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Effects of κ- and μ-opioid receptor agonists on Ca^{2+} channels in neuroblastoma cells: involvement of the orphan opioid receptor

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Abstract

The effects of μ-, δ- and κ-opioid receptor agonists, and orphanin FQ/nociceptin (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln), on K^+-induced $[Ca^{2+}]_i$ increase were examined in SK-N-SH cells. Exposure to K^+ (50 mM) resulted in a $[Ca^{2+}]_i$ rise, which was blocked (−85%) by furaldipine (1 μM) and increased (63%) by BayK 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate) (0.5 μM), indicating the involvement of L-type Ca^{2+} channels. The κ-opioid receptor agonists 3,4-dichloro-*N*-Methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U-50488H) (1-50 μM) and 5,7,8-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide (U-69593) (25 μM), and the μ-opioid receptor agonist sufentanil (100 nM–3 μM) inhibited the amplitude of K^+-induced $[Ca^{2+}]_i$ increase. The agonist of the orphan opioid receptor, orphanin FQ/nociceptin (1 μM), induced dual excitatory and inhibitory effects on the depolarisation-induced Ca^{2+} influx. The effects of the opioid receptor agonists were not blocked by the κ-opioid receptor antagonist nor-binaltorphimine (1 μM), only weakly prevented by naloxone (10–100 μM) and naltrexone (100 μM), and partially prevented by pertussis toxin (100 ng/ml, 24 h). The antagonist of the orphan opioid receptor, $[Phe^1\Psi(CH_2–NH)Gly^2]$nociceptin(1–13)$NH_2$ (1 μM), prevented the inhibitory effect of U-50488H, sufentanil and orphanin FQ. The present study provides pharmacological evidence for the presence of L-type Ca^{2+} channels in SK-N-SH cells, that are modulated by opioids through orphan opioid receptor activation.

Keywords: Opioid receptor; Orphanin FQ; Nociceptin; Ca^{2+} channels; Dihydropyridine

1. Introduction

The molecular mechanism by which opioids inhibit neurotransmitter release and reduce cellular excitability may involve blockade of voltage-dependent Ca^{2+} channels (North, 1993). It has been reported that activation of presynaptic μ- and δ-opioid receptors indirectly reduces voltage-dependent Ca^{2+} currents, by increasing K^+ conductance and shortening the action potential (North, 1993). In addition, activation of μ-, δ- and κ-opioid receptors may block directly Ca^{2+} influx, by inhibiting voltage-dependent Ca^{2+} channels in the nerve terminal (North, 1993). On the basis of electrophysiological studies, it has been proposed that the main Ca^{2+} current inhibited by opioids is a high-voltage-activated type, blocked by ω-conotoxin-GVIA, and insensitive to dihydropyridines (i.e., N-type) (Gross and Macdonald, 1987; Surprenant et al., 1990; Schroeder et al., 1991; Seward et al., 1991; Moises et al., 1994; Rhim and Miller, 1994). Nevertheless, a number of pharmacological studies suggest that dihydropyridine-sensitive Ca^{2+} channels (L-type) are involved in several opioid receptor-mediated effects, such as analgesia, respiratory depression, tolerance/dependence, abstinence, etc. (Hoffmeister and Tettenborn, 1986; Bongianni et al., 1986; Baeyens et al., 1987; Dierssen et al., 1990, 1991; Ruiz et al., 1993; Santillán et al., 1994, 1998; Barro et al., 1995; Díaz et al., 1995a,b). It has been reported that the association of L-type Ca^{2+} channel blockers with μ-opioid receptor agonists results in potentiation of the opioid effects and in inhibition of tolerance/dependence (Díaz et al., 1995a,b). Furthermore, amongst the adaptive responses brought about by chronic exposure to opioids, up-regulation of ^{3}Hdihydropyridine-binding sites plays an important role in tolerance development (Ramkumar and El-Fakahany, 1988; Díaz et al., 1995a).

The aim of this study was to investigate the possible coupling between opioid receptors and the dihydro-

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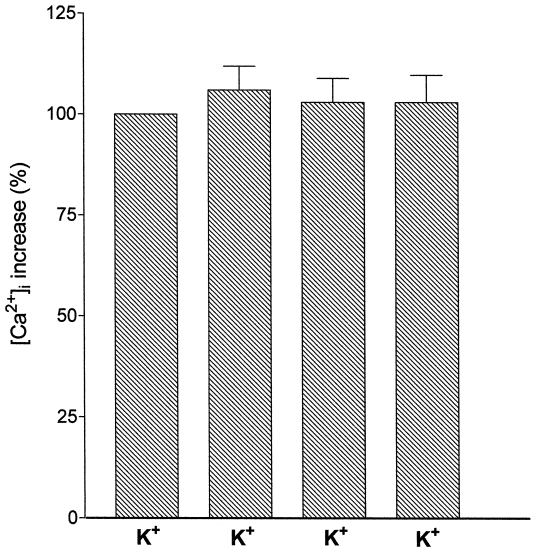

Fig. 1. $[Ca^{2+}]_i$ increase induced by superfusion of control cells ($n = 55$) with a depolarising solution containing 50 mM K^+. The stability of the response was tested by applying four consecutive depolarising pulses, at 5 min intervals.

pyridine-sensitive Ca^{2+} channel, in a homogeneous cell population in culture. The human neuroblastoma derived cell line SK-N-SH was selected because it displays opioid receptors, and has been well-characterised as a neuronal model (Yu et al., 1986; Lambert et al., 1989; Baumhaker et al., 1993; Fields et al., 1995). The effects of opioids on Ca^{2+} entry through voltage-activated Ca^{2+} channels were measured by Ca^{2+} imaging SK-N-SH cells loaded with fura-2.

