Neuropeptide Y Modulates Neurotransmitter Release and Ca\(^{2+}\) Currents in Rat Sensory Neurons

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Using \(^{125}\text{I}\)-labeled neuropeptide Y (NPY) and peptide YY (PYY), we demonstrated the existence of specific receptors for these peptides on rat dorsal root ganglion (DRG) cells grown in primary culture. Scatchard analysis of membrane homogenates indicated that the peptides bound to 2 populations of sites, with approximate affinities of 0.08 and 6.5 nm. Only low levels of binding were detected on sympathetic neurons cultured from the same animals or on a variety of neuronal clonal cell lines. The binding of \(^{125}\text{I}\)-NPY and \(^{36}\text{I}\)-PYY to DRG cell membranes was considerably reduced by the nonhydrolyzable analog of GTP, Gpp(NH)p. The major effect of Gpp(NH)p was to reduce the number of low-affinity NPY binding sites without altering the number of high-affinity binding sites. NPY potently inhibited Ca\(^{2+}\) currents recorded under voltage clamp in rat DRG cells. Both the transient and sustained portions of the Ca\(^{2+}\) current were inhibited. The inhibitory effects of NPY were completely blocked following treatment of the cells with pertussis toxin. Depolarization elicited a large influx of Ca\(^{2+}\) into DRG neurons as assessed using fura-2-based microspectrofluorimetry. This influx of Ca\(^{2+}\) could be partially inhibited by NPY. Furthermore, NPY effectively inhibited the depolarization-induced release of substance P from DRG cells in vitro. Thus, NPY may be an important regulator of sensory neuron function in vivo.

Neuropeptide Y (NPY) is a 36 amino acid peptide neurotransmitter (Tatemoto, 1982a) that is very widely distributed in both the central and peripheral nervous systems (Allen et al., 1983; Chronwall et al., 1985). In many instances, NPY is found to be colocalized with other neurotransmitters such as norepinephrine (Chronwall et al., 1984; Ekblad et al., 1984; Everitt et al., 1984; Jarvi et al., 1986; Wang et al., 1987). NPY is only one member of a family of peptides that also includes peptide YY (PYY) and the pancreatic polypeptides (PP), as well as several others (Tatemoto, 1982b; Tatemoto et al., 1982). NPY and PYY show considerable sequence homologies (Tatemoto et al., 1982). Furthermore, their pharmacological effects are also similar, suggesting that they may act at the same receptors (Lundberg and Tatemoto, 1982; Edvinsson, 1985; Saria and Beubler, 1985; Morley et al., 1985; Friel et al., 1986; Serfogzo et al., 1986; Zukowska-Grojec et al., 1986). PYY is found most in the glomerulosa containing endocrine cells of the gastrointestinal muscosa and may have endocrine or paracrine functions (Botcher et al., 1984). Circulating PYY has been detected, and its concentration changes under various dietary conditions (Pappas et al., 1986). In addition, however, PYY has also been detected in a unique population of neurons in the CNS (Ekman et al., 1986). These neurons are distinct from those that contain NPY and are less widely distributed.

Not only is NPY a very widely distributed neuropeptide, but it can be shown to produce a very large number of biological effects. Following its injection into the brain, for example, NPY produces many behavioral sequelae. One of the most striking of these is an enormous stimulation of feeding (Morley et al., 1985; Stanley et al., 1986). NPY also acts upon many peripheral systems, including a variety of smooth muscle preparations (Wahlestedt et al., 1985; Wahlestedt and Hakanson, 1986; Zukowska-Grojec et al., 1986), the heart (Lundberg et al., 1984), and intestinal epithelia (Friel et al., 1986; Hubel and Renquist, 1986). Interestingly, at several neuroeffector junctions, NPY appears to have both pre- and postsynaptic effects (Wahlestedt et al., 1985, 1986). Although PYY has been shown to inhibit adenylate cyclase in a number of cases, relatively little is known about the molecular basis of its action (Kassis et al., 1987; Westlund-Danielsson et al., 1987). Some studies have identified high-affinity binding sites for NPY in the nervous system (Unden and Bartfai, 1984; Chang et al., 1986; Harffstrand et al., 1986; Martel et al., 1986). However, nothing is known about the cellular transduction mechanisms involved in the production of NPY's neuronal effects. NPY receptors and immunoreactivity have been previously localized in the dorsal horn of the spinal cord (Hökfelt et al., 1981; Allen et al., 1983; Chronwall et al., 1985; Martel et al., 1986). It seemed, therefore, that NPY might act as a modulator of sensory neurotransmission. In the present study, we demonstrate that sensory neurons in vitro possess high concentrations of specific NPY receptors and that activation of such receptors leads to the inhibition of voltage-sensitive Ca\(^{2+}\) currents and of neurotransmitter release from these cells.

