Potassium Channels Regulated by Inositol 1,3,4,5-Tetakisphosphate and Internal Calcium in DDT1 MF-2 Smooth Muscle Cells*

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This study was carried out to determine the intracellular components responsible for the transmembrane current evoked by stimulation of H1-histaminergic receptors in DDT1 MF-2 smooth muscle cells. Histamine elicited an outward current that was reversed below the K+ equilibrium potential and passed voltage-independent K+ channels. A histamine concentration-dependent rise in outward current and in cytoplasmic-free Ca2+ with similar time courses was observed. The histamine-induced current was not found after depletion of internal Ca2+ stores, suggesting a coupling between internal Ca2+ and K+ current. The time course of the initial increase in inositol (1,4,5)-trisphosphate (Ins(1,4,5)P3) caused by histamine differs from that of the internal Ca2+ response. However, a significant concentration-dependent increase in inositol (1,3,4,5)-tetrakisphosphate (Ins(1,3,4,5)P4) was seen during the whole stimulating period. The role of internal Ca2+, Ins(1,4,5)P3, and Ins(1,3,4,5)P4 on the outward current was also examined by the addition of these substances directly to the cytoplasm. Internal application of Ca2+ increased the amplitude and duration of the histamine-induced current whereas internal EGTA suppressed the outward current. Internal Ins(1,4,5)P3 did not affect the histamine-induced K+ current. Ins(1,3,4,5)P4 inhibited the outward current, and the combination of Ins(1,3,4,5)P4 and Ca2+ abolished this response. The noradrenaline response evoked under normal conditions is not reflected by a change in transmembrane current or a change in Ins(1,3,4,5)P4 but is associated with an increase in Ins(1,4,5)P3 and internal Ca2+. Stimulation of α1-adrenoceptors, however, also evoked an outward current after the addition of Ins(1,3,4,5)P4 intracellularly. It is concluded that K+ channels, carrying the histamine outward current, are activated from the combined action of internal Ca2+ and Ins(1,3,4,5)P4.

Activation of transmembrane ion fluxes through stimulation of external receptor sites by transmitters or hormones is not yet fully understood. Agonist-receptor interaction may facilitate membrane currents by a direct action on ionic channels (Benham and Tsien, 1987) or indirectly via second messengers activating ionic channels from the inside (Hescheler et al., 1987). DDT1 MF-2 smooth muscle cells, derived from hamster vas deferens, possess histaminergic receptors of the H1 subtype, as demonstrated by receptor binding experiments (Mitsuhashi and Payan, 1988). An increase in intracellular Ca2+ upon H1 histaminergic receptor stimulation was observed in swine carotid artery (Rembold and Murphy, 1989), in rat aortic smooth muscle (Matsumoto et al., 1989), and in DDT1 MF-2 cells (Mitsuhashi and Payan, 1989). Stimulation of α1-adrenoceptors also increased internal Ca2+ in a variety of smooth muscle cell types (Chiu et al., 1987; Nelemans and Den Hertog, 1987a; Minneman, 1988) including DDT1 MF-2 cells (Nelemans et al., 1990). Intracellular Ca2+ originates from the extracellular space or from internal stores and functions to trigger the contractile apparatus in smooth muscle cells (Hartshorne, 1987). Ca2+ originating from the extracellular space may enter the cytoplasm, passing channel-like structures (Putney et al., 1989), or via channels coupled with the receptor (Benham and Tsien, 1987).