2. Methods

2.1. Cell culture

SK-N-SH neuroblastoma cells (obtained from the American Type Culture Collection, Rockville, MD, USA) were grown in Dulbecco's modified Eagle's medium (Sigma), supplemented with 10% foetal calf serum, 100 U/ml penicillin G, and 100 μg/ml streptomycin, in a humidified atmosphere of 5% CO_2, at 37°C, and fed every 3 days. Cells for the experiments were replated on 11 mm diameter glass coverslips and incubated 48 h prior to experiments. For treatment with pertussis toxin, cells were incubated with the toxin (100 ng/ml) for 24 h.

2.2. Intracellular Ca^{2+} concentration measurements

SK-N-SH neuroblastoma cells grown on glass coverslips were incubated with 4 μM fura-2 acetoxymethyl ester plus 0.2% pluronic F-127 (to make cells more permeable to fura-2), for 60 min, at room temperature, in standard incubation medium (composition in mM: NaCl, 145; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 0.2; NaHEPES, 10; glucose, 10; pH = 7.4). The coverslips were then placed under the microscope (Nikon, Diaphot) in a superfusion chamber, thermostated to 37ºC, and epi-illuminated, alternately, at 340 nm and 380 nm. Light emitted above 520 nm was recorded by an extended ISIS-M camera (Photonic Science, Robertsbridge, East Sussex, UK), and analysed using an Applied Imaging Magical image processor (Sunderland, Tyne and Wear, UK), with 64 Mb video RAM. Four video frames of each wavelength were averaged by hardware, with an overall time resolution of 3 s for each pair of images at alternate wavelengths. The resulting 340 nm images were normalised with respect to the 380 nm images (340/380) pixel by pixel to produce ratio images, and $[Ca^{2+}]_i$ was estimated from F_{340}/F_{380} (Grynkiewicz et al., 1985) by comparison with a calibration table, constructed by imaging fura-2 solutions with known Ca^{2+} concentrations using the same microscope objective. Other details were as described previously (Villalobos et al., 1992).

2.3. Drugs and chemicals

Fura-2/AM and pluronic F-127 were purchased from Molecular Probes (Eugene, OR, USA). The κ-opioid receptor agonists U-50488H (3,4-dichloro-*N*-Methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide) and U-69593 (5,7,8-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro [4,5]dec-8-yl]benzeneacetamide), the opioid receptor antagonists naloxone and naltrexone, and the κ-opioid receptor selective antagonist nor-binaltorphimine were purchased from RBI (Natick, MA, USA). The μ-opioid receptor agonist [D-Ala^2, *N*-$MePhe^4$,$Gly\text{-}ol^5$]enkephalin (DAGO), the δ-opioid receptor agonist [D-Ser^2-Leu,

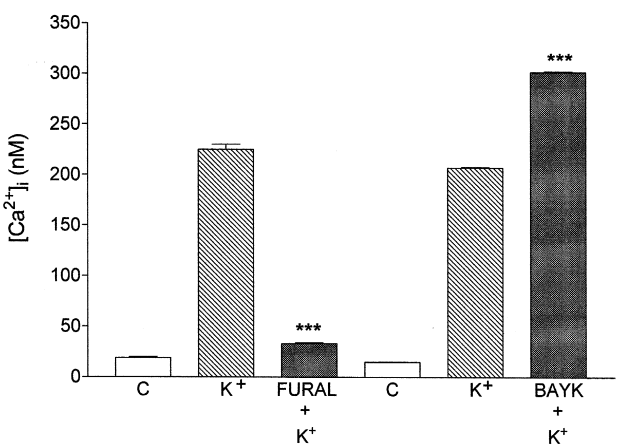

Fig. 2. Effects of dihydropyridines on $[Ca^{2+}]_i$ increase induced by 50 mM K^+. In the control test, superfusion of the cells with a solution containing 50 mM K^+ increased $[Ca^{2+}]_i$ to a peak level of 225 ± 18 nM. In the presence of the L-type Ca^{2+} channel blocker furaldipine (1 μM), the increase in $[Ca^{2+}]_i$ was reduced by −85%, whereas the Ca^{2+} channel activator BayK 8644 (0.5 μM) enhanced the amplitude of the response by 63%. Bars represent means ± S.E.M. $^{***}P < 0.001$ when compared with K^+ control response (paired *t*-test).