Materials and Methods

Preparation of \(^{125}\text{I}\)-NPY and \(^{125}\text{I}\)-PYY

The peptides were mono-iodinated using the chloramine T method. The reaction mixture contained 0.25 M phosphate buffer, 2.8 nM peptide, 0.5 nM \(^{125}\text{I}\) and 36 nM chloramine T. The reaction was stopped after 1 min with 525 nM sodium metabisulfite and transferred to a test tube containing 1.0 ml Sephadex G-25 equilibrated with the following buffer: 0.08 M Trizma HCl, 0.08 M NaCl, 0.02 M HCl, and 0.02% BSA, pH 8.6.
The iodinated peptide was extracted from the Sephadex in batch rinses, which were then pooled and purified on an Altex C18 ultrasphere ion-pairing column (5 μm particle size, 0.46 × 25 cm). The mobile phase consisted of acetonitrile and an aqueous buffer: 0.10 M phosphoric acid, 0.02 M triethylamine, 0.05 M NaClO4, all brought to pH 3.0 with NaOH. Several mono-iodinated peaks eluted at 34% (vol/vol) ACN and 0.02% sodium azide. The peak that offered maximal synthetic yield and specific binding to rat brain synaptosomes was selected as the choice peak and used in all future binding experiments.

**Cell culture**

Dorsal root ganglion (DRG) cells were cultured from neonatal rats as previously described (Perney et al., 1986).

**Binding studies**

**DRG cells.** Cells were grown for 2 weeks on 35 mm plates. The culture media was aspirated and replaced with binding buffer containing 137 mM NaCl, 5.4 mM KCl, 0.44 mM KH2PO4, 1.26 mM CaCl2, 0.81 mM MgSO4, 20 mM HEPES, 0.36% BSA, 1 mM EDTA, 0.1% bacitracin, 100 mg/liter streptomycin sulfate, 1 mg/liter aprotinin, 10 mg/liter trypsin inhibitor, and 1 μM captopril, pH 7.4. Enough buffer was added so that the total volume to 1 ml. Cold peptide was added to dilute the specific activity of the label at higher concentrations. The maximal concentration of label was 100,000 cpm/ml = 0.05 nm. The binding was initiated by adding iodinated label to the plates, which were then incubated with gentle shaking for 1 hr at 25°C (equilibrium conditions). The binding buffer was aspirated and the reaction terminated. The plates were quickly rinsed with 1 ml ice-cold binding buffer, aspirated, and layered with a final milliliter of buffer. The cells were scraped from the plate with a rubber policeman, transferred to a test tube, and counted for 125I in a gamma counter.

**DRG membranes.** Cells were grown for 2 weeks on 35 mm plates. The medium was aspirated and replaced with 0.5 ml of ice-cold binding buffer. The cells were scraped from the plate with a rubber policeman. The suspensions from each plate were then pooled and homogenized with a loose Teflon pestle (10-12 strokes). The resultant homogenate was stored on ice until the incubation began.

