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Effects of neuropeptide Y on L-type calcium current in guinea-pig ventricular myocytes

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1 Neuropeptide Y (NPY) reduces cell shortening at high concentrations in guinea-pig ventricular myocytes. We have studied the effects of the peptide on calcium current in cardiac myocytes.

2 We have recorded L-type calcium current in guinea-pig ventricular myocytes under conditions in which the effects of other overlapping currents have been minimised by using Na^+-free, K^+-free external solution and patch-clamp electrodes containing Cs^+.

3 Peak inward calcium current is reduced by NPY at concentrations in excess of 1 nM, and maximal inhibition (31%) was found at and above concentrations of 100 nM. The IC_{50} value for NPY inhibition of peak calcium current was 1.72 nM.

4 NPY had no effect on the voltage-dependence of calcium current amplitude, on the time course of current inactivation, or on the voltage-dependence of the steady-state gating variables.

5 NPY did not reduce the calcium current in the presence of 8-Br-cyclic AMP, and it was also without effect when GTP-γ-S or GDP-β-S were included in the patch pipette.

6 We conclude that in guinea-pig ventricular myocytes NPY acts at low concentration to reduce L-type calcium current, via a G-protein-mediated pathway and reduction in intracellular cyclic AMP.

Keywords: Neuropeptide Y; calcium current; guinea-pig; ventricular myocytes; patch-clamp

Introduction

Neuropeptide Y (NPY) is a 36-amino acid residue peptide (Tatemoto, 1982) which is distributed widely throughout the mammalian nervous system, and is found in abundance within the heart and the neurones associated with the coronary vasculature (Dalsgaard *et al.*, 1986). The peptide is localised both in sympathetic neurones (where it is co-localised with noradrenaline) (Dalsgaard *et al.*, 1986) and in non-adrenergic neurones (Hassall & Burnstock, 1984). It has a variety of actions on the cardiovascular system (Potter, 1988; Dumont *et al.*, 1992) which include peripheral vasoconstriction (Zukowska-Grojec *et al.*, 1987) and myocyte hypertrophy (Millar *et al.*, 1991). NPY has been shown to reduce coronary blood flow and myocardial contractility in isolated perfused hearts from rabbit (Allen *et al.*, 1983), guinea-pig (Franco-Cereceda *et al.*, 1985) and rat (Balasubramaniam *et al.*, 1988). Both positive and negative inotropic actions of NPY have been demonstrated in isolated cardiac tissue and in single cardiac myocytes (McDermott *et al.*, 1993). We have previously demonstrated that neuropeptide Y reduces unloaded cell shortening in single guinea-pig ventricular myocytes (Bryant *et al.*, 1991), an effect which was both dose-dependent and sensitive to pertussis toxin (PTX).

The location of presynaptic and postsynaptic NPY receptors suggest that *in vivo* NPY may have a variety of actions. NPY acts presynaptically on cardiac autonomic nerve terminals to modulate the release of acetylcholine and noradrenaline (Franco-Cereceda *et al.*, 1985; Potter, 1988). The release of NPY from sympathetic nerve terminals is dependent on frequency and duration of stimulation (Warner & Levy, 1989a), and attenuates the vagal effects in atrial myocardium (Warner & Levy, 1989b), hence, the presynaptic effects of NPY are dependent upon both sympathetic and parasympathetic tone.

The NPY receptor has been classified into various subtypes depending on the affinity of particular peptide fragments at the NPY binding site. Y_1 and Y_2 subtypes bind both NPY and peptide YY (PYY) with equal affinity (Michel, 1991). Y_1 receptors selectively bind the NPY analogue leu[31], pro[34] NPY (Wagner *et al.*, 1989) and the Y_2 receptor selectively binds NPY_{13-36} (Michel, 1991). In rat cardiac membranes, however, PYY shows a markedly lower affinity than NPY at the receptor, and on this basis a novel Y_3 subtype has been proposed (Balasubramaniam *et al.*, 1990; Michel, 1991). Recent studies have suggested that the NPY receptor on cardiac membranes may not be homogeneous (Sheriff and Balasubramaniam, 1992), with a high affinity binding site mediating the inhibitory effect of NPY_{17-36} via a PTX-sensitive G-protein and a low affinity binding site which mediates the stimulant effect of NPY_{17-36} via a cholera toxin-sensitive G-protein.