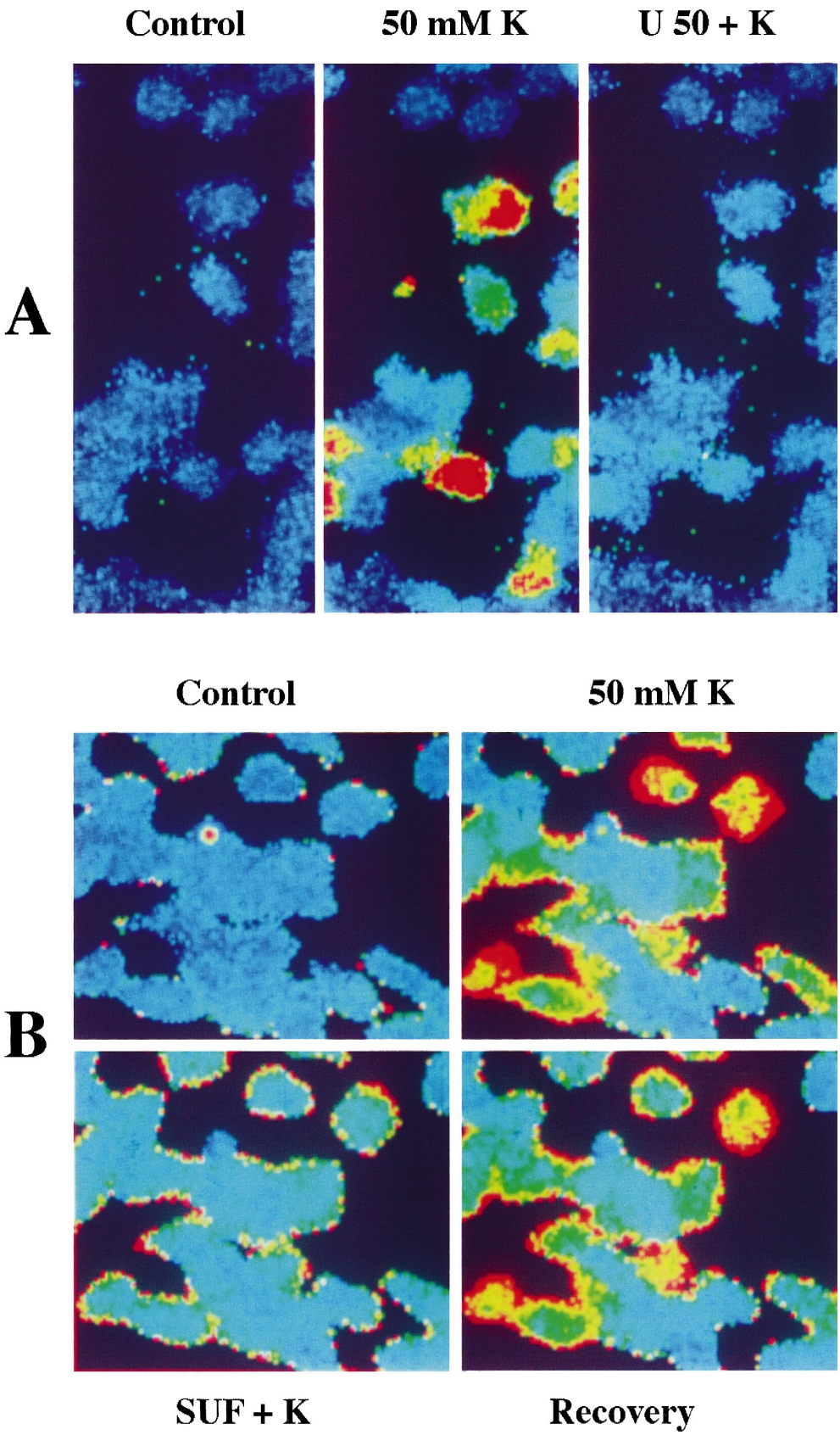

Fig. 3. Fura-2 Ca^{2+} imaging in SK-N-SH culture. (A) Left: control condition. Centre: exposure to 50 mM K^+ for 1 min. Right: response to 50 mM K^+ exposure in the presence of 25 μM U-50488H. (B) Top left: control condition. Top right: exposure to 50 mM K^+ for 1 min. Bottom left: response to 50 mM K^+ exposure in the presence of 3 μM sufentanil. Bottom right: recovery of the response to 50 mM K^+ after washing sufentanil.

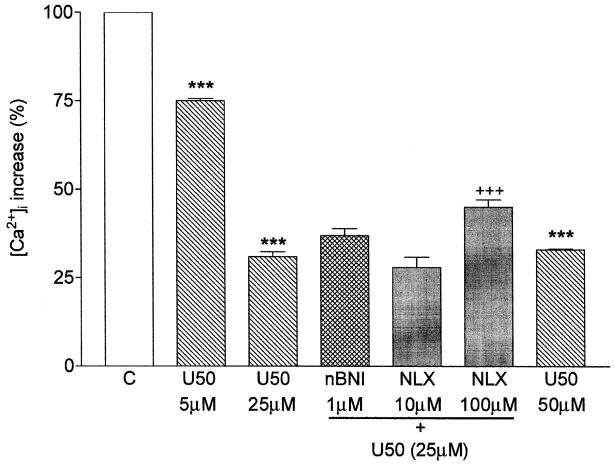

Fig. 4. Concentration-dependent inhibitory effect induced by U-50488H (5–50 μM) on $[Ca^{2+}]_i$ induced by depolarisation with 50 mM K^+; lack of antagonism with nor-binaltorphimine (1 μM) and partial antagonism with naloxone (NLX) (10–100 μM). Data are expressed as percentage of reduction in relation with the control response. Bars represent means ± S.E.M. $^{***}P < 0.001$ when compared with control response (Newman–Keuls test). $^{++}P < 0.001$ when compared with 25 μM U-50488H response (paired *t*-test).

enkephalin-Thr (DSLET), orphanin FQ/nociceptin (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln), and pertussis toxin were purchased from Sigma (Madrid, Spain). [Phe$^1\Psi$(CH$_2$–NH)Gly^2]Nociceptin(1–13)NH$_2$ was purchased from Tocris (Biogen Científica S.L., Madrid, Spain). Sufentanil (μ-opioid receptor agonist) was kindly supplied by Janssen Cylag (Madrid, Spain). Furaldipine (Ca^{2+} channel blocker) and BayK 8644 [methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate] (Ca^{2+} channel agonist) were kindly provided by Alter (Madrid, Spain), and Química Farmacéutica Bayer (Barcelona, Spain), respectively. All other chemicals were purchased from Sigma.

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. of at least three independent experiments. Statistical analysis was performed using Students's paired and unpaired tests and, for multiple comparisons, the Newman–Keuls test (GraphPAD InStat software). Significance level was set at $P < 0.05$.

