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Neomycin inhibits histamine and thapsigargin mediated Ca^{2+} entry in DDT_1 MF-2 cells independent of phospholipase C activation

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Abstract

The histamine H_1 receptor mediated increase in cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) was measured in the presence of the known phospholipase C (PLC) inhibitor, neomycin. Neomycin (1 mM) inhibited the histamine (100 μM) induced rise in $[Ca^{2+}]_i$ to the same extent as observed after blocking Ca^{2+} entry with $LaCl_3$. Likewise, the increase in $[Ca^{2+}]_i$ after re-addition of $CaCl_2$ (2 mM) to extracellular Ca^{2+} deprived and histamine pretreated cells was strongly reduced by neomycin. However, neomycin did not inhibit the histamine induced formation of inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) or the release of Ca^{2+} from internal stores. These results show that neomycin blocks histamine induced Ca^{2+} entry independent of phospholipase C activation. Inhibition of intracellular store Ca^{2+}-ATPase by thapsigargin (1 μM), elicited an increase in $[Ca^{2+}]_i$ due to a leakage from the stores, subsequently followed by store-dependent Ca^{2+} entry. Thapsigargin induced Ca^{2+} entry was also completely blocked by neomycin. These results indicate that neomycin inhibits histamine and thapsigargin induced Ca^{2+} entry. This inhibition is most likely exerted at the level of plasma membrane Ca^{2+} channels.

Keywords: Histamine H_1 receptor; Neomycin; Ca^{2+} entry; Phospholipase C

1. Introduction

Stimulation of plasma membrane receptors is often associated with an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), caused by the release of Ca^{2+} from internal stores and the entry of Ca^{2+} across the plasma membrane. It has been firmly established that agonist induced Ca^{2+} release is mediated by inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) (Streb et al., 1983; Berridge, 1993). Several mechanisms have been proposed to explain receptor mediated Ca^{2+} entry, including: opening of a ligand-gated Ca^{2+} channel (Benham and Tsien, 1987); activation of a Ca^{2+} channel by a GTP binding heterotrimeric protein (Matthews et al., 1989) and activation by a second messenger, such as $Ins(1,4,5)P_3$ (Kuno and Gardner, 1987; Restrepo et al., 1990; Mozhayeva et al., 1991), inositol 1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$, Morris et al., 1987; Lückhoff and Clapham, 1992) or arachidonic acid (Keyser and Alger, 1990; Van der Zee et al., 1995). Moreover, it was suggested that the filling state of intracellular Ca^{2+} stores determines the rate of Ca^{2+} entry (Putney, 1986). This pathway is activated by the Ca^{2+}-ATPase inhibitor, thapsigargin (Thastrup et al., 1990), causing an emptying of intracellular Ca^{2+} stores and possibly leading to the release of a cytosolic influx factor (Parekh et al., 1993; Randriamampita and Tsien, 1993; Thomas and Hanley, 1995). A physical link between luminal $Ins(1,4,5)P_3$ receptors and plasma membrane $Ins(1,3,4,5)P_4$ receptors was also suggested to activate Ca^{2+} entry (Irvine, 1992; Fadool and Ache, 1994).

In DDT_1 MF-2 smooth muscle cells, histamine H_1 receptor mediated $Ins(1,4,5)P_3$ and $Ins(1,3,4,5)P_4$ formation has been associated with Ca^{2+} release and Ca^{2+} entry (Molleman et al., 1991; Sipma et al., 1995a). Ca^{2+} entry was shown to be highly dependent on a continuous histamine H_1 receptor occupation, suggesting a strong store-independent component in histamine induced Ca^{2+} entry (Dickenson and Hill, 1992). In agreement, in a previous study based on patch-clamp measurements of Ca^{2+} activated K^+ currents, we showed that histamine evoked Ca^{2+}

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entry can occur in the absence of Ca^{2+} release and is therefore not completely dependent on the emptying of internal Ca^{2+} stores (Van der Zee et al., 1995). It was suggested that arachidonic acid is involved in histamine induced Ca^{2+} entry (Van der Zee et al., 1995). The aim of this study was to investigate whether phospholipase C activation is required for generating a messenger involved in the regulation of Ca^{2+} entry. The antibiotic and anti-arrhythmic drug neomycin (Anderson et al., 1995; Woodcock, 1995) is known to bind to phosphatydylinositol(4,5)bisphosphate, thereby inhibiting phospholipase C activation (Orsulakova et al., 1976; Schacht, 1976) and Ins(1,4,5)P_3 formation (Carney et al., 1985). Therefore, we determined the modulation of histamine H_1 receptor mediated and store-dependent Ca^{2+} entry by neomycin.

