Effects of Neuropeptide Y on Cell Length and Membrane Currents in Isolated Guinea Pig Ventricular Myocytes

S.M. Bryant, Kathryn O. Ryder, and G. Hart

Direct effects of neuropeptide Y were studied in left ventricular myocytes isolated from guinea pigs. Contraction was measured as the change in unloaded cell length using a photodiode array. Action potentials were elicited at 1 Hz in current-clamp mode, and membrane currents were measured using a switch-clamp amplifier with 2 M-KCl microelectrodes. At concentrations of \(10^{-4}\) M and above, neuropeptide Y reduced contraction in a concentration-dependent fashion. The reduction in contraction by the peptide was proportionately greater in the presence of isoproterenol, and the increase in contraction caused by isoproterenol was completely inhibited by \(10^{-5}\) M neuropeptide Y. In response to neuropeptide Y, action potential duration was shortened, and the time course of the shortening was similar to that of the reduction in contraction. Under voltage clamp, \(1 \times 10^{-5}\) M neuropeptide Y reduced peak L-type calcium current by 32% and shifted the myocyte current–voltage relation during a slow ramp in a manner that suggested a reduction in the background rectifier K+ current. The effects of the peptide on membrane currents were greatly attenuated by preincubation of the cells with pertussis toxin (100 ng/ml). We conclude that neuropeptide Y reduces developed shortening, action potential duration, L-type calcium current, and background rectifier current in single guinea pig ventricular myocytes and that these effects are mediated, at least in part, via membrane G proteins. (Circulation Research 1991;69:1106–1113)

Neuropeptide Y (NPY) is a 36–amino acid peptide that is colocalized with norepinephrine and is widely distributed in mammalian heart. The peptide has a negative inotropic action on intact heart. Although this action may in part be attributable to coronary vasoconstriction, studies have suggested that there may also be a direct negative inotropic effect on the cardiac myocytes. Rigel et al. have shown that NPY reduces developed force in isolated dog atrial and ventricular trabeculae, and Piper et al. have described a negative inotropic effect of the peptide on isolated ventricular myocytes from the rat. However, Wahlestedt et al. found no effect of \(5 \times 10^{-7}\) M NPY on guinea pig papillary muscle paced at 0.4 Hz, and the peptide is also without effect on isolated human atrium. In contrast, a positive inotropic effect of NPY has been described in spontaneously active guinea pig atrium and in cultured chick ventricular myocytes. In view of this apparent discrepancy, we have sought to confirm a postsynaptic action of NPY on contraction in single myocytes isolated from guinea pig ventricle.

In cardiac as well as in other tissues, NPY inhibits the increment in adenylate cyclase activity obtained in response to catecholamines. This inhibition is sensitive to pertussis toxin (PTX), which suggests that it is mediated via an inhibitory G protein. G proteins have been shown to play a part in the regulation of calcium channels in the heart. In neuronal tissue, NPY reduces calcium current (\(I_{Ca}\)). These data raise the possibility that some of the actions of NPY on the force of contraction in the heart may take place through changes in sarcolemmal membrane currents. Therefore, we have studied the effects of NPY using the voltage-clamp technique while simultaneously measuring unloaded cell length using a photodiode array.

Materials and Methods

Cells were isolated from the left ventricles of female Dunkin-Hartley guinea pigs weighing 400–600 g by using a modified enzyme technique and were layered on a glass coverslip, which formed the base of a perfusion chamber situated on the stage of
an inverted microscope (Nikon Diaphot), positioned on an isolation table (Photon Control Ltd., Cambridge, UK). Superfusate flow rate was 1–2 ml/min, and solution lines were switched using solenoid valves (Lee Products Ltd., Gerrards Cross, UK) just before entry to the bath. The temperature of the bath was maintained at 32±1°C by a heating coil, surrounding the input line, which was controlled by a feedback circuit using a sensing thermocouple positioned between the heating coil and the chamber. The level of solution in the bath was controlled using a feedback circuit, the sensor for which was a piece of photographic film, positioned on the meniscus and connected by a stainless-steel rod to an Akers transducer (SensoNor a.s., Horten, Norway).23

Micropipettes were pulled from filamented boro-silicate glass (Clarke Electromedical, Pangbourne, UK) and filled with 2 M KCl, giving a resistance of 15–25 MΩ. Voltage clamping was achieved using a single microelectrode switch-clamp technique (Axoclamp II-A, Axon Instruments, Inc., Foster City, Calif.). The clamp switching frequency was 3.5–7.5 kHz. An agar bridge formed the indifferent electrode.

