Distinct Components of Morphine Effects on Cardiac Myocytes are Mediated by the $\kappa$ and $\delta$ Opioid Receptors

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Morphine exerts direct effects on cultured cardiac myocytes from neonatal rats. These effects are mediated via the $\delta$ and the $\kappa$ opioid receptors, as $\mu$ opioid receptors are not present in neonatal cardiomyocyte cultures. Binding parameters to the $\delta$ and $\kappa$ opioid receptors were determined in membrane preparations from these cultures by heterologous competition to $[3H]$diprenorphine binding, with [D-Pen², D-Pen⁵]-enkephalin (DPDPE) and trans-(dl)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate (U-50,488H) as specific displacers respectively. To define the components of morphine effects mediated via activation of either the $\delta$ or the $\kappa$ opioid receptor alone, cardiac myocytes were exposed to morphine in the presence of specific antagonists to the $\delta$ or $\kappa$ opioid receptor respectively. Activation of the $\kappa$ opioid receptors by morphine caused a transient increase in $Ca^{2+}$ influx, leading to increase in amplitudes of $[Ca^{2+}]_i$ transients and contraction, with no change in the intracellular pH. Activation of the $\delta$ opioid receptors alone by morphine caused a decrease in the amplitude of contraction. This decrease was mediated by a decrease in the intracellular pH leading to reduced responsiveness of the myofilaments to $Ca^{2+}$. There was no change in $Ca^{2+}$ influx and in the amplitude of $[Ca^{2+}]_i$ transients. The effects mediated through the $\delta$ opioid but not through the $\kappa$ opioid receptors were pertussis toxin sensitive, indicating coupling of the $\delta$ opioid receptors to pertussis toxin sensitive GTP-binding proteins. The overall effects of morphine on the neonatal cardiac myocytes were the sum of the effects exerted by morphine when it activated each of the opioid receptors alone.

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Introduction

It has been recently found in our laboratory that morphine exerts direct effects on cardiac myocytes from neonatal rats. Exposure of cultured ventricular myocytes from neonatal rats to morphine caused an increase in cytosolic free $Ca^{2+}$ ([Ca²⁺]) transients, and an increase in Ca²⁺ influx. The increase in [Ca²⁺], transients was not accompanied by an increase in the amplitude of systolic cell motion (ASM), indicating reduced myofibril responsiveness to Ca²⁺. Intracellular pH measurements revealed that morphine caused acidosis. The effect of morphine on the intracellular pH but not on Ca²⁺ influx was inhibited by pertussis toxin, protein kinase inhibitor K323a, phorbol-ester and ethylisopropylamiloride (EIPA), indicating that the pathway is mediated via GTP-binding proteins and by altered activity of protein kinase C (PKC) and the Na⁺/H⁺ exchanger (Ela et al., 1993).

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Effects of opioid receptor agonists on cardiac myocytes of various origins have been reported. Enkephalin peptides and their synthetic analogs produced transient positive inotropic effects and increased Ca\(^{2+}\) influx in cultured cardiac myocytes from chick embryos. These effects could be blocked by naloxone. The presence of opioid receptors capable of specific binding of \(^{3}H\)naloxone has been demonstrated in these cells (Laurent et al., 1985; 1986). In isolated cardiac myocytes from adult rats, agonists of the \(\delta\) and \(\kappa\) opioid receptors caused negative inotropic effects due to decreased [Ca\(^{2+}\)], transients. These agonists also caused an increase in the level of inositol 1,4,5-trisphosphate (IP\(_3\)) (Ventura et al., 1991a; 1992). Recently it has been reported that stimulation of \(\delta\) opioid receptors in ventricular myocytes from adult rat reduced the \(\alpha\)-type Ca\(^{2+}\) channel current (Xiao et al., 1993). On the other hand, stimulation of \(\kappa\) opioid receptors in similar cells increased the level of cytosolic Ca\(^{2+}\) (Tai et al., 1992, Ventura et al., 1994).