2. Materials and methods

2.1. Cell culture

DDT_1 MF-2 smooth muscle cells, derived from a Syrian hamster vas deferens were cultured in Dulbecco's modified essential medium (DMEM) supplemented with 7 mM $NaHCO_3$, 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (Hepes, pH 7.2) and 10% fetal calf serum at 37°C in 5% CO_2.

2.2. Measurement of Ins(1,4,5)P_3

DDT_1 MF-2 cells were grown in monolayers in 9.6 cm^2 plastic wells and an experimental protocol described earlier (Sipma et al., 1995b) was followed. The medium was replaced by 2 ml DMEM at 20°C, 30 min before starting the experiment by adding agonists. After removing the medium, reactions were stopped with 400 μM 5% trichloroacetic acid and placed on ice for at least 45 min. Samples were washed 3 times with 800 μl water saturated diethylether and neutralised with KOH (25 μl, 0.2 M).

Mass measurements of Ins(1,4,5)P_3 were performed as described earlier, using a standard curve of Ins(1,4,5)P_3 in ether extracted trichloroacetic acid-solution (Molleman et al., 1991). In brief, samples were assayed in 25 mM Tris/HCl (pH 9.0), 1 mM EDTA, 1 mg bovine serum albumin, [^{3}H]Ins(1,4,5)P_3 (3,3 Ci/mmol, 2000 cpm/assay) and about 1 mg binding protein for 15 min. The binding protein was isolated from fresh beef liver (Chilvers et al., 1989). Bound and free radioactivities were separated by centrifugation. The radioactivity in the pellet was determined by liquid scintillation counting.

2.3. Measurements of intracellular Ca^{2+}

$[Ca^{2+}]_i$ was measured by Fura-2 fluorescence. Individual glass coverslips covered with a monolayer of DDT_1 MF-2 cells were placed in 10 cm^2 plastic petri dishes and 2 ml buffered salt solution (BBS) containing NaCl (145 mM), KCl (5 mM), $MgSO_4$ (0.5 mM), $CaCl_2$ (1 mM), D-glucose (10 mM), Hepes (10 mM, adjusted to pH 7.4) were added. Fura-2 was loaded in the cytosol by incubation with Fura-2/AM (3 μM) for 45 min at 37°C in BBS supplemented with 1% bovine serum albumin. The coverslip with cells was washed quickly 3 times by placing it in fresh BBS and left in BBS (22°C) for 10 min. Thereafter the coverslip was mounted in a specially designed holder and placed in a quartz cuvette. Total volume (BBS) in the cuvette was 2 ml and agonists and inhibitors were added in 20 μl portions without opening the cuvette chamber. Measurements were performed at 22°C. Under Ca^{2+} free conditions the cells were washed and Fura-2 fluorescence was measured in BBS without $CaCl_2$ but supplemented with 0.1 mM EGTA. Excitation wavelengths were 340 nm and 380 nm and the emission wavelength was 510 nm. The ratios of emitted light at 510 nm was acquired every 1.0 s. These ratios were converted to Ca^{2+} levels using the classical equation described by Grynkiewicz et al. (1985). The R_{max} of the equation was measured in the presence of 2.0 mM $CaCl_2$ and 10 μM ionomycin. The R_{min} was measured in the presence of 10 μM ionomycin and 50 mM EGTA (adjusted to pH 8). The autofluorescence of the cell was determined as fluorescence remaining in the presence of 5 mM $MnCl_2$ and 10 μM ionomycin.