Neuropeptide Y may alter contraction in the heart through several mechanisms, among which are changes in L-type calcium current (McDermott *et al.*, 1993). In neuronal tissue the action of NPY on both N-type and L-type calcium channels is inhibitory (Valentijn *et al.*, 1994; Ewald *et al.*, 1988). We have demonstrated inhibition of L-type calcium current in guinea-pig myocytes using the switch-clamp technique, but only a single high concentration was used (Bryant *et al.*, 1991). This inhibition was sensitive to prior incubation of the myocytes with pertussis toxin. Other workers have found that calcium current is increased by NPY in rat ventricular myocytes, an effect which was not blocked by the peptide fragment antagonist NPY_{18-36} and which was insensitive to pertussis toxin (Millar *et al.*, 1991). Weak stimulation of the calcium current by NPY has been shown in rabbit atrial cells (Dinanian *et al.*, 1992). The stimulatory effect of isoprenaline on the calcium current appears to be reduced by NPY in myocytes from both guinea-pig and rat, which is consistent with involvement of inhibitory G-proteins in at least part of the effects of the peptide on cardiac myocytes (Kassis, *et al.*, 1987; Piper *et al.*, 1989; Millar *et al.*, 1991; Bryant *et al.*, 1991).

Although these different effects of NPY on calcium current may be attributable to the different species used and to different experimental conditions, such results call for further experiments to characterize the actions of the peptide in more detail. We have set out to investigate the effects of low con-

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centrations of NPY on L-type calcium current in guinea-pig ventricular myocytes, by use of the patch-clamp technique. In order to isolate the calcium current from other, overlapping membrane currents we have chosen experimental conditions in which most other membrane currents were either blocked or absent. The results show an inhibitory action of the peptide on calcium current which appears to be mediated via G-proteins and inhibition of adenylate cyclase. Preliminary results have been published in abstract from (Bryant & Hart, 1994).

Methods

Cell isolation

Single left ventricular myocytes were isolated by an enzymatic dispersion technique (Ryder *et al.*, 1993). Female Dunkin-Hartley guinea-pigs weighing 300–600 g were given 500 iu heparin intraperitoneally before being killed by cervical dislocation. The heart was quickly removed and washed in warmed Tyrode solution (for composition see below), supplemented with $CaCl_2$ (750 μM). The aorta was cannulated and connected to a Langendorff apparatus. The heart was perfused for 5 min with nominally Ca^{2+}-free Tyrode solution containing EGTA, 0.1 mM, at 37°C. The solution was then switched to the same, supplemented with collagenase (0.5 mg ml^{-1}, collagenase type I, Worthington Biochemical Corporation, New Jersey, U.S.A.), protease (0.1 mg ml^{-1}, Pronase E, Sigma Chemical Company, Poole) and $CaCl_2$ (50 μM), for a period of 10 min. The left ventricle was dissected from the heart, chopped and placed into fresh enzyme solution (without protease). The cells were harvested at 5 and 10 min intervals after gentle mechanical agitation, washed twice in Tyrode solution supplemented with bovine serum albumin (5 mg ml^{-1}) and centrifuged at 400 r.p.m. for 1 min, before being re-suspended in Dulbecco's modified Eagle's medium (Gibco BRL, Paisley) and stored at room temperature. Cells were used within 12 h of isolation.