3. Results

In order to depolarise cells, and to determine if Ca^{2+} influx through voltage-activated channels could alter $[Ca^{2+}]_i$, extracellular K^+ was increased from 5 to 50 mM for 1 min. In the control group ($n = 55$), superfusion of the cells with the solution containing 50 mM K^+, increased $[Ca^{2+}]_i$ to a peak level of 280 ± 16 nM. The stability of the response was tested by applying three consecutive depolarising pulses, at 5 min intervals. In all cases, the response elicited was almost identical (Fig. 1). After withdrawal of the high-K^+ solution, $[Ca^{2+}]_i$ returned to basal resting levels within 1 min. In the presence of the L-type Ca^{2+} channel blocker furaldipine (1 μM) ($n = 12$), the increase in $[Ca^{2+}]_i$ induced by 50 mM K^+ was reduced by −85% ($P < 0.001$), in a reversible fashion (Fig. 2). On the other hand, the Ca^{2+} channel agonist BayK 8644 (0.5 μM) ($n = 240$) enhanced by 63% the amplitude of the high-K^+ induced increase in $[Ca^{2+}]_i$ ($P < 0.001$) (Fig. 2). These results indicate the involvement of L-type Ca^{2+} channels in the depolarisation-mediated Ca^{2+} influx.

The amplitude of the high-K^+ induced increase in $[Ca^{2+}]_i$ was also used as an index for analysis of the effect of opioids. After recording a control response to 1 min superfusion with the high-K^+ solution, the cell was superfused for 5 min with a solution containing the opioid receptor agonist, and then the depolarising pulse was repeated in the presence of the opioid. To evaluate the participation of specific opioid receptors, this test was repeated in the presence of different opioid receptor antagonists, such as naloxone (10–100 μM), naltrexone (10–100 μM), the selective κ-opioid receptor antagonist nor-binaltorphimine (1 μM), and the antagonist of the orphanin receptor [Phe$^1\Psi$(CH$_2$–NH)Gly^2]Nociceptin(1–13)NH$_2$ (1 μM). Some experiments, with a similar experimental protocol, were performed in cells pre-treated with pertussis toxin (100 ng/ml, 24 h) to assess the involvement of a receptor coupled to G_i or G_o proteins.

The κ-opioid receptor agonist U-50488H (5–50 μM; $n = 253$) induced a concentration-dependent inhibitory effect on the amplitude of high-K^+ induced increase of

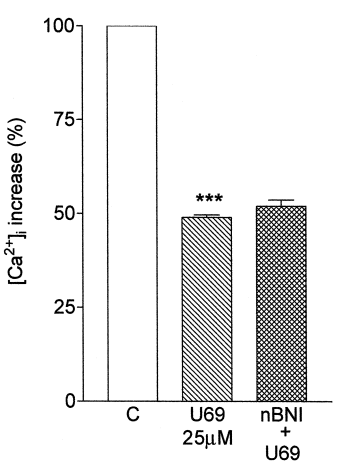

Fig. 5. Inhibitory effect induced by 25 μM U-69593 on $[Ca^{2+}]_i$ increase induced by depolarisation with 50 mM K^+ and the lack of antagonism with 1 μM nor-binaltorphimine. Data are expressed as percentage of reduction in relation with the control response. Bars represent means ± S.E.M. $^{***}P < 0.001$ when compared with 25 μM U-69593 control response (paired *t*-test).

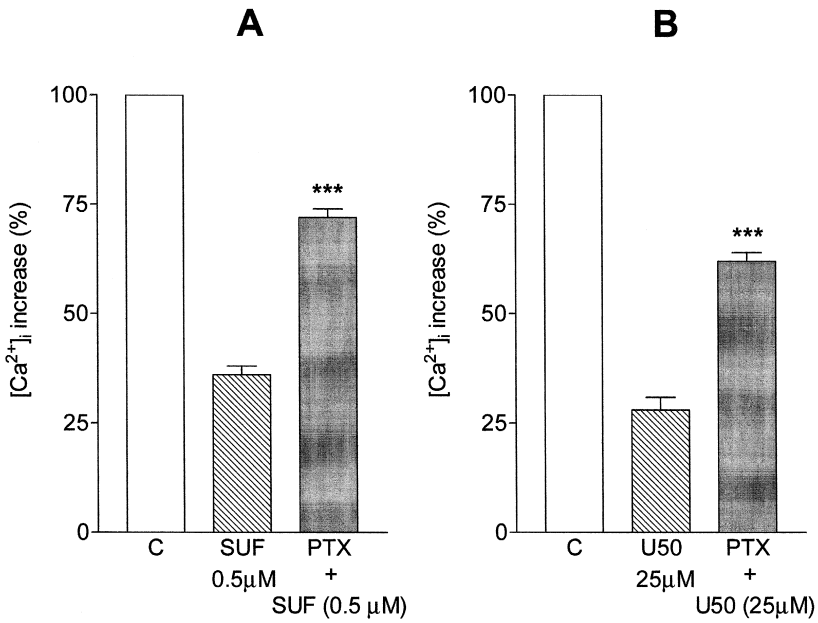

Fig. 6. Inhibitory effect induced by 0.5 μM sufentanil (A) and 25 μM U-50488H (B) on $[Ca^{2+}]_i$ increase induced by depolarisation with 50 mM K^+ and the antagonism with pertussis toxin (200 ng/ml, 24 h). Data are expressed as percentage of reduction in relation with the control response. Bars represent means ± S.E.M. ***$P < 0.001$ when compared with 0.5 μM sufentanil or 25 μM U-50488H control response (Student's *t*-test).