2.4. ^{3}[H]Arachidonic acid release

Arachidonic acid release was measured as described previously (Van der Zee et al., 1995). In brief, cells grown in 6-well plastic petri dishes (Costar) were labelled with 0.5 μCi ^{3}[H]arachidonic acid/10^6 cells/well in serum free culture medium (1 ml) for 3 h at 37°C. To eliminate unincorporated activity, cells were washed with buffered salt solution (BSS) containing: NaCl (145 mM), KCl (5 mM), $CaCl_2$ (1.4 mM), $MgSO_4$ (0.5 mM), glucose (10 mM), Hepes (10 mM, adjusted to pH 7.4), twice with BSS supplemented with 1% bovine serum albumin (essentially fatty acid free) and once again with BSS before equilibration for 25 min at 22°C. Complete washing was performed within 45 s. Cells were pretreated with neomycin (1 mM) during the equilibration period and during 20 min before the washing procedure. After this, cells were exposed to the indicated agonists, the solution was collected at the indicated time-points and ^{3}[H]arachidonic acid release was determined by liquid scintillation counting.

2.5. Data analysis

Data are represented as means ± S.E.M. Values were considered significantly different from control when $P < 0.05$ as determined using Student's unpaired t-test. A sigma plot logistic curve fit program (Jandel Scientific, USA) was used to determine EC_{50} values and to analyze

binding parameters obtained from the Ins(1,4,5)P_3 radioligand binding assay.

2.6. Chemicals

Fura-2/AM and Ins(1,4,5)P_3 sodium salt were obtained from Boehringer (Germany). Thapsigargin, neomycin sulphate and bovine serum albumin (essentially fatty acid free) were purchased from Sigma (USA). Histamine dihydrochloride was from Fluka (Switzerland). D-[2-^{3}H]Inositol 1,4,5-trisphosphate and [^{3}H]arachidonic acid were from Du Pont-New England Nuclear (USA). Lanthanum chloride, Hepes and all other chemicals were from Merck (Germany).

3. Results

The histamine H_1 receptor mediated increase in $[Ca^{2+}]_i$ was measured in the presence of neomycin. Histamine (100 μM) evoked a rapid increase in $[Ca^{2+}]_i$, reaching a maximum after about 30 s, which was mainly caused by Ca^{2+} release from internal stores. This initial rise in $[Ca^{2+}]_i$ was followed by a slowly declining phase, due to Ca^{2+} entry from the extracellular space (Fig. 1A, Table 1). Pretreatment of cells with neomycin (1 mM, 45 min) slightly reduced the initial rise in $[Ca^{2+}]_i$ and abolished the slowly declining component of the histamine induced response (Fig. 1B, Table 1). Similar results were obtained after blocking Ca^{2+} entry with $LaCl_3$ (Table 1), known to act directly on plasma membrane Ca^{2+} channels (Den Hertog et al., 1992).

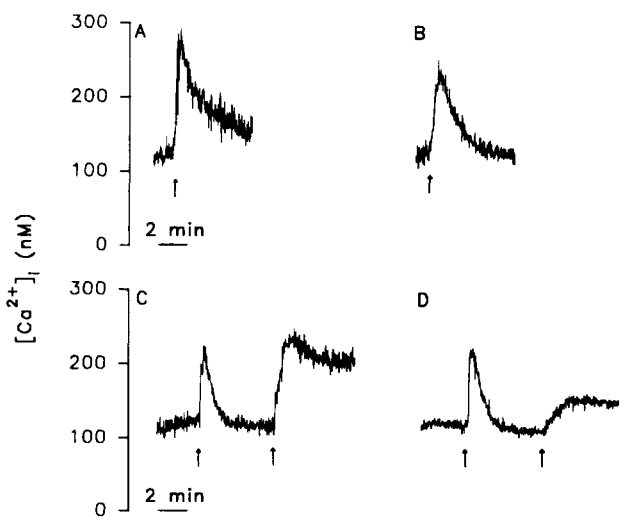

Fig. 1. The effect of neomycin on histamine H_1 receptor mediated changes in $[Ca^{2+}]_i$. The histamine (100 μM) induced increase in $[Ca^{2+}]_i$ was measured in the presence of extracellular Ca^{2+} in (A) non-pretreated cells and (B) in cells pretreated with neomycin (1 mM, 45 min). The arrow indicates the addition of histamine. The histamine (100 μM, first arrow) induced increase in $[Ca^{2+}]_i$ was also measured in the absence of extracellular Ca^{2+} in (C) non-pretreated cells and (D) in cells pretreated with neomycin (1 mM, 45 min). Extracellular Ca^{2+} (2 mM) was added after 5 min of stimulation of the cells with histamine (second arrow). Each tracing represents a typical result out of at least 6 experiments.