Whole-cell voltage-clamp

For electrophysiological recordings the cells were layered onto the floor of a perfusion chamber which was situated on the stage of an inverted microscope (Nikon, Japan). The cells were superfused with a Tyrode solution (see below) at a flow rate of 1–2 ml min^{-1}. All experiments were performed at 35±1°C. Calcium current was recorded in whole-cell configuration by use of the Axopatch-1C amplifier (Axon Instruments, Foster City, CA, U.S.A.). Micro-pipettes were fabricated out of borosilicate glass capillaries (GC200TF-15, Clark Electromedical Instruments, Pangbourne) and pulled on a horizontal electrode puller (BH-CC-PC, Mecanex SA, Switzerland). Electrode resistance was 2–4 MΩ measured with standard filling solution (for composition, see below). Gigaohm seals were formed in normal Tyrode solution. After rupture of the cell membrane the superfusate was switched to a Na^+-free external solution and the cell was allowed to equilibrate for a minimum of 10 min before membrane current was recorded. Calcium current was elicited from a holding potential of −45 mV, by a 200 ms (or 500 ms) step depolarisation to 0 mV. Data were recorded on magnetic tape for subsequent off-line analysis (RACAL, store 7DS, Southampton). The signals were played back through a six-pole Bessel filter and sampled at 1 kHz by a 12-bit analogue-to-digital converter (CED1401, Cambridge). Digitised records were analysed by the VCLAMP software package (CED, Cambridge). To minimize the effects of run-down of the calcium current which may result in over-estimation of the inhibitory effect of NPY a rigid protocol was observed, whereby the amplitude of the current after perfusion with the peptide for two minutes was compared with the control amplitude just before solution change. Myocytes were exposed to only one concentration of NPY except when the lowest concentration of the peptide was used when a maximal concentration of the peptide was subsequently applied as a control.

Solutions

The solution used for cell isolation consisted of (mM): NaCl 130, KCl 5.4, $MgCl_2$ 3.5, $NaHPO_4$ 0.4, glucose 10, HEPES 5. The pH was adjusted to 7.3 at 37°C with NaOH. The solution used for superfusion consisted of (mM): NaCl 134, KCl 5.4, $MgSO_4$ 1.2, glucose 11.1, HEPES 5, $CaCl_2$ 1.8. The pH was adjusted to 7.4 with NaOH. The electrode filling solution consisted of (mM): $CsCl_2$ 120, $MgCl_2$ 1, Mg-ATP 4, EGTA 10, HEPES 5, and the pH was adjusted to 7.2 with CsOH. Overlap from other membrane currents was minimised by using the following external solution for current recording (mM): tetraethylammonium chloride 140, $MgCl_2$ 1, $CsCl_2$ 10, glucose 10, HEPES 10, $CaCl_2$ 1.8, and the pH was adjusted to 7.4 with TEA-OH. K^+ currents were therefore eliminated by replacing internal and external K^+ with Cs^+ and by the use of external TEA^+. Intracellular EGTA was used to buffer the calcium transient, to block Ca^{2+}-activated currents and in conjunction with absence of external Na^+, to reduce Na-Ca exchange current.

Drugs

Neuropeptide Y (human, Novabiochem) was made freshly for each experiment as an aqueous stock solution at a concentration of 0.1 mM, and was added to the perfusate in appropriate dilution. 8-Bromoadenosine 3′:5′-cyclic monophosphate (8-Br-cyclic AMP, 10 mM; Sigma Chemical Corporation, Poole) was added to the perfusate when indicated. Guanosine-5′-o-thiotriphosphate (GTP-γ-S), 1 mM (Boehringer Mannheim U.K., Lewes) and guanosine-5-o-thiodiphosphate (GDP-β-S), 10 mM (Boehringer Mannheim U.K., Lewes) were added to the pipette filling solution when required.

Statistics

Data are expressed as mean±s.e. and statistical significance has been assessed at the 95% limit by Student's paired and unpaired *t* tests.

Results

Effects of NPY on whole-cell calcium current

Peak L-type calcium current is reduced by application of NPY. Figure 1a shows the time course of the effect of 100 nM NPY on peak calcium current in a representative recording. Recovery of current amplitude is observed in this cell on wash off of the peptide. Figure 1b shows L-type calcium current following a step depolarisation from −45 mV to 0 mV. In this representative experiment application of 100 nM NPY reduced peak inward calcium current by 30%. In a total of 9 cells, calcium current measured at 0 mV decreased from −1.4±0.2 nA to −0.99±0.17 nA following application of 100 nM NPY ($P<0.001$). When the current records before and after NPY were normalised with respect to peak inward current they superimpose (Figure 1b, bottom panel), illustrating that the peptide does not affect the time course of calcium-independent inactivation of the current. The time course of inactivation was fitted to the sum of two exponential functions and the mean time constants are as follows: fast component, control, 8.8±1.3 ms, NPY, 7.8±0.9 ms ($n=9$, $P=0.22$, paired *t* test); slow component, control, 60.2±6.1 ms, NPY, 59.3±4.9 ms ($n=9$, $P=0.87$, paired *t* test).