Cell length was measured using an S-series photodiode array (Reticon, Wokingham, UK)24 with a sweep time of 4 msec.

Voltage, current, and length signals were recorded on magnetic tape (Racial Store 7 recorder, Hythe, UK) for later analysis. The signals were played back through a six-pole Bessel filter and sampled by a 12-bit analog-to-digital converter (model CED 1401, Cambridge Electronic Design Ltd., Cambridge, UK). Analysis of the digitized records was performed on a Compaq Deskpro 286 computer using software provided by Dr. J. Dempster, Strathclyde University.

Results were compared using Student’s t test for paired samples and are expressed as mean±SEM.

Solutions

Superfusate composition was as follows (mM): Na+ 136, K+ 5.4, Mg2+ 1.2, Ca2+ 1.8, glucose 11.1, and HEPES 5.0, titrated with NaOH to pH 7.4. NPY was obtained from Cambridge Research Biochemicals, Cambridge, UK. PTX was obtained from Sigma Chemical Co., St. Louis, Mo.

Results

Effects of NPY on Action Potentials and Contraction

Action potentials were elicited at a frequency of 1 Hz by current injection in current-clamp mode, and results from a representative cell are shown in Figure 1A. After exposure to 1×10^-5 M NPY, the plateau of the action potential is depressed, and action potential duration (APD) is reduced. There was no significant change in resting membrane potential (Table 1). Cell length is shown in the top of Figure 1A, and cell shortening appears as a downward deflection. The change in cell length accompanying depolarization is substantially diminished by the peptide. These changes were consistent in all cells studied (Table 1).

![Figure 1](image)

**Figure 1.** Effects of neuropeptide Y on action potentials and contraction in a representative single myocyte. Panel A: Cell length (top recording) and membrane potential (bottom recording) showing responses to stimulation at 1 Hz in current-clamp mode. ●, After application of 1×10^-5 M neuropeptide Y. Panel B: Action potential duration at 90% repolarization (APD90, top recording) and cell length at peak contraction (bottom recording) displayed as a function of time. The start of the time bar marks the point at which 1×10^-5 M neuropeptide Y was applied.

| Table 1. Effects of Neuropeptide Y on Guinea Pig Ventricular Myocytes |
|--------------------------|-----------------|-----------------|-----------------|
|                        | Myocytes        | RP (mV)         | APD50 (msec)    | APD90 (msec)    | Contraction (μm) |
| Control                |                 | 246±20          | 281±20          | 18.4±3.2       |
| NPY                    |                 | 219±20*         | 256±20*         | 11.5±2.5†      |

Values are mean±SEM for eight cells in each column and nine in the last column (Contraction). RP, resting potential; APD50 and APD90, action potential duration measured at 50% and 90% repolarization, respectively; NPY, after application of 1×10^-5 M neuropeptide Y.

*p<0.05, †p<0.001 compared with control by paired t test.
Dose–Response Curves for NPY

The effects of NPY on contraction and on APD are concentration dependent. Figure 2 shows the effects of incremental concentrations of NPY on contraction, expressed as a percentage of the change in cell length accompanying regular action potentials at a frequency of 1 Hz. There is a significant reduction in developed shortening at $1 \times 10^{-5}$ M NPY. Increasing concentrations produced graded reductions, and the maximal effect does not appear to have been reached. The effects of higher concentrations were not tested.