Table 1
Effect of neomycin or $LaCl_3$ on the histamine or thapsigargin induced increase in $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} in DDT$_1$ MF-2 cells

	Increase in $[Ca^{2+}]_i$ (nM)	
	Maximal increase	Sustained phase
Treatment		
Histamine	160 ± 16	57 ± 9
Neomycin + histamine	122 ± 12 [a]	10 ± 5 [a]
$LaCl_3$ + histamine	115 ± 9 [a]	3 ± 2 [a]
Thapsigargin	130 ± 12	26 ± 5
Neomycin + thapsigargin	43 ± 3 [a]	0 ± 0 [a]
$LaCl_3$ + thapsigargin	41 ± 5 [a]	0 ± 0 [a]

The histamine (100 μM) or thapsigargin (1 μM) induced increase in $[Ca^{2+}]_i$ was measured in non-pretreated cells and in cells pretreated with neomycin (1 mM, 45 min) or $LaCl_3$ (50 μM, 2 min). The sustained increase in $[Ca^{2+}]_i$ was measured 2 min after the addition of histamine or 10 min after the addition of thapsigargin. Basal $[Ca^{2+}]_i$ values; non-pretreated: 130 ± 4 nM; neomycin: 120 ± 6 nM; $LaCl_3$: 126 ± 6 nM. [a] Different from value obtained from non-pretreated cells, $P < 0.01$. Data are expressed as means ± S.E.M. of at least 6 experiments.

In the absence of extracellular Ca^{2+}, histamine (100 μM) elicited a transient rise in $[Ca^{2+}]_i$ (Fig. 1C, Table 2), as observed in the presence of $LaCl_3$. This increase in $[Ca^{2+}]_i$, which is due to the release of Ca^{2+} from Ins(1,4,5)P_3 sensitive internal stores, was not affected by pretreatment of cells with neomycin (Fig. 1D, Table 2). In agreement, the maximal histamine induced Ins(1,4,5)P_3 formation, measured after 1 min of stimulation of cells (Sipma et al., 1995a) was not affected by neomycin (1 mM, Fig. 2). Re-addition of extracellular Ca^{2+} (2 mM), 5

Table 2
Effect of neomycin on histamine or thapsigargin induced Ca^{2+} release and Ca^{2+} entry in DDT$_1$ MF-2 cells

	Increase in $[Ca^{2+}]_i$ (nM)	
	Absence of Ca^{2+}	2 mM Ca^{2+}
Treatment		
None		73 ± 9
Neomycin		43 ± 2 [a]
$LaCl_3$		38 ± 3 [a]
Histamine	113 ± 8	138 ± 10
Neomycin + histamine	105 ± 8	51 ± 6 [a]
Thapsigargin	41 ± 3	203 ± 21
Neomycin + thapsigargin	46 ± 4	53 ± 8 [a]

The histamine (100 μM) or thapsigargin (1 μM) induced increase in $[Ca^{2+}]_i$ were measured in the absence of extracellular Ca^{2+} (Ca^{2+} release) in non-pretreated cells and in cells pretreated with neomycin (1 mM, 45 min) or $LaCl_3$ (50 μM, 2 min). Ca^{2+} entry was measured as maximal increase in $[Ca^{2+}]_i$ after the addition of 2 mM Ca^{2+} to the solution 5 min after challenge of the cells with histamine or thapsigargin. Basal $[Ca^{2+}]_i$ values in the absence of extracellular Ca^{2+}; non-pretreated: 113 ± 4 nM; neomycin: 111 ± 4 nM. [a] Different from value obtained from non-pretreated cells, $P < 0.01$. Data are expressed as means ± S.E.M. of at least 6 experiments.

min after the challenge of cells with histamine, gave rise to an initial rapid increase in $[Ca^{2+}]_i$ (overshoot), followed by a maintained elevated level after about 2 min (Fig. 1C, Table 2). Neomycin inhibited this rise in $[Ca^{2+}]_i$ induced by the re-addition of extracellular Ca^{2+} (Fig. 1D, Table 2). It was observed that in the absence of histamine, the addition of extracellular Ca^{2+} also elicited a substantial increase in $[Ca^{2+}]_i$ (Table 2), without the transient overshoot (not shown). This unstimulated rise in $[Ca^{2+}]_i$ observed on the addition of extracellular Ca^{2+} to neomycin pretreated cells was similar as that measured after blocking Ca^{2+} channels with $LaCl_3$ (Table 2).