The different results obtained in the three cell types described above raise the question of whether the response of cardiac myocytes to morphine is different from responses to specific \(\delta\) and \(\kappa\) agonists. Morphine has a higher selectivity for \(\mu\) receptors, but at the concentrations used (10\(^{-5}\)–10\(^{-6}\)M) it binds also to \(\delta\) and \(\kappa\) receptors [\(\mu\) receptors are not present in cultured cardiac myocytes (Zimlichman et al., 1996)]. It is not yet known whether possible “cross-talk” between these receptors may lead to a different response from that induced by binding to each receptor alone.

Focusing on this question, we have determined, using specific antagonists, which components of the effects of morphine are mediated via the \(\delta\) or the \(\kappa\) opioid receptors. We have found that the overall effects of morphine on neonatal cardiac myocytes consist of the sum of the effects exerted by morphine via each of the opioid receptors alone.

**Material and Methods**

**Cell Culture**

Cultures of ventricular myocytes from neonatal rats were prepared as described previously (Ela et al., 1993). Briefly myocardial cells were isolated from ventricular fragments of the hearts of one day old Sabra rats by serial trypsinization as described (Hallaq et al., 1989) and suspended in Ham F10 media containing 20% serum and antibiotics. The cell suspensions were enriched with myocytes by pre-plating on tissue culture plastic Petri dishes for 1 h (during this time, the fibroblasts become attached to the Petri dish). The myocyte-enriched suspension was collected and diluted to 5 \times 10\(^5\) cells/ml. For measurements of contractility and [Ca\(^{2+}\)], the cells were plated on circular glass coverslips (25 mm). For measurements of pH, the cells were plated on rectangular coverslips (50 \times 12 mm). For measurements of 45Ca\(^{2+}\) influx, the cells were plated on multi-well plates (12 wells per plate). The cells were maintained in humidified 5% CO\(_2\)–95% air atmosphere at 37°C for 4 or 5 days, before performing the experiments.

**Membrane preparation and binding assay**

Membranes were prepared from cultured cardiac myocytes 4 days after plating. The cultures were washed with phosphate-buffered saline, pH 7.4, the cardiomyocytes were collected in the same buffer and then homogenized in 20 volumes of 50 mM Tris-HCl buffer, pH 7.4 using Polytron homogenizer (Kinematica, Lucerne). The homogenate was centrifuged at 1000 \(\times\) g for 10 min and the pellet was discarded. The supernatant was centrifuged at 40 000 \(\times\) g for 20 min and the pellet, containing the crude membranes, was resuspended in Tris buffer with a Dounce homogenizer. Binding assay was carried out as previously described (Zimlichman et al., 1996). In brief, aliquots of crude membranes were incubated with 1 nM \[^{3}H\]di- preorphine (34 Ci/mmol, Amersham, Buckinghamshire) at 25°C for 60 min. To determine the parameters of \(\delta\) binding sites, the incubation was done in the presence of a saturating concentration (100 nM) of a specific \(\kappa\) agonist and a range of concentrations of a specific \(\delta\) agonist as a displacer. To determine the parameters of \(\kappa\) binding sites, the \(\delta\) agonist was present at a saturating concentration (100 nM) and the \(\kappa\) agonist at a range of concentrations as a displacer. We have used the specific \(\delta\) and \(\kappa\) agonists [D-Pen\(^2\), D-Pen\(^5\)]-enkephalin (DPDPE) and trans-(dl)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate (U-50,488H), respectively. The amount of protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. B\(_{max}\) s and K\(_i\) s values were calculated from three independent experiments (9 points each) with the Inplot 4 computer program (Graph Pad Software, San Diego, CA) and drawn with the SigmaPlot 4.11 computer program (Jandel Scientific, Corta Madera, CA, USA) as previously pre-plated
Measurement of the amplitude of cell motion