Examples of current records elicited by depolarisation to different potentials from a holding potential of −45 mV are shown in Figure 2a. NPY (100 nM) reduced the peak current at

all potentials at which net current was inward. The voltage-dependence of the calcium current was not altered by the peptide (Figure 2b).

The effects of 100 nM NPY on the steady-state activation and inactivation variables for the calcium current were investigated. Activation (d_∞) curves were constructed by plotting relative chord conductance as a function of voltage. Steady-state inactivation was measured as the ratio of calcium current at the test potential (0 mV) in the absence of a prepulse to the current recorded following a 200 ms prepulse to each conditioning potential. Figure 3 shows that there was no significant shift in the voltage-dependence of either gating variable following exposure to 100 nM NPY. Half-activation potentials were -17.0 ± 1.2 mV (control), -16.0 ± 1.9 mV (NPY, $n=11$, $P=0.3$, paired t test). Half-inactivation potentials were -20.5 ± 0.8 mV (control), -19.4 ± 0.7 mV (NPY, $n=9$, $P=0.06$, paired t test). The slopes of the Boltzmann relationships were not significantly different before and after exposure to NPY.

Dose-response relationship

The presence of 'run-down' under patch conditions (Kameyama *et al.*, 1988) is likely to result in an over-estimation of the inhibitory effects of the peptide, if exposure to multiple concentrations were to be made in each cell. Therefore, we exposed each cell to a single concentration of the peptide. The lowest concentration of the peptide used 0.1 nM reduced I_{Ca} by $2.8\pm2.7\%$ ($n=5$), in these experiments a maximal concentration of NPY (100 mM) subsequently applied as a test agonist, reduced I_{Ca} by $30\pm4\%$ ($n=5$). The dose-response curve (Figure 4) is therefore a composite of the effects of NPY on a total of 38 cells. Reduction in calcium current amplitude at 0 mV was found at all concentrations greater than 1 nM. The effect was maximal at concentrations in excess of 1 μM, and the mean reduction in calcium current at this concentration was $31\pm4\%$ ($n=9$). From these data the IC_{50} value for NPY on calcium current amplitude was 1.72 nM.

Effects of NPY in the presence of exogenous cyclic AMP stimulation

Cyclic AMP-dependent phosphorylation has been shown to regulate L-type calcium current in ventricular myocytes (Trautwein and Hescheler, 1990). In order to explore the possibility that NPY acts to reduce calcium current via a reduction in cyclic AMP, cells were superfused with the membrane-permeable cyclic AMP analogue 8-bromoadenosine 3':5'-cyclic monophosphate (8-Br-cyclic AMP) (Beebe *et al.*, 1988). Addition of 8-Br-cyclic AMP to single myocytes in Na^+-free solution caused a sustained increase in baseline calcium current of $128\pm13\%$ ($n=6$, $P=0.05$). In Figure 5a peak amplitude of calcium current at 0 mV is plotted with respect to time for a representative cell; application of 8-Br-cyclic AMP caused a sustained increase in the amplitude of the current. In the presence of 8-Br-cyclic AMP, subsequent addition of NPY has little effect on calcium current amplitude in this cell. Two minutes after myocytes were exposed to 100 nM NPY in the presence of 8-Br-cyclic AMP the mean change in peak calcium current amplitude was $4\pm7\%$ ($n=6$). In a control series of experiments NPY applied to a cohort of cells not exposed to 8-