Because NPY is colocalized and coreleased with norepinephrine,25 we elected to test the effects of incremental doses of the peptide in the presence of a $\beta$-agonist. In four cells, prior application of $1 \times 10^{-8}$ M isoproterenol increased cell shortening from 18.2±2.2 μm to 22.2±2.2 μm ($p=0.008$). The dose–response curve in the presence of isoproterenol is shown as filled circles in Figure 2. Exposure to NPY in the presence of isoproterenol produced relatively greater inhibition of contraction at each concentration of NPY than in the absence of the $\beta$-agonist; the curve is shifted downward in a parallel fashion. The difference between the dose–response curves in Figure 2 may be accounted for by inhibition of the increment in contraction produced by isoproterenol. It appears that this increment has been inhibited by the lowest concentration of NPY tested ($1 \times 10^{-6}$ M).

Effects of NPY on Membrane Currents

A shortening of APD implies a change in the balance of membrane currents in a more outward (or less inward) direction during the plateau phase of the action potential. Therefore, we investigated the effects of NPY using the voltage-clamp technique. The holding potential was set at $-45$ mV to inactivate the sodium channel and the T-type Ica. Depolarizing pulses were applied from this potential to 0 mV at a frequency of 0.2 Hz, and results from a representative cell are shown in Figure 3. In the bottom panel, it can be seen that the holding current before the depolarizing clamp step has shifted inward after exposure to $1 \times 10^{-5}$ M NPY. Soon after the onset of the depolarizing step, there is an inward peak of membrane current as L-type Ica activates rapidly.26,27 After exposure to NPY, the peak Ica amplitude is substantially reduced. Inactivation of this current then takes place slowly, and at more positive potentials, an outward current (Iox) develops during the later part of the clamp step.28 The mean data from seven cells are summarized in Table 2. Note that contraction amplitude is also reduced. We shall now address each of these parameters in more detail.

Calcium Current

The voltage-dependence of the L-type Ica has been studied, and the results are shown in Figure 4. Figure 4A displays a family of current recordings from a single cell, elicited by depolarization to the potentials shown from a holding potential of $-45$ mV. At each potential, peak Ica is reduced. The mean current–voltage relation from four cells is plotted in Figure 4B. The circles represent peak Ica at increments of 10 mV and show
that $I_{Ca}$ has been reduced by the peptide. The potential at which $I_{Ca}$ was maximal did not change in the presence of NPY, suggesting that there is no shift in the voltage dependence of the activation properties of the channel. There was also no shift in the reversal potential for $I_{Ca}$. The time course of $I_{Ca}$ inactivation at +5 mV was fitted to the sum of two exponentials. The time constants for $I_{Ca}$ inactivation were not significantly different after $1 \times 10^{-5}$ M NPY (Table 2).

### Table 2. Effects of Neuropeptide Y in Voltage-Clamp Mode

<table>
<thead>
<tr>
<th>Myocytes</th>
<th>$I_{holding}$ (nA)</th>
<th>Peak $I_{Ca}$ (nA)</th>
<th>$\tau_1$ (msec)</th>
<th>$\tau_2$ (msec)</th>
<th>Contraction (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.48±0.06</td>
<td>-2.2±0.2</td>
<td>8.3±1.3</td>
<td>73±2</td>
<td>7.1±1.0</td>
</tr>
<tr>
<td>NPY</td>
<td>0.26±0.07*</td>
<td>-1.5±0.2</td>
<td>10.2±1.4</td>
<td>85±6</td>
<td>4.6±0.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM for seven cells in each column and eight in the last column (Contraction). $I_{holding}$, holding current at $-45$ mV; Peak $I_{Ca}$, peak early inward current on depolarization from the holding potential to 0 mV; $\tau_1$ and $\tau_2$, fast and slow time constants, respectively, of $I_{Ca}$ inactivation; NPY, after application of $1 \times 10^{-5}$ M neuropeptide Y. *$p<0.001$, †$p<0.01$ compared with control by paired t test.

### Delayed Potassium Current

Current at the end of 500-msec depolarizing clamp steps consists largely of $I_K^{26}$, although at the more negative end of the potential range tested, steady-state “window” $I_{Ca}$ will contribute to the observed membrane current. The 500-msec isochronal current–voltage relation is plotted as squares in Figure 4B. The graph suggests a trend toward increased $I_K$ after NPY, but this does not reach statistical significance. We also assessed $I_K$ by measuring the amplitude of the decaying $I_K$ tail on repolarization to $-45$ mV. After a clamp step to $+40$ mV for 500 msec, the tail amplitudes were 0.20±0.03 nA before NPY and 0.19±0.02 nA after NPY ($n$=4), confirming a lack of significant effect of the peptide on the delayed rectifier.