Changes in the contractile state of the cultured myocytes following the addition of the drugs were determined by measuring the amplitude of systolic cell motion, using a phase contrast video motion detector system (Hallaq et al., 1989). Circular glass coverslips with attached cardiac myocytes were placed in a glass-bottomed cell chamber and were superimposed constantly with buffered salt solution (BSS) containing (mM): NaCl, 140; KCl, 5; CaCl₂, 1; MgCl₂, 1; glucose, 10; Na₂HPO₄, 1; HEPES, 10, pH 7.4. The cell chamber was mounted on the stage of the phase contrast inverted microscope. The temperature of the solution in the cell chamber was maintained at 37°C. The beating rate was kept constant by field stimulation of the cells via two platinum electrodes based in the superimposed solution. The amplitude of contraction was measured by recording the movement of a microsphere at baseline and after drug addition. The amplitude of cell motion is expressed as the percentage of the value in the same cell before drug addition.

Measurement of [Ca²⁺], transients

Measurement of [Ca²⁺], transients was done in cells loaded with the fluorescent Ca²⁺ indicator indo-1. Coverslips with attached cultured cells were incubated for 45 min at room temperature in BSS containing indo-1 acetoxy-methylester (indo-1/AM) (5 μM) and probenecid (3 mM), then washed and incubated again in BSS containing probenecid (3 mM) for 30 min before measurement. The coverslip with the loaded cardiac myocytes was then placed in the cell chamber which was mounted on the stage of the inverted microscope. The cell chamber was superfused with BSS, maintained at 37°C and the cells in the chamber were field stimulated as described above. The drugs were dissolved in BSS and added by superfusion. Simultaneous measurements of calcium transients and cell motion were made as described by Peeters et al. (1987) using an FM-1000 dual wavelength fluorescence microphotometer (Rincon Inc., San Paulo, CA, USA). The FM-1000 system was connected to the inverted microscope and to the video motion detector system as described previously (Ela et al., 1993). The amplitude of systolic...
cell motion (ASM) was recorded simultaneously with the recording of the indo-1 fluorescence ratio at 410/480 nm. Before measurements, autofluorescence of unloaded cultures was determined and this value was subtracted from the fluorescence of the indo-1 loaded cultures before the computation of the ratio. Measurements were done on groups of a few attached cells which contracted synchronously, before and at different time intervals after drug addition. Each measurement continued for approximately 5 s between measurements the cells were kept in the dark to minimize indo-1 bleaching. It has been suggested that in rat cardiac myocytes loaded with indo-1, calibration of \([\text{Ca}^{2+}]_i\) is not precise due to subcellular compartmentalization of indo-1. Because the fluorescence ratio was shown to be a monotonic function of \([\text{Ca}^{2+}]_i\), we have presented indo-1 signals in uncalibrated form as suggested by Spurgeon et al. (1990).

### Measurement of \(46\text{Ca}^{2+}\) influx rates

Cardiac myocytes, plated in 12-well multiwell plates, were preincubated in BSS for 1 h at 37°C, then placed in a shaker at 37°C. The BSS solution was then exchanged with fresh BSS at 37°C to which the examined drugs were subsequently added. Before drug addition and at different time intervals thereafter, \(46\text{Ca}^{2+}\) was added (4 µCi/well). The influx of \(\text{Ca}^{2+}\) was terminated 15 s later by the removal of the medium and washing the cells in the well with 3 portions of 2 ml BSS at 0°C. Subsequently, 0.4 ml of NaOH (0.2 M) was added to each well, and the plates were incubated at 37°C for 30 min. Aliquots were then taken from each well for protein determination by the method of Lowry et al. (1951) and for measurement of the radioactivity in toluene triton scintillation fluid.

### Measurement of intracellular pH

Intracellular pH was measured using the pH-sensitive fluorescent dye \(2',7'\text{-bis(carboxyethyl-5(6)-carboxyfluorescein)}\) (BCECF). Cardiac myocytes attached to rectangular coverslips were incubated in BSS containing 20 µM BCECF-acetoxymethoxy ester (BCECF/AM) for 45 min, then washed with BSS to remove extracellular dye. The coverslip with the loaded cells was inserted diagonally into a cuvette containing 3 ml BSS at 37°C which was placed in a temperature-controlled chamber of a fluorescence spectrophotometer. The pH was measured as described previously (Ela et al., 1993). Fluorescence emission at 535 nm was recorded as a function of time, before and after drug addition, in cells excited at 495 nm. Excitation at 450 nm was done before and after the measurements. Calibration was done as described previously (Ela et al., 1993).