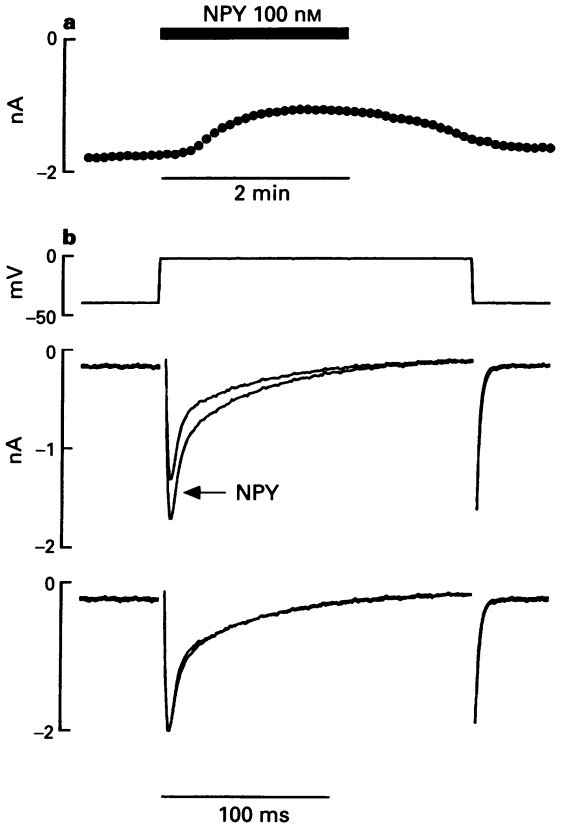

Figure 1 Effects of 100 nM neuropeptide Y (NPY) on L-type calcium current in a guinea-pig ventricular myocyte. (a) Time course of the effect of NPY (100 nM) on the peak L-type calcium current of a representative cell. NPY superfusion for 2 min (denoted by the solid bar) reduced peak current amplitude. Recovery of the current amplitude was observed in this cell when the peptide was washed off. (b) Top panel, membrane voltage, (holding potential, −45 mV; test potential, 0 mV); centre panel, membrane currents before and after exposure to NPY. Bottom panel, the current records from the centre panel have been normalised with respect to maximal peak inward current. The peptide does not alter current kinetics under these conditions.

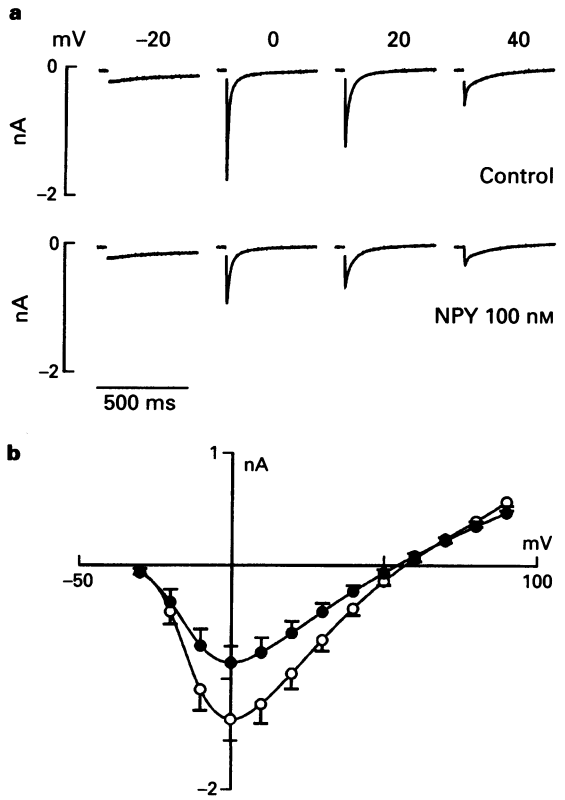

Figure 2 Effects of neuropeptide Y (NPY) on the current-voltage relationship in a guinea-pig ventricular myocyte. (a) Original current records elicited by 500 ms step depolarisation from −45 mV to the potentials at the top of the figure. Upper records, before NPY; lower records, after exposure to 100 nM NPY. (b) Current-voltage relationship for calcium current before (open symbols) and after (closed symbols) exposure to 100 nM NPY. Mean data from 11 cells; vertical lines show s.e.mean.