Besides Ca^{2+} entry that is dependent on occupation of histamine H_1 receptors (Dickenson and Hill, 1992; Van der Zee et al., 1995), histamine may also provoke Ca^{2+} entry regulated by the filling state of internal Ca^{2+} stores (Putney, 1986). Inhibition of intracellular Ca^{2+}-ATPase pumps by thapsigargin (1 μM, Thastrup et al., 1990) caused a slowly evolving and sustained increase in $[Ca^{2+}]_i$ (Fig. 3A, Table 1). In the absence of extracellular Ca^{2+}, the Ca^{2+} response was transient and much smaller and re-addition of extracellular Ca^{2+} caused a strong and rapid increase in $[Ca^{2+}]_i$ (Fig. 3C, Table 2). Reduction of the thapsigargin induced rise in $[Ca^{2+}]_i$ was also observed in the presence of $LaCl_3$ (50 μM). Moreover, when $LaCl_3$ was added 5 min after thapsigargin, $[Ca^{2+}]_i$ rapidly declined to the basal unstimulated level (not shown). These results suggest store-dependent Ca^{2+} entry in DDT_1 MF-2 cells. Since arachidonic acid generation after stimulation with histamine is partly responsible for Ca^{2+} entry in DDT_1 MF-2 cells (Van der Zee et al., 1995), we determined the formation of arachidonic acid after stimulation with thapsigargin. Thapsigargin failed to induce arachidonic acid formation (basal: 318 ± 12 dps/10^6 cells; thapsigargin 1 μM, 5 min: 327 ± 25 dps/10^6 cells; histamine 100 μM, 15 s: 429 ± 11 dps/10^6 cells*, $P < 0.05$, $n = 8$). Neomycin strongly inhibited both the thapsigargin evoked

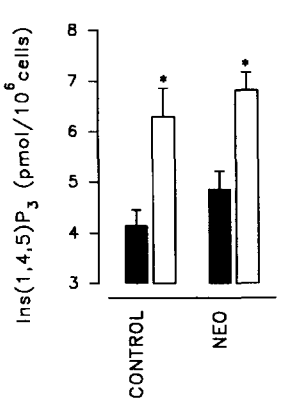

Fig. 2. Histamine H_1 receptor mediated formation of $Ins(1,4,5)P_3$. Basal $Ins(1,4,5)P_3$ (solid bars) and the histamine (100 μM, 60 s) induced formation of $Ins(1,4,5)P_3$ (open bars) was measured in non-pretreated cells (CONTROL) and in cells pretreated with neomycin (NEO, 1 mM, 45 min). * Different from the respective unstimulated value, $P < 0.05$. Data are expressed as means ± S.E.M. of 6 experiments.