### Results

Binding parameters of \(\delta\) and \(\kappa\) opioid receptor agonists to the \(\delta\) and \(\kappa\) opioid receptors were determined in crude membrane preparations from cultured neonatal rat cardiomyocytes. It was found previously that \(\mu\) opioid receptors are not present in cardiac myocyte membranes from cultured neonatal rats (Zimlichman et al., 1996) and from adult rats (Ventura et al., 1989). Determination of \(\delta\) and \(\kappa\) opioid receptor binding parameters was performed by heterologous competition with \([3H]\)diprenorphine binding, with DPDPE and U-50,488H as specific displacers respectively (Fig. 1). Two binding sites fitted best each of the competition curves as detected by the Inplot 4 program. The parameters are shown in Table 1.

Morphine acts on ventricular myocytes from neonatal rats by two distinct pathways distinguishable by their sensitivity to pertussis toxin: (1) Increased \(\text{Ca}^{2+}\) influx leading to increased \([\text{Ca}^{2+}]_i\), transients; (2) Decreased intracellular pH leading to reduced myofibril responsiveness to \(\text{Ca}^{2+}\) (Ela et al., 1993).

Further experiments were designed to determine which of the opioid receptors mediated each component of the effect of morphine. Experiments were done in the presence of naltrindole, a selective antagonist of \(\delta\) opioid receptors (Portoghese et al., 1988), to determine the components mediated through the \(\kappa\) opioid receptors, and in the presence of norbinaltorphimine dihydrochloride (nor-BNI), a selective antagonist of the \(\kappa\) opioid receptors (Portoghese et al., 1987), to determine the components mediated through the \(\delta\) opioid receptors.

Exposure of ventricular myocytes to nor-BNI alone did not affect the rate of \(\text{Ca}^{2+}\) influx, but the presence of nor-BNI together with morphine abolished the morphine-induced increase in \(\text{Ca}^{2+}\) influx (Fig. 2). On the other hand, the \(\delta\) antagonist naltrindole did not affect the morphine-induced increase in \(\text{Ca}^{2+}\) influx. An increase to 356% of control level was observed in the presence of morphine and naltrindole. Thus, the morphine-induced increase in \(\text{Ca}^{2+}\) influx is mediated via the \(\kappa\) opioid receptors.

Preincubation of ventricular myocytes for 10 min with naltrindole (1 nM) alone did not affect the intracellular pH. However, the subsequent exposure
Table 1. Binding parameters of \(\delta\) and \(\kappa\) opioid receptor agonists to the \(\delta\) and \(\kappa\) opioid receptors in membranes from cultured neonatal rat cardiomyocytes.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>(K_{i,1}) (nm)</th>
<th>(K_{i,2}) (nm)</th>
<th>(B_{\text{max},1}) (fmol/mg protein)</th>
<th>(B_{\text{max},2}) (fmol/mg protein)</th>
<th>(B_{\text{max}}) total (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-50,488H</td>
<td>0.22 ± 0.33</td>
<td>32 ± 5</td>
<td>11.0 ± 3.0</td>
<td>13.2 ± 2.2</td>
<td>24.0 ± 5.2</td>
</tr>
<tr>
<td>DPDPE</td>
<td>0.73 ± 0.07</td>
<td>140 ± 13</td>
<td>2.4 ± 0.3</td>
<td>10.5 ± 1.5</td>
<td>12.9 ± 2.8</td>
</tr>
</tbody>
</table>

Determination of \(\delta\) and \(\kappa\) opioid receptor binding parameters was performed by heterologous competition to \([3H]\)diprenorphine binding, with DPDPE and U-50,488H as specific displacers respectively (see Materials and Methods and Fig. 1). The values were determined by the Inplot 4 program.