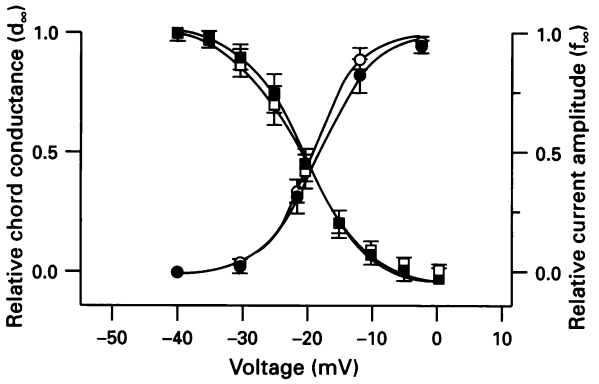

Figure 3 Effects of neuropeptide Y (NPY) on steady-state kinetic variables for calcium current. (○, ●), Activation variable (d_∞); (□, ■) inactivation variable (f_∞). Open symbols, control; solid symbols, after 100 nM NPY. For details of protocols used, see text. The curves are the Boltzmann equation plots for the data.

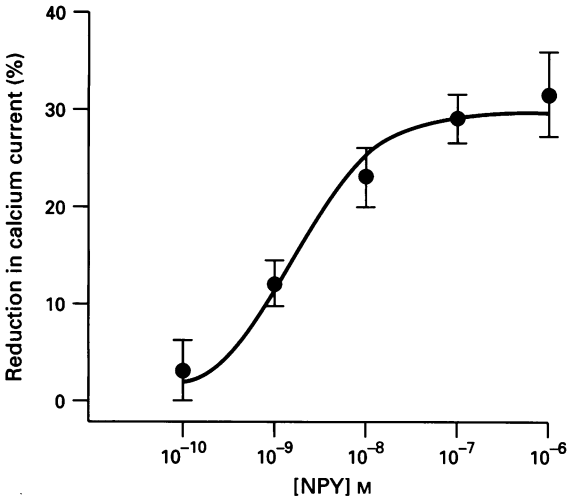

Figure 4 Concentration-dependence of the action of neuropeptide Y (NPY) on calcium current. Concentration-response curve for the inhibitory effect of NPY on L-type calcium current. Mean data from 38 cells are combined as follows: 0.1 nM, $n=5$; 1 nM, $n=7$; 10 nM, $n=5$; 100 nM, $n=17$; 1 μM, $n=9$. The curve is the Michaelis-Menten plot to the data. Vertical lines show s.e.mean.

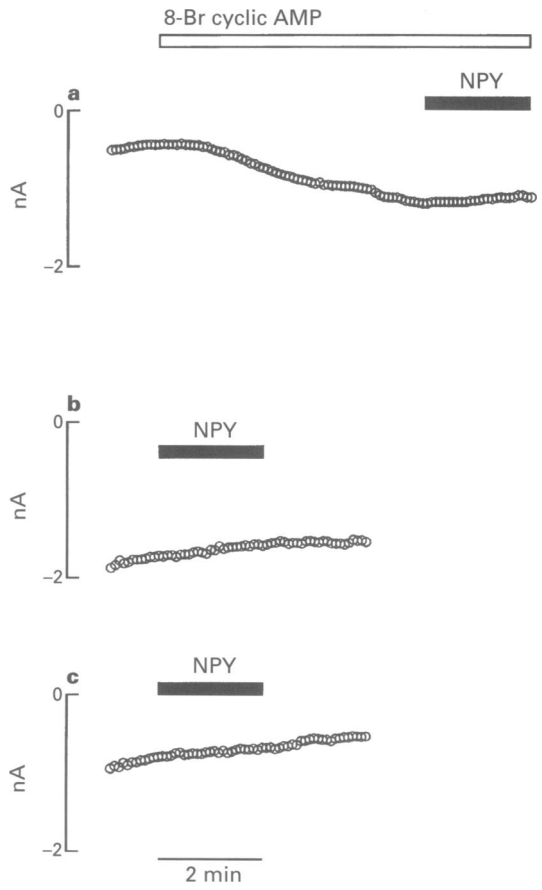

Figure 5 Peak inward calcium current recorded during step depolarisation from −45 mV to 0 mV, plotted as a function of time for 3 separate experiments. These data are example records taken from three individual myocytes. (a) Superfusion of 8-Br-cyclic AMP (10 mM) caused an increase in the amplitude of calcium current. Neuropeptide Y (NPY; 100 nM) added in the presence of 8-Br-cyclic AMP did not reduce the amplitude of the calcium current (b and c). Application of NPY (100 nM) did not significantly reduce the amplitude of the calcium current recorded from single myocytes with either GDP-β-S, 10 mM (b) or GTP-γ-S, 1 mM (c) included in the patch pipette, as compared to control conditions (see Figure 1).