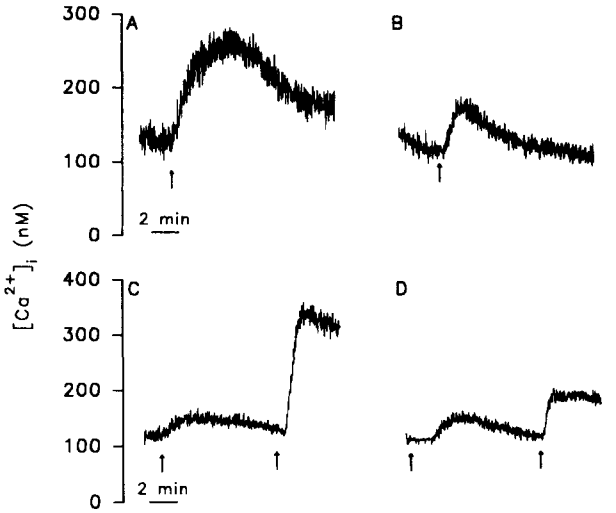

Fig. 3. The effect of neomycin on thapsigargin induced changes in $[Ca^{2+}]_i$. The thapsigargin (1 μM) induced rise in $[Ca^{2+}]_i$ was measured in the presence of extracellular Ca^{2+} in (A) non-pretreated cells and (B) in cells pretreated with neomycin (1 mM, 45 min). The arrow indicates the addition of thapsigargin. The thapsigargin (1 μM, first arrow) induced increase in $[Ca^{2+}]_i$ was also measured in the absence of extracellular Ca^{2+} in (C) non-pretreated cells and (D) in cells pretreated with neomycin (1 mM, 45 min). Extracellular Ca^{2+} (2 mM) was added after 5 min of stimulation of the cells with thapsigargin (second arrow). Each tracing represents a typical result out of at least 6 experiments.

rise in $[Ca^{2+}]_i$ when extracellular Ca^{2+} was available (Fig. 3B, Table 1) and after the addition of Ca^{2+} to the Ca^{2+} free solution (Fig. 3D, Table 2). The response to thapsigargin in the absence of extracellular Ca^{2+} (Fig. 3C) was not affected by neomycin (Fig. 3D, Table 2). The Ca^{2+} channels activated by both histamine (see also Dickenson and Hill, 1992) or thapsigargin are not permeable to Mn^{2+}, since the quenching-rate of Fura-2, induced by basal Mn^{2+} entry (Hallam and Rink, 1985) was not increased by histamine or thapsigargin (not shown).

4. Discussion

In this study, we investigated the involvement of phospholipase C activity to generate a messenger regulating Ca^{2+} entry in DDT_1 MF-2 cells. It is shown that histamine H_1 receptor mediated Ca^{2+} entry was completely abolished in the presence of neomycin and $LaCl_3$. However, this action on Ca^{2+} entry of neomycin is independent on phospholipase C activity, reflected by the unaffected histamine induced $Ins(1,4,5)P_3$ formation in DDT_1 MF-2 cells.

Since histamine evokes Ca^{2+} release from internal stores, it is supposed to cause Ca^{2+} entry that is dependent on the filling state of the store (Putney, 1986). Store-dependent Ca^{2+} entry induced by thapsigargin that inhibits the intracellular store Ca^{2+} ATPase (Thastrup et al., 1990; Bian et al., 1991) was also completely inhibited by

neomycin. Basal Ca^{2+} entry, measured after addition of $CaCl_2$ to extracellular Ca^{2+}-deprived cells is also inhibited by neomycin and $LaCl_3$. This basal Ca^{2+} entry might be caused by the 'leaking out' of cytoplasmic Ca^{2+}, resulting in a reduced filling-state of intracellular stores, consequently provoking store-dependent Ca^{2+} entry. Remarkably, Dickenson and Hill (1992) did not detect an increase in $[Ca^{2+}]_i$ upon the addition of extracellular Ca^{2+} to unstimulated and extracellular Ca^{2+}-deprived cells at 37°C. In contrast, a marked increase in $[Ca^{2+}]_i$ was observed under our experimental conditions (22°C). Since intracellular store-Ca^{2+} ATPase pumps are more activated at 37°C than at 22°C (Squier et al., 1988; Kalabokis and Hardwicke, 1988), apparently the rates of Ca^{2+} entry and uptake equalize at 37°C, leading to a no-net increase in $[Ca^{2+}]_i$. The Ca^{2+} entry remaining in the presence of neomycin and $LaCl_3$ (Table 2) most likely reflects the transition from the basal $[Ca^{2+}]_i$ in the absence to that in the presence of extracellular Ca^{2+} and might be mediated by non-specific cation channels or Na^+/Ca^{2+} exchange. These mechanisms were not activated by histamine or thapsigargin.

Direct histamine H_1 receptor-dependent Ca^{2+} entry and the indirect store-dependent mechanism stimulated by thapsigargin (and histamine) were inhibited by neomycin. Neomycin most likely inhibits Ca^{2+} entry by blocking the plasma membrane Ca^{2+} channels, as suggested also for hepatocytes (Altin and Bygrave, 1987). Therefore, neomycin is not a suitable tool to study the effects of messengers generated downstream of phospholipase C activation on receptor mediated and capacitive Ca^{2+} entry in DDT_1 MF-2 cells. Interestingly, in ischemic hearts, the inositol phosphate formation induced by reperfusion was strongly reduced by neomycin as well as by removing Ca^{2+} from the reperfusion solution (Anderson et al., 1995). In accordance with our results, we suggest that neomycin may act as a anti-arrhithmic drug (Woodcock, 1995) by preventing Ca^{2+} entry, rather than by a direct inhibition of $Ins(1,4,5)P_3$ formation.