$[Ca^{2+}]_i$ (Figs. 3 and 4). This inhibition reached a maximum value of −70% of the control response, at a concentration of 25 μM. Similar results were obtained with the κ-opioid receptor agonist U-69593, at a concentration of 25 μM ($n = 43$)(Fig. 5). Incubation of cells for 24 h ($n = 51$) with pertussis toxin (100 ng/ml) partially blocked the U-50488H (25 μM) inhibitory effect on the $[Ca^{2+}]_i$ increase induced by K^+ (Fig. 6). However, the response was not blocked by nor-binaltorphimine (1 μM; $n = 28$) or by naloxone (10–100 μM; $n = 49$) (Figs. 4 and 5). The antagonist of the orphan opioid receptor [$Phe^1\Psi(CH_2$–$NH)Gly^2$]Nociceptin(1–13)NH_2 (1 μM) completely prevented the inhibitory effect of U-50488H in 62% of all cells tested ($n = 52$) (Fig. 7). The remaining 38% of the cells recovered the response to the high-K^+ solution after washing-out the opioid, indicating the reversibility of the effect.

The μ-opioid receptor agonist sufentanil (100 nM–3 μM; $n = 111$) also induced a concentration-dependent inhibitory effect on the amplitude of high-K^+ induced in-

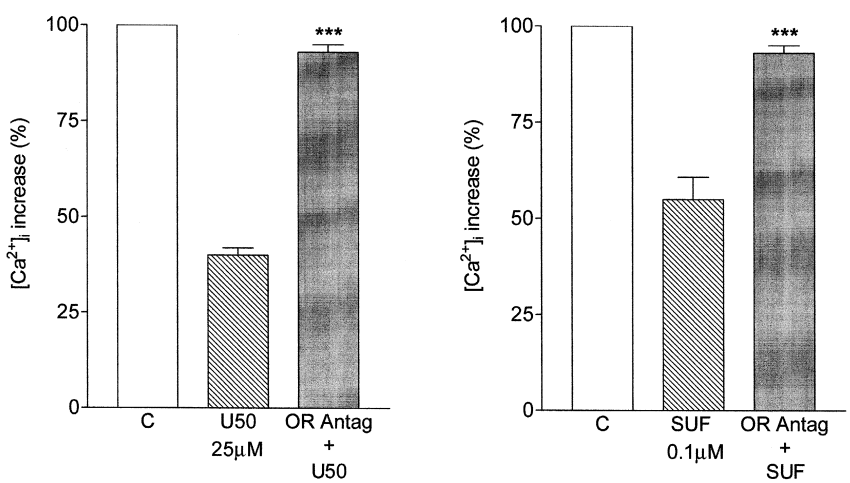

Fig. 7. Inhibitory effect induced by 25 μM U-50488H (A) and 0.1 μM sufentanil (B) on $[Ca^{2+}]_i$ increase induced by depolarisation with 50 mM K^+ and the antagonism with the antagonist of orphan opioid receptors [$Phe^1\Psi(CH_2$–$NH)Gly^2$]Nociceptin(1–13)NH_2 (1 μM) (OR Antag). Data are expressed as percentage of reduction in relation with the control response. Bars represent means ± S.E.M. ***$P < 0.001$ when compared with 25 μM U-50488H or 0.1 μM sufentanil control response (Student's *t*-test).

crease in $[Ca^{2+}]_i$ (Figs. 3 and 8). This inhibition was almost complete (−94% of the control response), at a concentration of 3 μM. The effect was weakly antagonised by naloxone ($n = 36$) and naltrexone ($n = 42$), and partially prevented by pertussis toxin ($n = 48$) (Figs. 6 and 8). The antagonist of the orphan opioid receptor [Phe$^1\Psi$(CH$_2$–NH)Gly^2]Nociceptin(1–13)NH$_2$ (1 μM) completely prevented the inhibitory effect of sufentanil (0.1 μM) in 74% of the cells tested ($n = 29$) (Fig. 7). The remaining 26% of the cells recovered the response to the high-K^+ solution after washing-out the opioid, indicating the reversibility of the effect.

Unlike sufentanil, the μ-opioid receptor agonist DAGO (1–10 μM; $n = 28$) had no effect on the depolarisation-induced Ca^{2+} influx (results not shown). The δ-opioid receptor agonist DSLET (100 nM–10 μM; $n = 31$) did not have any effect (results not shown).

The agonist of the orphan opioid receptor orphanin FQ/nociceptin (1 μM) induced dual effects on the depolarisation-induced Ca^{2+} influx. In 60% of all cells tested ($n = 30$), we observed a 40 ± 7% inhibition of the response, whereas in the remaining 40% of the cells, depolarisation-induced Ca^{2+} influx was increased to 143 ± 12% (Fig. 9). The reproducibility of the response was tested by applying two consecutive pulses of the drug. The excitatory or inhibitory response elicited in a given cell was almost identical. Both, the inhibitory and the excitatory effects of orphanin FQ/nociceptin, were completely prevented by pre-treatment with the antagonist of orphan opioid receptors [Phe$^1\Psi$(CH$_2$–NH)Gly^2]Nociceptin(1–13)NH$_2$ (1 μM) (Fig. 9).

The antagonist of the orphan opioid receptor [Phe^1-Ψ(CH$_2$–NH)Gly^2]Nociceptin(1–13)NH$_2$ showed per se little effect on depolarisation-induced Ca^{2+} influx. In 55% of all cells tested ($n = 29$), we observed a 13.8 ± 3.5% inhibition, and in 45% of the cells, the response was slightly increased (+14.9 ± 5.1%).