The incubation microfuge tubes were filled to a volume of 500 μl with buffer, label, and cold peptide. To initiate the reaction, 500 μl of the tissue homogenate was added to each tube, followed by shaking at 25°C for 1 hr (equilibrium conditions). The binding buffer was aspirated and the reaction terminated. The plates were quickly rinsed with 1 ml ice-cold binding buffer, aspirated, and layered with a final milliliter of buffer. The cells were scraped from the plate with a rubber policeman, transferred to a test tube, and counted for 125I in a gamma counter.

Protein assays were performed using the Biorad method (Bradford reagent).

**Binding data analysis**

In all binding experiments, the total bound ligand was analyzed with a nonlinear least squares curve fitting program (Yamaoka et al., 1981), which interpreted the binding as having both saturable (specific) and nonsaturable (nonspecific) components according to adsorption isotherm models.

**Microspectrofluorimetry**

Measurements of [Ca2+]i in single DRG cells were carried out using the dye fura-2 as previously described (Thayer et al., 1986).

**Substance P release**

The evoked release of substance P from DRG cells was measured by radioimmunoassay as previously described (Perney et al., 1986).

**Electrophysiology**

DRG neurons cultured from 1-d-old rat pups were voltage-clamped in the whole-cell patch-clamp configuration (Hamill et al., 1981; Marty and Neher, 1983). Ionic conditions optimized isolation of Ca2+ currents: K+ channel blockers tetrathylammonium (TEA+) and cesium (Cs+) replaced, respectively, Na+ on the outside and K+ on the inside of the neurons, so Na+ was present, and pulses were to the Cl- equilibrium potential. External solutions contained (in mM) 10 CaCl2, 140 TEACl, 1 MgCl2, and 10 glucose. Internal solutions contained (in mM) 10 EGTA, 140 CsCl, 1 MgCl2, and an ATP regenerating system (14 creatine phosphate, 50 U/ml creatine phosphokinase) to retard run-down of Ca2+ currents (Forscher and Oxford, 1985). Both solutions were buffered to pH 7.4 with 10 mM HEPES.

Voltage-clamp pulses (350 msec) to 0 mV were given alternately from −40 and −80 mV at 10 sec intervals. The sustained Ca2+ current ("L" type) was elicited from the −40 mV holding potential and the transient Ca2+ currents ("N" and "T" types) (Nowycky et al., 1985) were additionally elicited from the −80 mV holding potential.

Inhibition of sustained and transient Ca2+ currents by various concentrations of NPY was calculated as follows. Total and sustained Ca2+ currents were plotted as a function of time and the pre-exposure time course was extrapolated as a single exponential to estimate the expected magnitudes of sustained and transient (total minus sustained) currents at the end of exposure. The percentage reduction of actual sustained and transient currents compared with these expected magnitudes was then calculated.

The patch-clamp amplifier was the Yale Mark V with 1 GΩ headstage. An Indec 1123 computer system was used to produce voltage-clamp pulses and record data. Calcium currents were filtered at 500 Hz and digitally sampled at 400 μsec intervals. Leakage and capacitative currents were subtracted from current records by modeling the sum of the appropriate number (8 or 4) of currents produced by 10 mV hyperpolarizing pulses from holding potential and subtracting the modeled current from the raw current traces. NPY was applied by bath superfusion from a polyethylene tube positioned approximately 2 mm from the voltage-clamped neurons.

**Statistics**

Significance levels were determined using the 2 tailed Student's t test.

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**Table 1. Binding parameters for DRG cells**

| System          | n  | Bmax(nM) | Kd(nM) | Bmax(μM) | Kd(μM) |
|-----------------|----|----------|--------|----------|--------|
| 125I-NPY        |    |          |        |          |        |
| Intact cells    | 3  | 307 ± 19 | 0.62 ± 0.15 | -        | -      |
| Membranes       | 3  | 26 ± 10  | 0.08 ± 0.03 | 971 ± 143 | 6.5 ± 4.0 |
| Membranes + Gpp(NH)p | 3 | 15 ± 10  | 0.12 ± 0.05 | 228 ± 130 | 1.5 ± 0.6 |
| 125I-PYY        |    |          |        |          |        |
| Intact cells    | 1  | 220      | 0.43   | -        | -      |
| Membranes       | 1  | 20       | 0.03   | 2170     | 1.2    |