Figure 2. Effects of morphine, in the presence and absence of nor-BNI, on the rates of \(45\text{Ca}^{2+}\) influx into neonatal cardiac myocytes. The cardiac myocytes, loaded with BCECF, were incubated in BSS at 37°C. The arrow indicates the addition of morphine (1 \(\mu\)M) (▲), or morphine + nor-BNI (1 nM) (●). At the indicated times, \(45\text{CaCl}_2\) (4 \(\mu\)Ci/well) was added for 15 s, followed by washing the cells and determination of the radioactivity within the cells as described in Materials and Methods. Data are means ± S.E. of six measurements.

Figure 3. Effects of morphine in the presence and absence of naltrindole on the intracellular pH in the neonatal cardiac myocytes. The cardiac myocytes, loaded with BCECF, were incubated in BSS at 37°C. The arrows indicate the addition of morphine alone (1 \(\mu\)M) (—▲—), or naltrindole alone (1 nM) at 0 time followed by the addition of morphine (1 \(\mu\)M) + naltrindole (1 nM) at 10 min (—■—). Intracellular pH was determined as described in Materials and Methods. A representative experiment is shown. Similar results were obtained in five experiments.
It appears that the overall effect of morphine on the ASM (no change) is the sum of the positive and negative effects mediated via the $\kappa$ and $\delta$ opioid receptors, respectively. It is not clear why nor-BNI alone and naltrindole alone caused small positive inotropic effects (by 7%). The effect of naltrindole may be caused by antagonizing endogenous opioid peptides which are produced in cultured ventricular myocytes (Springhorn and Claycomb, 1989).

It has been found previously that preincubation with pertussis toxin abolished the morphine-induced decrease in intracellular pH (Ela et al., 1993 and Fig. 3). We examined therefore the effect of morphine on ASM and on $[Ca^{2+}]_{i}$ transients after preincubation with pertussis toxin. Similar to the results obtained in the presence of naltrindole but to a larger extent, a positive inotropic effect was induced by morphine, reaching 135% of the control level, in cells preincubated with pertussis toxin (Fig. 6). On the other hand, preincubation with pertussis toxin did not affect the increase in $[Ca^{2+}]_{i}$ transients induced by morphine (Fig. 6).

**Discussion**

It was found in the present study that $[^3H]$dipronorphine, an analogue of morphine, showed specific binding to membranes of cultured cardiac myocytes from neonatal rats and could be displaced by $\delta$ and $\kappa$ opioid receptor agonists. Analysis of the displacement curves suggested the presence of $\delta$ and $\kappa$ opioid receptors with two subclasses of binding sites for each receptor type ($K_i$ values below nanomolar and at the nanomolar range). The presence of $\delta$ and $\kappa$ opioid receptors on the sarcolemma of isolated cardiac myocytes from adult rats was found previously in binding studies with labeled $\delta$ and $\kappa$ agonists (Ventura et al., 1989). In the study of Ventura et al., (1989) a single class of binding sites was detected for each receptor, with $K_i$ values in the low nanomolar range. On the other hand, Jin et al. (1995) reported in membrane homogenate from adult rat hearts the presence of two subclasses of $\kappa$-binding sites, similar to $\kappa_{1a}$ and $\kappa_{1b}$ reported by Rothman et al. (1990). The differences in binding parameters between the results of the various experiments may originate from the differences in the type of cells (neonatal vs adult), in the methods of membrane preparation or in the experimental procedures.