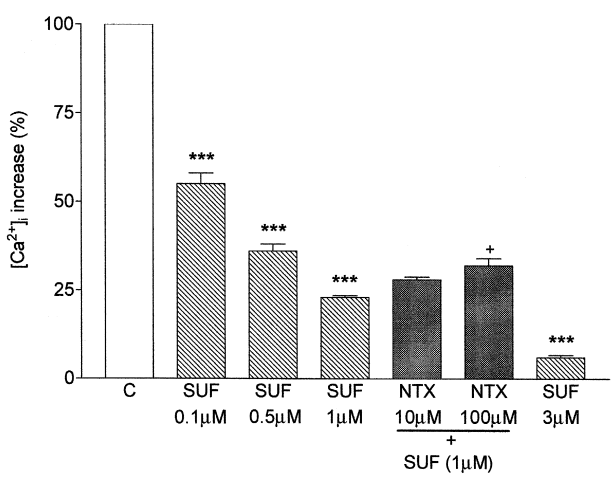

Fig. 8. Concentration-dependent inhibitory effect induced by sufentanil (0.1–3 μM) on $[Ca^{2+}]_i$ increase induced by depolarisation with 50 mM K^+ and the partial antagonism with naltrexone (NTX) (10–100 μM). Data are expressed as percentage of reduction in relation with the control response. Bars represent means ± S.E.M. $^{***}P < 0.001$ when compared with control response (Newman–Keuls test). $^{+}P < 0.05$ when compared with 1 μM SUF response (paired *t*-test).

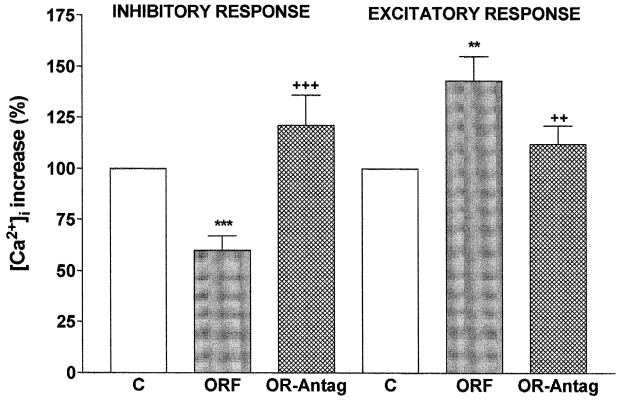

Fig. 9. Inhibitory (18 out of 30 cells) and excitatory (12 out of 30 cells) effects induced by 1 μM orphanin FQ (ORF) on $[Ca^{2+}]_i$ increase induced by depolarisation with 50 mM K^+ and the antagonism with the antagonist of orphan opioid receptors [Phe$^1\Psi$(CH$_2$–NH)Gly^2]Nociceptin(1–13)NH$_2$ (1 μM) (OR Antag). Data are expressed as percentage of change in relation with the control response. Bars represent means ± S.E.M. $^{***}P < 0.001$ when compared with orphanin FQ control responses (paired *t*-test).

4. Discussion

The present study provides pharmacological evidence for the presence of L-type Ca^{2+} channels in undifferentiated SK-N-SH cells, modulated by opioid drugs through orphan opioid receptor activation.

In this human neuroblastoma cell line, the increase in $[Ca^{2+}]_i$ evoked by depolarisation with a solution containing 50 mM K^+ was prevented by furaldipine and potentiated by BayK 8644, suggesting that it is due to the entry of Ca^{2+} through dihydropyridine-sensitive channels. Therefore, the amplitude of the high-K^+ induced increase in $[Ca^{2+}]_i$ was used as an index to analyse the effect of opioids on Ca^{2+} influx via L-type channels. Our results demonstrate that the depolarisation-induced Ca^{2+} transient was depressed concentration-dependently by the μ-selective opioid receptor agonist sufentanil, and by the κ-opioid receptor agonists U-50488H and U-69593, but not by the δ-opioid receptor agonist DSLET.

The SK-N-SH cell line was initially reported to contain μ- and δ-receptors in a ratio 5:1 (Yu et al., 1986). However, it has been described phenotypic heterogeneity in this range due to differences in culture conditions (Baumhaker et al., 1993). Under the specified conditions of the present study, the δ-opioid receptor agonist DSLET had no effect on the depolarisation-induced Ca^{2+} influx in SK-N-SH cells, indicating the lack of involvement of δ-opioid receptors. On the other hand, parallel experiments performed on

NG108-15, a cell line which contain δ-opioid receptors exclusively, demonstrated a dose-dependent inhibitory effect of DSLET on L-type Ca^{2+} currents (unpublished observations).

Our results show that pertussis toxin prevents the inhibitory effect on Ca^{2+} influx through L-type channels induced by sufentanil and by U-50488H, indicating the involvement of specific receptors coupled to G_i/G_o proteins. The inability of the selective κ-opioid receptor antagonist *n*-binaltorphimine, and the extremely low efficacy of the non-selective opioid receptor antagonists naloxone and naltrexone to inhibit the effects, indicate that the opioids are not acting through the classical μ-, δ- and κ-opioid receptors. The interesting finding of the present study is the observation that the effects of sufentanil and U-50488 are blocked in about 70% of investigated cells by [$Phe^1\Psi(CH_2–NH)Gly^2$]Nociceptin(1–13)NH_2, a new selective and competitive antagonist at the orphan opioid receptor (Guerrini et al., 1998). We, therefore, conclude that the inhibitory effects of sufentanil and U-50488 on Ca^{2+} influx through L-type Ca^{2+} channels are mediated by the orphan opioid receptor. Both, the cell line SK-N-SH and its subclone SH-SY5Y, express the orphan opioid receptor in a density corresponding to approximately 45% of total opioid binding (Cheng et al., 1995, 1997; Wu et al., 1997). The orphan opioid receptor is a G protein-coupled receptor with functional as well as structural homology to opioid receptors (Mollerau et al., 1994; Cheng et al., 1995). This receptor has a low affinity (in the μM range) for prototypic opioid receptor agonists and antagonists (Zhang and Yu, 1995; Fawzi et al., 1997), which is consistent with the high concentrations of opioid drugs used in this study.