Br-cyclic AMP but taken from the same isolations and at the same time, reduced I_{Ca} by 31±3% ($n=6$).

Effects of non-hydrolyzable GTP analogues

In previous experiments we have described partial but not complete inhibition of the effect of NPY on calcium current by pre-incubation of the cells with pertussis toxin which leaves open the possibility that either NPY action cannot be fully accounted for by G-protein inhibition, or alternatively the G-protein system was not completely inactivated by pertussis toxin. Therefore, in order to inhibit more completely the G-protein system we have used the guanine nucleotide analogues GTP-γ-S and GDP-β-S.

Inclusion of 10 mM GDP-β-S in the patch pipette solution, a concentration sufficient to block completely the stimulatory effect of isoprenaline in these cells (Kozlowski *et al.*, 1992), reduced the inhibitory effect of NPY on the calcium current (Figure 5b). Mean data show that addition of 100 nM NPY to the superfusate was accompanied by a small reduction in peak calcium current from −1.48±0.2 nA to −1.36±0.3 nA. This 8% reduction in calcium current is significantly different from the 31% reduction in calcium current seen in control conditions ($P<0.001$) (see Figure 1).

Figure 5c shows peak calcium current recorded at 0 mV from a representative cell when 1 mM GTP-γ-S was included in the patch pipette filling solution. In this cell inclusion of GTP-γ-S into the patch pipette abolished the calcium current responsiveness to NPY. Mean calcium current amplitude under control conditions was −0.88±0.20 nA, and after addition of 100 nM NPY the current amplitude was −0.83±0.20 nA (n=4).

Discussion

The experiments described in this paper show that in guinea-pig ventricular myocytes NPY acts in low concentration to reduce calcium current amplitude. This action appears to be mediated by G-proteins and inhibition of adenylate cyclase.

The potential importance of NPY is illustrated by the finding that elevated plasma levels of NPY-like immunoreactivity have been associated with increased mortality (Ullman *et al.*, 1995) and congestive heart failure (Maisel *et al.*, 1989), and in heart failure the peptide may play a pathophysiological role (Sheriff & Balasubramaniam, 1992). Although much is known about its receptor binding characteristics

(Sheriff & Balasubramaniam, 1992; Dumont *et al.*, 1992) and biochemical actions (McDermott *et al.*, 1993), the physiological mechanisms underlying the postsynaptic effects of the peptide in the heart are less well known. There is substantial evidence for inhibition of calcium current in neuronal tissue, via (directly-coupled) G-proteins (Ewald *et al.*, 1988; Dumont *et al.*, 1992). We have previously shown inhibition of L-type calcium current in ventricular myocytes (Bryant *et al.*, 1991) but these experiments were done using switch clamp and the effects of low concentrations and the current gating properties were not studied. In the present experiments the threshold of inhibition of calcium current by NPY is as low as 0.1 nM (Figure 4), and the IC_{50} value of 1.72 nM compares with the value of 6 nM for inhibition of rat neuronal high-threshold calcium current (Wiley *et al.*, 1993). The IC_{50} for [^{125}I]-NPY binding to rat sarcolemmal membrane is 60 nM and the IC_{50} value in the rat for inhibition of isoprenaline-stimulated adenylate cyclase activity is <1 nM (Sheriff & Balasubramaniam, 1992). Maximal inhibition of L-type calcium current under these experimental conditions (with external Na^+ substituted by TEA) was seen at concentrations of 100 nM and above. Although compounds containing a quaternary ammonium group are known to have weak muscarinic activity, acetylcholine has been found to have little effect on I_{Ca} in guinea-pig myocytes (Trautwein & Hescheler, 1990). Therefore, TEA may be expected to have either no effects or a very small effect resulting in an underestimation of the inhibitory action of NPY on I_{Ca}. The 31% maximal reduction in peak calcium current (Figure 4), however, corresponds closely to the 32% reduction obtained previously in guinea-pig ventricular myocytes on exposure to 10 μM NPY but without extracellular TEA (Bryant *et al.*, 1991).