Consistent with the binding results it was found in the present study that the effect of morphine on neonatal rat cardiac myocytes is composed of two
Morphine Effects on Cardiomyocytes

The effects of morphine on cardiomyocytes were mediated through the δ and κ opioid receptors. In the presence of a specific antagonist to the δ opioid receptors, morphine caused, via the κ receptors, a transient increase in Ca²⁺ influx, leading to increased [Ca²⁺], transients, and increased amplitude of contraction, with no effect on the intracellular pH. In the presence of a specific antagonist to the κ opioid receptor the effects of morphine were mediated through the δ opioid receptors and consisted of a decrease in the amplitude of contraction, caused by a decrease in the intracellular pH leading to reduced responsiveness of the myofilaments to Ca²⁺ (Fabiato and Fabiato, 1978). There was no change in Ca²⁺ influx and in the amplitude of [Ca²⁺], transients. Preincubation with pertussis toxin led to effects similar to those obtained in the presence of naltrindole: no decrease in intracellular pH and a positive inotropic effect in response to morphine. On the other hand, preincubation with pertussis toxin did not affect the increase in [Ca²⁺], transients induced by morphine via the κ opioid receptors. Our results indicate, therefore, that the δ opioid receptors in cardiac myocytes, but not the κ opioid receptors, are coupled to pertussis toxin sensitive GTP-binding proteins.

The overall effects of morphine on neonatal cardiac myocytes consisted of the sum of the effects exerted by morphine via each of the opioid receptors alone. The direct effects of specific δ and κ opioid receptor agonists on the functions of cardiac myocytes have been previously studied in isolated cardiac myocytes from adult rats (Ventura et al., 1992). In these cells the κ opioid receptor agonist U-50,488H caused a transient increase in contraction which was followed by a sustained decrease. These changes were accompanied by alkalosis and increased responsiveness of the myofilaments to Ca²⁺. The δ opioid receptor agonist DPDPE caused a sustained decrease in contractility without an initial increase. Changes similar to these in the contractile amplitudes were observed in [Ca²⁺], transients. Marked and sustained increase in inositol 1,4,5-trisphosphate (IP₃) was observed following κ and δ opioid receptor stimulation. It was suggested that the negative inotropic responses to the κ and δ opioid receptor agonists were mediated by sarcoplasmic reticulum Ca²⁺ depletion (Ventura et al., 1992).

The differences between the responses to opioid receptor stimulation in neonatal and adult rat cardiac myocytes may result from the differences in the developmental stage of the cardiomyocytes. In this regard, developmental differences in the responses to opioid receptor stimulation have been reported in nerve cells from chicks and from rats.
Neonatal ventricular myocytes are immature cells with less developed sarcoplasmic reticulum than adult cells. Studies on the effects of ryanodine and nicardipine on the inotropic response of cardiac muscles of neonatal and adult rats indicated that contraction of adult rat myocardium is highly dependent on Ca\(^{2+}\) release from the sarcoplasmic reticulum, while that of the neonatal rat is more dependent on trans-sarcolemma Ca\(^{2+}\) influx (Tanaka and Shigenobu, 1989). Comparison of the effects of thapsigargin on the amplitude of systolic motion in neonatal and adult cardiomyocytes has shown that inhibition of the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase by thapsigargin in adult cells decreased the amplitude of contraction by 76.3%, whereas complete inhibition of the function of the sarcoplasmic reticulum in neonatal myocytes reduced the amplitude by only 34% of control (Ela et al., 1994; Novakova et al., 1995). These differences may help to explain the different response to morphine in neonatal and adult rat cardiac myocytes. Because in neonatal cardiac myocytes contraction depends mainly on the trans-sarcolemma Ca\(^{2+}\) influx, effects of \(\delta\) and \(\kappa\) receptor ligands on sarcoplasmic reticulum Ca\(^{2+}\) release may not be observed, or alternatively, do not exist in neonatal cardiac myocytes.

Differences in responses to activation of other receptors in cardiomyocytes from neonatal rats and adult rabbits and rats have been reported recently (Kohmoto et al., 1993). Exposure of cultured neonatal rat ventricular myocytes to endothelin-1 (10 nM), angiotensin II (10 nM) or 12-O-tetradecanoylphorbol-13-acetate (TPA) (80 nM) decreased both the amplitude of cell contraction and the \([Ca^{2+}]_i\) transients. The same ligands induced marked increases in the amplitude of contraction with no change in \([Ca^{2+}]_i\) transients in adult rabbit cardiomyocytes. A similar increase in contraction amplitude induced by endothelin-1 has been observed in adult rat ventricular myocytes (Kramer et al., 1991). The mechanisms mediating these differences have not yet been elucidated. Differences were also found in the responses of ventricular myocytes from neonatal and adult rats to the activation of \(\sigma\) receptors (Novakova et al., 1995).