Supporting the presence in SK-N-SH of orphan opioid receptors functionally coupled to L-type voltage-sensitive Ca^{2+} channels, nociceptine/orphanin FQ, the putative endogenous ligand for the orphan opioid receptor (Meunier et al., 1995), reduces the amplitude of the high-K^+ induced increase in $[Ca^{2+}]_i$. It has been previously reported in SH-SY5Y and SK-N-SH cells that nociceptine/orphanin FQ inhibits voltage-dependent Ca^{2+} channels, and attenuates forskolin-induced cAMP accumulation through pertussis toxin-sensitive G proteins (Connor et al., 1996; Cheng et al., 1997; Wu et al., 1997). In dissociated rat pyramidal neurons, Knoflach et al. (1996) demonstrated, by electrophysiological procedures, that orphanin FQ was able to inhibit L-type Ca^{2+} channel currents. Under our experimental conditions, orphanin FQ/nociceptin displayed dual inhibitory and excitatory effects on Ca^{2+} influx through L-type channels. Like the other opioid drugs tested, in most cells, the response to orphanin FQ/nociceptin was a reduction of Ca^{2+} influx, but, in a significant proportion of investigated cells, orphanin FQ/nociceptin produced a potentiation of K^+-induced increase in $[Ca^{2+}]_i$. It has been previously reported that orphanin FQ/nociceptin, in the presence of the cholinergic agonist carbachol, produced an increase in $[Ca^{2+}]_i$ in SH-SY5Y cells (Connor et al., 1996). In this regard, stimulation of Ca^{2+} entry as a consequence of μ-, δ- and κ-opioid receptor activation has been reported to occur in a number of neuronal cell types, including the SK-N-SH cell line (Keren et al., 1994; Tang et al., 1995; Yin et al., 1997).

In summary, we conclude that the inhibitory effects on Ca^{2+} influx through L-type channels induced by the μ-opioid receptor agonist sufentanil, the κ-opioid receptor agonists U-50488H and U-69593, and by the ligand for the orphan opioid receptor nociceptin/orphanin FQ in SK-N-SH cells are mediated, under our experimental conditions, through orphan opioid receptors. It is worth noting that the antagonism by pertussis toxin of an opioid drug-induced effect is widely used to define its specific nature, without testing naloxone or any other opioid blocking agent. On the contrary, some of the effects of opioid drugs defined as non-opioid on the basis of the lack of antagonism by naloxone, could be mediated through the pertussis toxin-sensitive, naloxone-insensitive ORL1 receptors coupled to the same effectors than typical opioid receptors.

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References

Baeyens, J.M., Esposito, E., Ossowska, G., Samanin, R.D., 1987. Effects of peripheral and central administration of Ca^{2+} channel blockers in the naloxone-precipitated abstinence syndrome in morphine-dependent rats. Eur. J. Pharmacol. 137, 9–13.

Barro, M., Ruiz, F., Hurlé, M.A., 1995. κ-opiod receptor mediated antinociception in rats is dependent on the functional state of dihydropyridine-sensitive Ca^{2+} channels. Brain Res. 672, 148–152.

Baumhaker, Y., Gafni, M., Keren, O., Sarne, Y., 1993. Selective and interactive down-regulation of mu- and delta-opioid receptors in human neuroblastoma SK-N-SH cells. Mol. Pharmacol. 44, 461–467.

Bongianni, F., Carla, V., Moroni, F., Pellegrini-Sanpietro, D.E., 1986. Ca^{2+} channel inhibitors suppress the morphine-withdrawal syndrome in rats. Br. J. Pharmacol. 88, 561–567.

Cheng, J., Standifer, K.M., Tublin, P.R., Su, W., Pasternak, G., 1995. Demonstration of κ_3-opioid receptors in the SH-SY5Y human neuroblastoma cell line. J. Neurochem. 65, 170–175.

Cheng, Z.J., Fan, G.H., Zhao, J., Zhang, Z., Wu, Y.L., Jiang, L.Z., Zhu, Y., Ma, L., 1997. Endogenous opioid receptor-like receptor in human neuroblastoma SK-N-SH cells, activation of inhibitory G protein and homologous desensitization. Neuroreport 8, 1913–1918.

Connor, M., Yeo, A., Henderson, G., 1996. The effect of nociceptin on Ca^{2+} channel current and intracellular Ca^{2+} in the SH-SY5Y human neuroblastoma cell line. Br. J. Pharmacol. 118, 205–207.

Díaz, A., Ruiz, F., Flórez, J., Pazos, A., Hurlé, M.A., 1995a. Regulation of dihydropyridine-sensitive Ca^{2+} during opioid tolerance and supersensitivity in rats. J. Pharmacol. Exp. Ther. 274, 1538–1544.

Díaz, A., Ruiz, F., Flórez, J., Hurlé, M.A., Pazos, A., 1995b. μ opioid receptor regulation during opioid tolerance and supersensitivity in rat central nervous system. J. Pharmacol. Exp. Ther. 274, 1545–1551.

Dierssen, M.M., Flórez, J., Hurlé, M.A., 1990. Ca^{2+} channel modulation by dihydropyridines modifies sufentanil-induced antinociception in acute and tolerant conditions. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 559–565.

Dierssen, M.M., Ruiz, F., Flórez, J., Hurlé, M.A., 1991. Ca^{2+} channel modulation by dihydropyridines modifies sufentanil-induced respiratory depression in cats. Eur. J. Pharmacol. 198, 149–155.

Fawzi, A.B., Zhang, H., Weig, B., Hawes, B., Graziano, M.P., 1997. Nociceptin activation of the human ORL1 receptor expressed in Chinese hamster ovary cells, functional homology with opioid receptors. Eur. J. Pharmacol. 336, 233–242.