In a previous study we reported that histamine induced Ca^{2+} entry still occurred if the Ca^{2+} release process and the concomitant store-dependent Ca^{2+} entry was inhibited (Van der Zee et al., 1995). Histamine induced arachidonic acid formation was supposed to be in involved in histamine H_1 receptor mediated Ca^{2+} entry. Moreover, histamine induced Ca^{2+} entry was shown to be dependent on histamine H_1 receptor occupation in DDT_1 MF-2 cells (Dickenson and Hill, 1992). Store-dependent Ca^{2+} entry however, as activated by thapsigargin, is not mediated by arachidonic acid, because thapsigargin does not induce arachidonic acid formation.

In conclusion, neomycin inhibits the plasma membrane Ca^{2+} channels that can be activated by a histamine H_1 receptor-dependent pathway and the channels activated by a mechanism that is dependent on the filling-state of intracellular Ca^{2+} stores. The inhibitory action of neomycin on histamine induced Ca^{2+} entry is not dependent on phospholipase C activity.

References

Altin, J.G. and F.L. Bygrave, 1987, The influx of Ca^{2+} induced by the administration of glucagon and Ca^{2+} mobilizing agents to the perfused rat liver could involve at least two separate pathways, Biochem. J. 242, 43.

Anderson, K.E., A.M. Dart and E.A. Woodcock, 1995, Inositol phosphate release and metabolism during myocardial ischemia and reperfusion in rat heart, Circ. Res. 76, 261.

Benham, C.D. and R.W. Tsien, 1987, A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle, Nature 328, 275.

Berridge, M.J., 1993, Inositol phosphates and cell signalling, Nature 341, 197.

Bian, J.H., T.K. Gosh, J.C. Wang and D.L. Gill, 1991, Identification of intracellular Ca^{2+} pools. Selective modification by thapsigargin, J. Biol. Chem. 266, 8801.

Carney, D.H., D.L. Scott, E.A. Gordon and E.F. LaBelle, 1985, Phosphoinositides in mitogenesis: neomycin inhibits thrombin-stimulated phosphoinositide turnover and initiation of cell proliferation, Cell 42, 479.

Chilvers, E.R., R.A.J. Challiss, P.J. Barnes, S.R. Nahorski, 1989, Mass changes of inositol 1,4,5-trisphosphate in trachealis muscle following agonist stimulation, Eur. J. Pharmacol. 164, 587.

Den Hertog, A., B. Hoiting, A. Molleman, J. van den Akker, M. Duin and A. Nelemans, 1992, Calcium release from separate receptor-specific intracellular stores induced by histamine and ATP in a hamster cell line, J. Physiol. 454, 591.

Dickenson, J.M. and S.J. Hill, 1992, Histamine H_1-receptor-mediated calcium influx in DDT_1 MF-2 cells, Biochem. J. 284, 425.

Fadool, D.A. and B.W. Ache, 1994, Inositol 1,3,4,5-tetrakisphosphate-gated channels interact with inositol 1,4,5-trisphosphate-gated channels in olfactory receptor neurons, Proc. Natl. Acad. Sci. USA 91, 9471.

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

Hallam, T.J. and T.J. Rink, 1985, Agonists stimulate divalent cation channels in the plasma membrane of human platelets, FEBS Lett. 186, 175.

Irvine, R.F., 1992, Inositol phosphates and Ca^{2+} entry: towards a proliferation or a simplification?, FASEB J. 6, 3085.

Kalabokis, V. and P. Hardwicke, 1988, Variation of scallop sarcoplasmic reticulum Ca^{2+} ATPase activity with temperature, J. Biol. Chem., 263, 9178.

Keyser, D.O. and B.E. Alger, 1990, Arachidonic acid modulates hippocampal calcium current via protein kinase C and oxygen radicals, Neuron 5, 545.