### Steady-State Current at Negative Potentials

In view of the inward shift in holding current at $-45$ mV (Table 2), we have examined the effect of NPY on quasi–steady-state currents over a wider range of potentials by using a ramp voltage protocol. The membrane was depolarized positive to $+50$ mV to discharge the gated conductances activated over this range, and then a negative ramp was applied at a rate of 6.6 mV/sec. In the potential range negative to $-10$ mV, the configuration of the current–voltage relations is determined largely by the background rectifier K+ current ($I_{Kb}^{29}$) and is plotted in Figure 5. The control curve shows the expected N-shaped relation with anomalous rectification.$^{30,31}$ After NPY, there is an outward shift of current at very negative potentials. The curves cross at $-85$ mV, corresponding to the reversal potential for $K^+$. At potentials between $-85$ and $-30$ mV, the current after NPY is more inward. This result suggests that the conductance of the background $I_{Kb}$ channel is reduced by the peptide. The crossover at $-30$ mV suggests that there may also be a small hyperpolarizing shift of the activation variable after NPY.

### Length–Voltage Relations

The changes in length associated with depolarizing voltage-clamp steps to different potentials are shown in Figure 6A. On depolarization, there is a phasic contraction followed at the more positive potentials by a second component of contraction. The amplitude of the twitch increases with potential and then declines at more positive potentials. NPY appears to reduce both of these components of length change. Figure 6B shows the phasic component as a function

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**Figure 4.** Effects of neuropeptide Y (NPY) on membrane currents from a holding potential of $-45$ mV. Panel A: Family of current recordings showing the effects of depolarizations lasting 500 msec from the holding potential to the potentials shown above the recordings. The top four recordings were obtained in control solution; the bottom four recordings were obtained after application of $1 \times 10^{-5}$ M NPY. Panel B: Current–voltage relations for peak early current (circles) and current at the end of 500-msec depolarizing clamp steps (squares). Open symbols represent control; closed symbols represent the presence of $1 \times 10^{-5}$ M NPY. Standard error bars are shown for $n=4$ cells.
of voltage. The peptide reduces contraction at each potential without having any overall effect on the voltage dependence of the U-shaped relation.

Effects of NPY After Pretreatment With Pertussis Toxin

Piper et al.\(^1\) have shown in rat myocytes that the effect of NPY on the increment in contractility after isoproterenol is sensitive to PTX. In neural tissue the inhibition of L-type \(I_{\text{Ca}}\) by NPY is blocked by PTX pretreatment.\(^1\) We have sought to find out whether the effects of NPY on contraction and membrane current in the heart are influenced by PTX in the absence of catecholamine stimulation. Cells were preincubated with 100 ng/ml PTX at 37°C for 3 hours.\(^1\) (Exposure to significantly higher concentrations, as reported in Reference 21, was associated with deterioration of the cells.) There was no significant difference in electrical parameters or in contractility between the preincubated and control groups before application of the peptide (Table 3). NPY (1×10\(^{-5}\) M) was then added, and the percentage changes in the PTX-treated group after the peptide are compared with those in an untreated group in Table 4. The shortening of APD at 90% repolarization seen in control cells after NPY is not evident in cells preincubated with PTX. We have shown two measurements of membrane current as representative of the effects of NPY on \(I_{\text{Ca}}\) and \(I_{\text{K1}}\). The fall in peak \(I_{\text{Ca}}\), normally found with this concentration of NPY is halved, and the inward shift in holding current is greatly reduced. However, the inhibition of contraction is not so clearly affected by PTX pretreatment, which reduces the fall in developed length measured under voltage clamp but has no apparent effect on the length change accompanying action potentials. Possible reasons for this discrepancy will be discussed later. There was no change in resting potential in either group after application of the peptide.