Bmax is reported as fmol/mg. Kd is reported as μM. Absence of a detectable binding site is indicated (−). [Gpp(NH)p] = 0.1 mM. Values are reported as means ± SE.
Materials

NPY, PYY, substance P, and Gpp(NH)p were purchased from Sigma (St. Louis). Pertussis toxin was purchased from List Biological Laboratories, Inc. (Campbell, CA). ¹²⁵I was purchased from Amersham (Arlington Heights, IL). All other chemicals were reagent grade.

Results

Both ¹²⁵I-NPY and ¹²³I-PYY exhibited a high degree of specific binding to intact cultured rat DRG cells (Table 1). Binding curves obtained using intact cells indicated the presence of a single site with a $K_d$ of 0.62 ± 0.15 nM and a $B_{max}$ of 307 ± 19 fmoI/mg protein for ¹²⁵I-NPY (Fig. 1, Table 1). A 2-site model did not yield a significantly better fit of the data. Considering the relatively large amount of nonspecific binding inherent in whole-cell binding studies, it is possible that a second site was obscured. However, a 1-site model appears to be the most parsimonious interpretation of the data. The characteristics of the binding curves changed when membranes isolated from DRG cells were used. Scatchard plots became clearly curvilinear, indicating the presence of more than one kind of binding site (Fig. 2, Table 1). Thus, for both ligands binding studies on isolated membranes revealed the presence of 2 binding sites. For example, studies with ¹²⁵I-NPY indicated the presence of sites with $K_d$'s of 0.08 ± 0.03 and 6.5 ± 4.0 nM.

When DRG cell membranes were treated with Gpp(NH)p, a nonhydrolyzable analog of GTP, the specific binding of ¹²⁵I-NPY and ¹²³I-PYY decreased considerably (Figs. 3, 4). Concentrations of Gpp(NH)p as low as 10⁻⁶ M were effective in this respect. The decrease in binding associated with the effects of Gpp(NH)p appeared to be due to the disappearance of many of the lower-affinity binding sites, so that the binding more closely resembled the whole-cell profile. It is not clear what the basis of this decline in the $B_{max}$ of the lower-affinity sites might be. One possibility is that the sites are transformed into a further form with such a low affinity that it is difficult to detect owing to limitations of the binding assay (i.e., relatively high nonspecific binding in the low-affinity concentration range).

In contrast to DRG neurons, only low levels of binding sites were observed on neurons cultured from the superior cervical ganglia of neonatal rats, on normal or differentiated PC12 cells, or on NG108-15 or F-11 clonal neuronal cell lines (Table 2).

We next investigated the functional consequences of activation of the NPY receptors located on DRG neurons. When DRG cells were depolarized by raising [K⁺], from 5 to 50 mM, the release of substance P into the bathing medium was stimulated (Perney et al., 1986). This release could be significantly inhibited by 10⁻⁷ M NPY (Fig. 5). It has been previously demonstrated that several other neurotransmitters can also block the evoked release of substance P from DRG cells, an effect that is associated with the inhibition of DRG Ca²⁺ currents (Mudge et al., 1979; Dunlap and Fischbach, 1981). We therefore examined the effects of NPY on DRG Ca²⁺ currents recorded using the whole-cell patch-clamp method. When cells were held at hyperpolarized potentials (−80 mV), steps to 0 mV evoked a large Ca²⁺ current that exhibited both transient and sustained components. This pattern is similar to that previously observed in chick DRG cells (Nowycky et al., 1985). When cells were held at a relatively depolarized potential (−40 mV), steps to 0 mV evoked Ca²⁺ currents which exhibited little inactivation. It has been shown previously using chick DRG cells that the overall form of the DRG Ca²⁺ current is due to the activation of 3 separate types of voltage-sensitive Ca²⁺ channels (Nowycky et al., 1985). The sustained portion of the current is primarily due to the activation of L channels, whereas the transient portion of the current is due to the activation of N and T channels. These latter channel types are mostly inactivated when cells are held at −40 mV.

