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