An additional cause for the difference between the results of the two groups may be the differences in the experimental procedures, since the results of Ventura et al. (1991b; 1992) were obtained at 23°C and the results of the present study were obtained at 37°C.

Our results show that activation of \(\kappa\) receptors in neonatal rat cardiac myocytes increased the influx of Ca\(^{2+}\) and the amplitude of \([Ca^{2+}]_i\) transients. This result is consistent with the finding that \(\kappa\) agonists cause arrhythmia in perfused hearts (Wong and Lee, 1987; Wong et al., 1990) and increase the level of cytosolic free Ca\(^{2+}\) in isolated rat ventricular myocytes (Tai et al., 1992; Ventura et al., 1994). The mechanism of the response to \(\kappa\) receptor activation is not known. It was previously reported that exposure of adult rat cardiomyocytes to the \(\kappa\) agonist U-50,488H causes a marked increase in inositol 1,4,5-trisphosphate (IP\(_3\)) and in inositol 1,3,4,5-tetrakisphosphate (IP\(_4\)) formation (Ventura et al., 1991a). It has been found in various types of cells that IP\(_3\) (Shirakawa and Miyazaki, 1995) and IP\(_4\) (Mochizuki-Oda et al., 1994) increase the rates of Ca\(^{2+}\) influx across cell membranes (see also Berridge, 1993), either directly or indirectly via a diffusible messenger activated by depletion of IP\(_3\) – Ca\(^{2+}\) stores (Randriamampita and Tsien, 1993; Parekh et al., 1993). If similar mechanisms operate in cardiac myocytes, the observed increase in Ca\(^{2+}\) influx in neonatal cardiac myocytes following \(\kappa\) opioid receptor activation may be mediated through a similar pathway. The increase in the amplitude of \([Ca^{2+}]_i\) transients caused by \(\kappa\) receptor activation, appears to be mediated by the increase in Ca\(^{2+}\) influx (present study) together with mobilization of Ca\(^{2+}\) from intracellular stores by IP\(_4\) (Ventura et al., 1992). However, in neonatal cardiomyocytes the contribution of the second component to the amplitude of \([Ca^{2+}]_i\) transients is relatively smaller than in adult cardiac myocytes (Tanaka and Shigenobu, 1989).

Activation of the \(\delta\) opioid receptors led to a negative inotropic response in cardiomyocytes from both adult and neonatal rats but the mechanisms mediating these effects appear to be different. In adult cardiomyocytes activation of \(\delta\) receptors led to depletion of sarcoplasmic reticulum Ca\(^{2+}\) (Ventura et al., 1992), whereas in neonatal cardiac myocytes this activation led to a decrease in intracellular pH and consequently to reduced responsiveness of the myofilaments to Ca\(^{2+}\). This response was mediated via pertussis toxin sensitive GTP-binding proteins, protein kinase C and the Na\(^+\)/H\(^+\) exchanger (Ela et al., 1993 and the present study). A different response was reported in chick embryo cardiomyocytes, in which \(\delta\) receptor agonists caused an increase in contractility mediated by an increase in the level of cAMP (Laurent et al., 1985; 1986). It appears that the responses and the signal transduction pathways coupled to \(\delta\) opioid receptor activation in cardiac myocytes are different in various species and may depend on the developmental stage.
In conclusion in the present study we show that morphine activates both κ and δ opioid receptors in cardiac myocytes from neonatal rats. Activation of each of the opioid receptors alone leads to specific and different effects which are mediated via a different signal transduction pathway. The overall effect of morphine on cardiac myocytes from neonatal rats appears to be the sum of the effects mediated by the δ and κ opioid receptors with no evidence for “cross-talk” between the signals from the two receptors.

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