Fields, A., Gafni, M., Oron, Y., Sarne, Y., 1995. Multiple effects of opiates on intracellular Ca^{2+} level and on Ca^{2+} uptake in three neuronal cell lines. Brain Res. 687, 94–102.

Gross, R.A., Macdonald, R.L., 1987. Dynorphin A selectively reduces a large transient (N-type) Ca^{2+} current of mouse dorsal root ganglion neurons in cell culture. Proc. Natl. Acad. Sci. U.S.A. 84, 5469–5473.

Grinkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450.

Guerrini, R., Calo, G., Rizzi, A., Bigoni, R., Bianchi, C., Salvadori, S., Regoli, D., 1998. A new selective antagonist of the nociceptin receptor. Br. J. Pharmacol. 123, 163–165.

Hoffmeister, F., Tettenborn, D., 1986. Ca^{2+} agonists and antagonists of the dihydropyridine type: antinociceptive effects, interference with opiate-μ-receptor agonists and neuropharmacological actions in rodents. Psychopharmacology 90, 299–307.

Keren, O., Garty, M., Sarne, Y., 1994. Dual regulation by opioids of ^{3}Hnorepinephrine release in the human neuroblastoma cell line SK-N-SH. Brain Res. 646, 319–323.

Knoflach, F., Reinscheid, R.K., Civelli, O., Kemp, J.A., 1996. Modulation of voltage-gated calcium channels by orphanin FQ in freshly dissociated hippocampal neurons. J. Neurosci. 16, 6657–6664.

Meunier, J.-C., Mollerau, C., Toll, L., Saudreau, C., Moisand, C., Alvinerie, P., Butour, J.-L., Guillemot, J.-C., Ferrara, P., Monsarrat, B., Mazarguil, H., Vassart, G., Permentier, M., Costentin, J., 1995. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature 377, 532–535.

Moises, H.C., Rusin, K.I., Macdonald, R.L., 1994. μ-opioid receptors-mediated reduction of neuronal Ca^{2+} current occurs via a G_o-type GTP-binding protein. J. Neurosci. 14, 3842–3851.

North, R.A., 1993. Opioid actions on membrane ion channels. In: Herz, A., Akil, H., Simon, E. (Eds.), Handbook of Experimental Pharmacology: Opioids I. Springer-Verlag, Berlin, pp. 773–797.

Ramkumar, V., El-Fakahany, E.E., 1988. Prolonged morphine treatment increases rat brain dihydropyridine-binding sites: possible involvement in development of morphine dependence. Eur. J. Pharmacol. 146, 73–83.

Rhim, H., Miller, R.J., 1994. Opioid receptors modulate diverse types of Ca^{2+} channels in the nucleus tractus solitarius of the rat. J. Neurosci. 14, 7608–7615.

Ruiz, F., Dierssen, M., Flórez, J., Hurlé, M.A., 1993. Potentiation of acute respiratory depression and reversion of tolerance by the Ca^{2+} antagonist nimodipine in awake rats. Naunyn Scmied. Arch. Pharmacol. 348, 633–637.

Santillán, R., Maestre, J.M., Hurlé, M.A., Flórez, J., 1994. Enhancement of opiate analgesia by nimodipine in cancer patients chronically treated with morphine. A preliminary report. Pain 58, 129–132.

Santillán, R., Hurlé, M.A., Armijo, J.A., de los Mozos, R., Flórez, J., 1998. Nimodipine-enhanced opiate analgesia in cancer patients requiring morphine dose escalation: a double-blind, placebo-controlled study. Pain 76, 17–26.

Schroeder, J.E., Fischbach, P.S., Zheng, D., Mccleskey, E.W., 1991. Activation of mu opioid receptors inhibits transient high- and low-threshold Ca^{2+} currents, but spares a sustained current. Neuron 6, 13–20.

Seward, E., Hammond, C., Henderson, G., 1991. μ-opioid receptor-mediated inhibition of the N-type Ca^{2+} channel current. Proc. R. Soc. London, B. 244, 129–135.

Surprenant, A., Shen, K.Z., North, R.A., Tatsumi, H., 1990. Inhibition of Ca^{2+} currents by noradrenaline, somatostatin and opioids in guinea pig submucosal neurons. J. Physiol. 431, 585–608.

Tang, T., Kiang, J.G., Cote, T., Cox, B.M., 1995. Opioid-induced increase in $[Ca^{2+}]_i$ in ND8-47 neuroblastoma X dorsal root ganglion hybrid cells is mediated through G protein-coupled δ-opioid receptors and desensitized by chronic exposure to opioid. J. Neurochem. 65, 1612–1621.

Villalobos, C., Fonteriz, R., Lopez, M.G., Garcia, A.G., Garcia-Sancho, J., 1992. Inhibition of voltage-gated Ca^{2+} entry into GH3 and chromaffin cells by imidazole antimycotics and other cytochrome *P*450 blockers. FASEB J. 6, 2742–2747.

Wu, Y.L., Pu, L., Ling, K., Zhao, J., Cheng, Z.J., Ma, L., Pei, G., 1997. Molecular characterisation and functional expression of opioid receptor-like1 receptor.

Yin, D.L., Ren, X.H., Zheng, Z.L., Pu, L., Jiang, L.Z., Ma, L., Pei, G., 1997. Etorphine inhibits cell growth and induces apoptosis in SK-N-SH cells: involvement of pertussis toxin-sensitive G proteins. Neurosci. Res. 29, 121–127.

Yu, V.C., Richards, M.L., Sadee, W., 1986. A human neuroblastoma cell line expresses μ- and δ-receptor sites. J. Biol. Chem. 261, 1065–1070.

Zhang, S., Yu, L., 1995. Identification of dynorphins as endogenous ligands for an opioid receptor-like orphan receptor. J. Biol. Chem. 270, 22772–22776.