NPY has no significant effect on the time course of the calcium-independent component of calcium current inactivation (Figure 1) or on the voltage-dependence of calcium current gating properties (Figure 3). The results in Figure 2 showing a decrease in whole-cell calcium current would be consistent with NPY acting to reduce the open probability of single calcium channels. No data are presently available on the action of the peptide on single channels in heart muscle. In Figure 1b there is no change in background current at 0 mV in the presence of NPY. This is in contrast to the inward shift in holding current which we described previously in physiological external solutions (Bryant *et al.*, 1991) and which is attributable to a reduction in the inward rectifier current I_{K1}. This current was absent under the present experimental conditions.

We have previously shown that pre-incubation of ventricular myocytes with pertussis toxin greatly reduces the effect of NPY on the calcium current (Bryant *et al.*, 1991). The experiments with guanine nucleotide analogues were designed to produce much more complete inhibition of the G-protein system and the results are consistent with the inhibitory effect of NPY on calcium current being entirely mediated by G-protein transduction, under these conditions. The lack of effect of NPY on calcium current in the presence of 8-Br-cyclic AMP, a potent direct activator of adenylate cyclase, is also consistent with NPY acting to reduce calcium current via a reduction in cyclic AMP levels. The action of NPY on calcium current in the absence of catecholamine stimulation would suggest that under these circumstances, about 30% of basal calcium current is supported by available cyclic AMP levels.

The present data show that NPY reduced basal I_{Ca} by 31% an effect mediated via G-protein coupled inhibition of adenylate cyclase, and are in agreement with the effects of NPY on rat cardiac membranes (Kassis *et al.*, 1987). However, though adenosine and acetylcholine have been shown to reduced isoprenaline-stimulated I_{Ca} in guinea-pig ventricular myocytes, via a PTX-sensitive G-protein and inhibition of adenylate cyclase (Isenberg and Belardinelli, 1984; Belardinelli *et al.*, 1995), both are without effect on basal I_{Ca} (Trautwein & Hescheler, 1990). Reasons for this difference are unclear, although there are a number of possible explanations. Adenylate cyclase activity and G-protein regulation of the enzyme are dependent on experimental factors. Although we did not test the effect of adenosine (in our conditions) on I_{Ca}, under similar experimental conditions adenosine reduces basal I_{Ca} (by 35%) in ferret ventricular myocytes (Qu *et al.*, 1993) and I_{Ca} in human atrial myocytes by 30% (Pelzmann *et al.*, 1995). Moreover, Mewes *et al.*(1993) have recently demonstrated that agonist-free β-adrenoceptors may still be functionally active in single guinea-pig ventricular myocytes stimulating the L-type calcium current via G_s stimulation of adenylate cyclase. This mechanism could provide a substrate for the inhibitory action of G_i on adenylate cyclase in the absence of isoprenaline stimulation. Possible differences may also arise because G-protein mediated inhibition of adenylate cyclase can take place by at least two mechanisms (Fleming *et al.*, 1992). NPY receptors may be coupled to more than one second messenger system: therefore, although the effects of adenosine may be solely mediated via G_i the effects of NPY may mediated via several G-protein delimited pathways. Our data are consistent with the notion that part of the inhibitory action of NPY on I_{Ca} may be mediated via a PTX-insensitive pathway (Millar *et al.*, 1991; Sheriff & Balasubramaniam, 1992; Xiang & Brown, 1993), but we cannot exclude the possibility that the reduction of basal I_{Ca} may take place via G_i mediated inhibition of adenylate cyclase.

Although we have never observed an increase in calcium current secondary to NPY in guinea-pig ventricular myocytes we should like to emphasise that our data do not exclude the possibility that under different experimental conditions, such effects may result from different mechanisms. Increases in calcium current amplitude have been described in rat ventricular myocytes (Millar *et al.*, 1991), in rabbit atrial myocytes (Dinanian *et al.*, 1992), and in vascular smooth muscle cells (Xiong *et al.*, 1993). These actions may depend on such preparations having different relative activities of stimulatory and inhibitory G-proteins, and different basal levels of adenylate cyclase activity and calcium channel phosphorylation or different NPY receptor subtype densities. Further work is necessary to explore the physiological basis for the different effects of NPY in these preparations.

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