10⁻⁷ M NPY dramatically reduced the magnitude of the DRG Ca²⁺ current. Both phases of the Ca²⁺ current appeared to be reduced. Figure 6 illustrates the effects of NPY on the peak Ca²⁺ current evoked from −80 mV (transient plus sustained) and on the sustained current evoked from −40 mV. Inhibitory effects were observed at NPY concentrations as low as 10⁻¹⁰ M and half-maximal effects at about 10⁻⁹ M (Fig. 7). The Ca²⁺ current recovered following washout of the peptide. The effects produced by NPY were extremely reliable. Thus, the peptide inhibited the Ca²⁺ current in every cell tested.

The binding studies discussed above suggest that NPY receptors in DRG cells are associated with G-proteins. We therefore examined the effects of NPY following treatment of DRG cells...
20

NPY (nM)

0

1000

1200

200 400 600 800

NPY Bound (fmol/mg)

A.

125I-NPY Bound (fmol/mg)

B.

NPY Bound (fmol/mg)

NPY (nM)

0

10

20

30

40

0.0

0.5

1.0

150

0

50

100

150

Figure 2. Equilibrium binding of 125I-NPY to DRG membranes. Also see Table 1). A representative experiment is shown (n = 3). A, Direct plot. Solid line with data points represents measured total binding; dotted lines represent saturable and nonsaturable components as obtained by nonlinear least-squares computer analysis. B, Scatchard plot of saturable binding component in A. All points represent triplicate determinations.

with pertussis toxin (Fig. 8). Following such treatment the inhibitory effects of 10^{-7} M NPY were no longer apparent. Pertussis toxin blocked the effects of NPY on all phases of the Ca^{2+} current.

We next examined the effects of NPY on the voltage-dependent uptake of Ca^{2+} into single DRG neurons using fura-2 based microspectrofluorimetry. The resting [Ca^{2+}], in DRG neurons was approximately 10^{-7} M. When cells were depolarized by raising [K^+], from 5 to 50 mM, [Ca^{2+}] rapidly increased. Treatment of cells with 10^{-7} M NPY partially inhibited this depolarization-induced rise in [Ca^{2+}]. (Fig. 9). The inhibitory effects of NPY were no longer apparent if cultures were treated with pertussis toxin (n = 2).

Discussion

Receptors for NPY and PYY are extremely widely distributed both in the CNS and in the periphery (Chang et al., 1986; Harfstrand et al., 1986; Martel et al., 1986). In studies to be reported elsewhere we have found that both 125I-NPY and 125I-PYY exhibit curvilinear Scatchard plots and and appear to label 2
distinct binding sites in rat brain synaptosomes (Walker and Miller, 1987), a similar situation to that found in DRG membranes. Furthermore, biochemical studies indicate that both ligands can interact with both binding sites. It seems, therefore, that receptors for NPY and PYY may actually be the same molecule(s), biological specificity being derived from the presence or absence of the particular peptide agonist.