Discussion

These experiments show that NPY has a direct negative inotropic effect on isolated guinea pig ventricular myocytes. The action of the peptide is concentration dependent, and the effects on cell shortening take place concomitantly with a reduction in APD. The concentrations of NPY that were necessary to obtain a direct effect in these experiments were high compared with some studies in neural tissue. In other tissues, however, direct agonist effects have been reported over a concentration range similar to that used in these experiments. For exam-
ple, in vascular smooth muscle the threshold for the direct vasoconstrictor action of NPY varies with site and species and spans the range 10^{-9} to 10^{-6} M. The effects of NPY on the smooth muscle of the lower esophageal sphincter^32 take place over a closely similar concentration range to the actions that we have described in isolated ventricular myocytes. Therefore, we consider that we cannot dismiss the possibility of a direct physiological action of the peptide in heart. Hassall and Burnstock^33 have identified NPY in nonvascular neurons in the heart, which do not contain norepinephrine, and it appears that these neurons make direct synaptic contact with myocytes. Therefore, NPY may have a role in modulation of membrane currents and contractility at the level of the individual cardiac myocyte.

In much of its distribution, NPY is colocalized and coreleased with norepinephrine and acts postsynaptically on norepinephrine^12 and acetylcholine^25 release as well as postsynaptically in potentiating norepinephrine-evoked vasoconstriction. The actions of the peptide in modulating the response to catecholamines are usually seen at much lower concentrations than those necessary for any direct effects. The data in this paper are in accord with this experience. Figure 2 suggests that the concentration of NPY required to inhibit the increment in myocyte contraction produced by isoproterenol may be substantially lower than the concentration that inhibits contraction in the absence of extrinsic \( \beta \)-stimulation.

We have found the onset of action of the peptide to be much more rapid than that found in canine atria and ventricular trabeculae by Rigel et al.,^10 who reported that the negative inotropy took as long as 13 minutes (mean) to reach steady state. They also observed a 30% inhibition of contractile force in ventricular trabeculae with 10^{-5} M NPY, which is about twofold greater than the reduction in contraction seen with 10^{-5} M NPY in our experiments. Possible explanations for both of these differences may lie in the ability of field stimulation to release catecholamines from intact muscle preparations or in the difference between force and cell shortening. If catecholamines are released over a period of time in intact muscle, the effects of NPY may take longer to reach steady state and (because of the increased sensitivity of the \( \beta \)-adrenoceptor-induced increment to the peptide) will be proportionately larger than in isolated, denervated myocytes. In this regard, it is slightly unexpected that Wahlestedt et al. found no effect of \( 5 \times 10^{-7} \) M NPY on the norepinephrine-stimulated component of contraction in paced guinea pig papillary muscle, although they found that the peptide greatly reduced the propranolol-sensitive increment in force generated by a train of rapid pulses.

The reduction in contraction in response to NPY is accompanied by a shortening of the action potential. The plateau potential is slightly reduced from the initial phase of repolarization after the upstroke (Figure 1), which would suggest that the membrane current change responsible occurs early in the action potential plateau. The substantial reduction in \( I_{\text{Ca}} \) amplitude that we have found is probably sufficient

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<th>Table 3. Effects of Pertussis Toxin on Electrical and Mechanical Properties of Ventricular Myocytes</th>
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<td><strong>Myocytes</strong></td>
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<tr>
<td>Control</td>
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Values are mean±SEM. APD_{90} and APD_{90} action potential duration measured at 50% and 90% repolarization, respectively; Contraction (AP), contraction elicited in association with an action potential by current injection (frequency, 1 Hz); Contraction (VC), contraction after voltage-clamp depolarization to 0 mV (frequency, 0.2 Hz); \( I_{\text{holding}} \), holding current at -45 mV; Peak \( I_{\text{Ca}} \), peak early inward current; PTX, preincubation with 100 ng/ml pertussis toxin at 37°C for 3 hours. Data from the two groups of cells are not significantly different (by unpaired \( t \) test).