Kuno, M. and P. Gardner, 1987, Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes, Nature, 326, 301.

Lückhoff, A. and D. Clapham, 1992, Inositol 1,3,4,5-tetrakisphosphate activates an endothelial Ca^{2+} permeable channel, Nature 355, 356.

Matthews, G., E. Neher and R. Penner, 1989, Second messenger-activated calcium influx in rat peritoneal mast cells, J. Physiol. 418, 105.

Molleman, A., B. Hoiting, M. Duin, J. Van den Akker, A. Nelemans and A. Den Hertog, 1991, Potassium channels regulated by inositol 1,3,4,5-tetrakisphosphate and internal Ca^{2+} in DDT_1 MF-2 smooth muscle cells, J. Biol. Chem. 266, 5658.

Morris, A.P., D.V. Gallacher, R.F. Irvine and O.H. Petersen, 1987, Synergism of inositol trisphosphate and tetrakisphosphate in activating Ca^{2+}-dependent K^+ channels, Nature 330, 653.

Mozhayeva, G.N., A.P. Naumov and Y.A. Kuryshev, 1991, Variety of Ca(2+)-permeable channels in human carcinoma A431 cells, J. Membr. Biol. 124, 113.

Orsulakova, A., E. Stockhorst and J. Schacht, 1976, Effect of neomycin on phosphoinositide labelling and calcium binding in guinea-pig inner ear tissues in vivo and in vitro, J. Neurochem. 26, 285.

Parekh, A.B., H. Terlau and W. Stumer, 1993, Depletion of $InsP_3$ stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger, Nature 364, 814.

Putney, J.W., Jr., 1986, A model for receptor-regulated calcium entry, Cell Calcium 7, 1.

Randriamampita, C. and R.Y. Tsien, 1993, Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx, Nature 364, 809.

Restrepo, D., T. Miyamoto, B.P. Bryant and J.H. Teeter, 1990, Odor stimuli trigger influx of calcium into olfactory neurons of the channel catfish, Science 249, 1166.

Schacht, J., 1976, Inhibition by neomycin of polyphosphoinositide turnover in subcellular fractions of guinea pig cerebral cortex in vitro, J. Neurochem. 27, 1119.

Sipma, H., M. Duin, B. Hoiting, A. Den Hertog and A. Nelemans, 1995a, Regulation of histamine- and UTP-induced increases in $Ins(1,4,5)P_3$, $Ins(1,3,4,5)P_4$ and Ca^{2+} by cyclic AMP in DDT_1 MF-2 cells, Br. J. Pharmacol. 114, 383.

Sipma, H., A. Den Hertog and A. Nelemans, 1995b, Ca^{2+}-dependent and -independent mechanisms of cyclic-AMP reduction: mediation by bradykinin B_2 receptors, Br. J. Pharmacol. 115, 937.

Squier, T.C., D.J. Bigelow, D.D. Thomas, 1988, The lipid fluidity directly modulates the overall protein rotational mobility of the Ca^{2+} ATPase in sarcoplasmic reticulum, J. Biol. Chem. 263, 9178.

Streb, H., R.F. Irvine, M.J. Berridge and I. Schulz, 1983, Release of Ca^{2+} from a nonmitochondrial intracellular store in Pancreatic acinar cells by inositol-1,4,5- trisphosphate, Nature 306, 67.

Thastrup, O., P.J. Cullen, B.K. Drobak, M.R. Hanley and A.P Dawson, 1990, Thapsigargin, a tumour promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+} ATPase, Proc. Natl. Acad. Sci. USA 87, 2466.

Thomas, D. and M.R. Hanley, 1995, Evaluation of calcium influx factors from stimulated jurkat T-lymphocytes by microinjection into Xenopus Oocytes, J. Biol. Chem. 270, 6429.

Woodcock, E.A., 1995, Inositol phosphates in the heart: controversy and consensus, J. Mol. Med. 73, 313.

Van der Zee, L., A. Nelemans and A. Den Hertog, 1995, Arachidonic acid is functioning as a second messenger in activating the Ca^{2+} entry process on H_1-histaminoceptor stimulation in DDT_1 MF-2 cells, Biochem. J. 305, 859.