One area in which both NPY receptors and immunoreactivity exist in high concentrations is in the dorsal horn of the spinal cord (Hökfelt et al., 1981; Allen et al., 1983; Chronwall et al., 1985). In the present study we have demonstrated that receptors exist on sensory neurons in vitro. It is likely, therefore, that much of the binding normally observed in the substantia gelatinosa is on the terminals of incoming sensory neurons. Such a result is consistent with the idea that one normal function of NPY might be to modulate sensory information flowing into the CNS. In support of this proposal, we have also demonstrated that NPY can reduce the depolarization-evoked release of substance P from DRG cells in vitro. Substance P is usually associated with small unmyelinated polymodal nociceptors (Aronin et al., 1983). Thus, NPY may be involved in the normal control
inhibition of Ca^{2+} currents by 10^{-9} M NPY. A representative experiment is shown (n = 5). DRG neurons were voltage-clamped in the whole-cell mode under ionic conditions optimal for isolation of Ca^{2+} currents (see Materials and Methods). Voltage-clamp pulses (350 msec) to 0 mV were alternately given at 10 sec intervals from holding potentials of -40 mV to evoke the sustained Ca^{2+} current (L type) and from -80 mV to evoke the transient Ca^{2+} currents (N and T types) also. The effect of a 3 min exposure to 10^{-9} M NPY (indicated by the horizontal bar above the time scale) is plotted as a function of time for total (squares) and sustained Ca^{2+} currents (diamonds). Traces at the top compare the sustained (left) and total (right) Ca^{2+} currents at the end of exposure to NPY with currents just prior to exposure. Scale bars for current traces are 250 pA and 50 msec. [See Ewald et al. (1988) for another example.]

A question of considerable interest is how these receptors function at a molecular level and, in particular, how their activation produces inhibition of substance P release. There are several ways in which this could be achieved. For example, a change in K^{+} permeability (Kandel and Schwartz, 1982) or in intracellular Ca^{2+} buffering (Boyle et al., 1984) could be involved. However, the most direct method would be by inhibition of the voltage-dependent influx of Ca^{2+} into the nerve terminal. It appears that such a mechanism is widely used in both vertebrate and invertebrate neurons (Miller, 1987). In vertebrate neurons, this phenomenon has, in fact, been most frequently studied in DRG cells (Dunlap and Fischbach, 1981; Werz and MacDonald, 1984; Dolphin et al., 1986). All the different neurotransmitters discussed above that block substance P release also suppress Ca^{2+} currents in DRG cell bodies. If such a mechanism also occurs in the nerve terminal, then it is presumably the basis for the inhibition of substance P release. It should be noted that not all agents that block substance P release from DRG neurons do so by blocking Ca^{2+} currents. Thus, opioids that act on \mu- or \delta-receptors, for example, appear to act by enhancing one or more DRG K^{+} conductances (Miller, 1984).

Table 2. \(^{125}\)I-NPY binding to membranes from various cell types

| Cell type                          | \(^{125}\)I-NPY bound (fmol/mg) |
|-----------------------------------|---------------------------------|
| Rat dorsal root ganglia           | 10.95 ± 2.98                    |
| Rat sympathetic ganglia           | 4.45 ± 1.35                     |
| HSWP fibroblasts                  | 0.23 ± 0.15                     |
| PC12 (rat pheochromocytoma), differentiated with dibutyryl cAMP and nerve growth factor | 0.21 ± 0.26 |
| F-11 (rat neuroblastoma \times DRG), differentiated with dibutyryl cAMP and nerve growth factor | 0.23 ± 0.13 |

Cell membranes were incubated with 20 pm \(^{125}\)I-NPY for 1 hr at 25°C. Specific binding was determined in the presence of 500 nm unlabeled NPY. In the case of DRG, specific binding was determined from equilibrium curves derived from nonlinear least-squares analysis. All points are reported as means ± SE (n = 3).
Figure 9. Inhibition of depolarization-induced peak increase in \([Ca^{2+}]_c\) in single DRG neurons by \((10^{-10} \text{ m NPY})\) (P < 0.025). Cells were depolarized by raising \([K^+]_o\) from 5 to 50 mM \((n = 8)\) (Thayer et al., 1987).