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<th>Table 4. Comparison of Changes on Exposure to Neuropeptide Y After Pretreatment with Pertussis Toxin</th>
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<td><strong>Myocytes</strong></td>
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<td>NPY</td>
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Values are mean±SEM. APD_{90}, action potential duration at 90% repolarization; Contraction (AP), contraction elicited in association with an action potential by current injection (frequency, 1 Hz); Contraction (VC), contraction after voltage-clamp depolarization to 0 mV (frequency, 0.2 Hz); \( I_{\text{holding}} \), holding current at -45 mV; Peak \( I_{\text{Ca}} \), peak early inward current; NPY, after application of \( 1 \times 10^{-5} \) M neuropeptide Y; PTX, pertussis toxin. Note that the measurements are percentage change from control baseline values (NPY) and, for a different group of cells, from baseline values after a 3-hour incubation with 100 ng/ml PTX (NPY after PTX).

\*\textit{p}<0.01, \textit{fp}<0.001 compared with NPY by unpaired \( t \) test.
per se to account for the reduction in APD, even in the face of a small fall in \( I_{K1} \), which would itself tend to lengthen the action potential.

An inhibitory effect of NPY on \( I_{Ca} \) in neuronal tissue is well characterized,²¹,²⁷ but we are unaware of any previous reports confirming a direct action on the cardiac L-type calcium channel. Although the conditions of these experiments allow the possibility of overlap from other currents to interfere with the \( I_{Ca} \) recordings, the extent by which such overlap may interfere with the changes produced by the peptide is likely to be small, given the lack of effect of NPY on \( I_K \) decay tails, elicited by repolarization from positive potentials. We have not found significant transient outward current (\( I_o \)) in these cells, which obviates another potentially overlapping current mechanism.

We have not sought an effect of the peptide on the T-type current, which carries a much smaller proportion of the gated calcium charge movement in these cells. From action potential work, NPY is thought to increase background \( K^+ \) permeability in neuronal tissue.²⁸ A reduction in background \( I_{K1} \) in the heart is, therefore, a new finding, which is strengthened by the similar behavior of \( I_{Ca} \) and \( I_{K1} \) after PTX pretreatment. Patch-clamp studies will be necessary to explore the effects on this current system in more detail.

The actions of NPY on \( I_{Ca} \) and the \( I_{K1} \) are greatly attenuated by pretreatment with PTX, suggesting the involvement of the membrane GTP-binding proteins, \( G_i \).²⁹ In neural tissue, the inhibition of N-type \( I_{Ca} \) by the peptide is also PTX sensitive.²¹ It is possible that the change in L-type \( I_{Ca} \) in heart is brought about via a second messenger system¹⁷ rather than through a direct effect of NPY on the channel-associated \( G_i \). Further experiments will be needed to distinguish between these possibilities in cardiac myocytes. Contraction was also diminished when studied within the voltage range of the calcium channel, but there was no change in the inhibition of the larger contraction that accompanies an action potential. This may suggest that the effects of NPY on contraction may involve other mechanisms as well as a reduction in \( I_{Ca} \). However, to avoid myocyte deterioration, the concentration of PTX used was not as high as others have found necessary to achieve maximal receptor uncoupling. Longer incubation periods were associated with deterioration in the cells and the appearance of transient inward currents on clamping. Clearly, incomplete inhibition of \( G \) protein responses may underlie the lack of significant change in contraction in current-clamp mode, but the disparity raises the possibility that the effects of NPY on the linkage between \( I_{Ca} \) and contraction may not be simple. Another possible explanation is furnished by Piper et al.,¹¹ who have found, using rat myocytes, that the relation between force and the second messenger system under the influence of NPY is nonlinear. However, in their experiments contraction was much more sensitive to increasing NPY concentration than cAMP accumulation. Further experiments will be necessary to distinguish among these and other explanations: our experiments were not designed to explore the effects of the peptide on the relation between membrane currents and force but rather to show that the effects of NPY are, at least in part, mediated via receptors coupled to the protein \( G_i \).

We conclude that NPY has direct effects on isolated ventricular myocytes, resulting in a shortening of APD, a reduction in \( I_{Ca} \) and \( I_{K1} \), and a fall in developed contraction.

References


Key Words: heart • neuropeptide Y • contraction • calcium current • inward rectifying K+ current
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