Binding studies with DRG membranes revealed the presence of 2 classes of NPY binding sites, as also found in the brain. The significance of this observation in physiological terms is difficult to assess. A reasonable argument can be made, however, that the effects of NPY on DRG Ca\(^{2+}\) currents are mediated by the lower-affinity sites. First, the EC\(_{50}\) for the effect of NPY on DRG Ca\(^{2+}\) currents is similar to the \(K_a\) of the low-affinity site or, indeed, to the \(K_a\) for the single site observed in whole-cell binding studies (approximately 1 nM). Second, in membrane preparations the properties of the low-affinity site are modulated by Gpp(NH)p, indicating that it is linked to a G-protein. The observation that pertussis toxin blocks the effects of NPY on the DRG Ca\(^{2+}\) current further indicates the involvement of a G-protein. With respect to the binding studies using DRG membranes, it may be considered strange that the major effect of Gpp(NH)p is on the \(B_{max}\), rather than the \(K_a\) of the low-affinity site. However, there are several precedents for such an observation, including studies on the labeling of NPY receptors reported by other authors (Unden and Bartfai, 1984; Chang et al., 1985; Nakajima et al., 1986). The fate of these sites is not clear. It is possible that they represent an inositol lipid-linked form of the receptor that can be shed from the membrane under the influence of a Gpp(NH)p activated enzyme such as phospholipase C (Hemperly et al., 1986). Indeed, there are now several examples of proteins that are anchored in the membrane by being covalently linked to inositol phospholipids. More likely, however, is a shift in the state of the receptor to one of very low affinity. Indeed, other studies we have conducted using brain membranes have demonstrated that the effects of Gpp(NH)p are reversible. However, the major point to be made is that DRG cells possess large numbers of NPY binding sites. Some of these appear to be linked to G-proteins. Furthermore, the affinity of such sites is consistent with the effects of NPY on DRG Ca\(^{2+}\) currents.

NPY is a very potent inhibitor of DRG Ca\(^{2+}\) currents and of the depolarization-induced entry of Ca\(^{2+}\) into DRG cells as assessed by fura-2-based microspectrofluorimetry. As with other reported inhibitory modulators of DRG Ca\(^{2+}\) currents, its effects are reversed upon treating cells with pertussis toxin (Holz et al., 1986). This indicates the involvement of a G-protein of the \(G_i\) or \(G_o\) type. NPY appears to inhibit both the sustained and transient portions of the DRG Ca\(^{2+}\) current. By analogy with other results on chick DRG cells, it may be supposed that the sustained portion of the rat DRG current is due primarily to the activation of L channels (Nowycky et al., 1985). This would be reasonable, as we have previously shown that the evoked release of substance P from rat DRG cells is dependent primarily on the activation of L channels. Thus, if NPY can inhibit substance P release, then presumably it should be able to inhibit L currents. However, although such a series of observations would be internally consistent, it cannot be assumed a priori that the various phases of the rat DRG Ca\(^{2+}\) current are due to the activation of channels with exactly the same properties as those underlying the chick DRG Ca\(^{2+}\) current. Indeed, it is quite clear that the inactivation kinetics of N-type currents do differ considerably in different types of neurons and possibly even in DRG cells from different species (Bean, 1987; Hirning et al., 1987). Thus, the ultimate proof that any particular type of Ca\(^{2+}\) channel is being modulated must await studies of this phenomenon at the single-channel level. Until that time, however, the above description of events seems the most reasonable and is at least consistent with other data in the literature.

The molecular mechanisms whereby NPY (or other modulators) block DRG Ca\(^{2+}\) currents are not known with any certainty. Some evidence suggests a role for the enzyme protein kinase C (Ranc and Dunlap, 1986; Ewald et al., 1988). In the present case, it is noteworthy that the effects of NPY on all phases of the Ca\(^{2+}\) current can be reversed by pertussis toxin. If, indeed, the various phases of the current are due to the activation of different populations of Ca\(^{2+}\) channels, this suggests that G-proteins of the \(G_i\) or \(G_o\) type must be involved in the NPY-induced modulation of all these various channel types.

NPY has been shown to modulate the release of neurotransmitters from other neurons as well, including some sympathetic neurons and hippocampal pyramidal cells (Lundberg and Stjarne, 1984; Colmers et al., 1987). It will be interesting to see if these cases also involve the modulation of neuronal Ca\(^{2+}\) currents.

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