There is substantial evidence that phosphatidylinositol metabolism plays a physiological role in the release of calcium from intracellular structures. In particular, the metabolites inositol trisphosphate (Ins(1,4,5)P3) and inositol tetrakisphosphate (Ins(1,3,4,5)P4) are considered to function as second messengers, contributing to the release of calcium from internal stores (Berridge and Irvine, 1989). It is noticed that GTP may function similar to Ins(1,3,4,5)P4 in DDT1 MF-2 cells (Mullaney et al., 1988; Ghosh et al., 1989). The localization of the Ca2+ stores involved in the cellular response to receptor stimulation and their sensitivity to inositol phosphates is still under discussion (Volpe et al., 1988; Putney et al., 1989; Berridge and Irvine, 1989). Besides their action on Ca2+ stores, inositol phosphates may also play a role in the activation of ionic channels in the plasma membrane. Certain K+ channels are regulated by inositol phosphates as observed in mouse lacrimal acinar cells (Morris et al., 1987; Changya et al., 1989). In fact, phosphatidylinositol metabolism was activated in several smooth muscle cells in the presence of histamine (Villalobos-Molina and Garcia-Sainz, 1983; Donaldson and Hill, 1985; Bielkiewicz-Vollrath et al., 1987; Hall and Hill, 1988) and by stimulation of α1-adrenoceptors (Nelemans and Den Hertog, 1987b; Minneman, 1988; Nelemans et al., 1990).

The present study shows that in particular Ins(1,3,4,5)P4, was formed within the time frame of the rise in internal Ca2+ and the outward current in the presence of histamine. Direct addition of Ins(1,3,4,5)P4, or Ca2+ to the cytoplasm revealed that both components are involved in generation of the outward K+ current. More specific, we demonstrate here for the

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first time that both Ins (1,3,4,5)P\textsubscript{4} and internal Ca\textsuperscript{2+} are necessary to activate K\textsuperscript{+} channels in the plasma membrane.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The DDT, MF-2 cells, derived from a Syrian hamster vas deferens (Norris et al., 1974) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 7 mM NaHCO\textsubscript{3}, 10 mM HEPES at pH 7.2, and 10% fetal calf serum at 37 °C in 95% O\textsubscript{2}, 5% CO\textsubscript{2} (Molleman et al., 1989). In electrophysiological measurements the cells were plated on glass coverslips, and in Ca\textsuperscript{2+} measurements and inositol phosphate determination the cells were brought into suspension.

**Membrane Currents**—Microelectrode and whole-cell patch-clamp measurements were performed at 20 °C as described earlier (Mollemann et al., 1989). Microelectrodes (Clark GC150F-15 glass, Reading, England) were filled with 1 M KCl and had a typical resistance of 50–80 megohms. Cells superfused at a constant flow rate (1 ml/min) by means of a multichannel peristaltic pump (IPS Ismatec, Zürich, Switzerland) were penetrated with a microelectrode using a nanostepper (model B, World Precision Instruments, New Haven, CT), and membrane potentials were recorded by a high resistance amplifier under voltage-clamp (List EPC-7, Federal Republic of Germany). Establishment of the whole-cell patch-clamp configuration was monitored via a CCD camera (Sony AX160K) mounted on the microscope (Olympus CK) and detected electrically by measuring the resistance and capacity. Membrane currents of the cells were recorded under voltage-clamp (List EPC-7, Federal Republic of Germany).

Data are presented as mean ± S.E. and considered to be significantly different (p < 0.05) from control values using Student’s t-test.

**Intracellular Calcium**—Cytoplasmic free Ca\textsuperscript{2+} levels were determined by indo-1 fluorescence, as reported previously (Hoiting et al., 1990; Nelemans et al., 1990). Cell suspensions at a density of 2 × 10\textsuperscript{6} cells/ml were loaded with indo-1 ester (2 μM) for 45 min at 37 °C. Recordings were made at an excitation wavelength of 340 nm and emission wavelengths of 400 and 480 nm at 20 °C using a fluorescence spectrophotometer (Hitachi). Cytoplasmic free Ca\textsuperscript{2+} concentrations were calculated (Hesketh et al., 1983) using 0.015% Triton X-100 as a permeabilizing agent.

**Solutions**—Electrophysiological measurements were performed in extracellular solution, and patch pipettes were filled with intracellular solution (Benham et al., 1985). Extracellular solution composition (in mM) was: NaCl, 125; KCl, 6; CaCl\textsubscript{2}, 1.2; MgCl\textsubscript{2}, 2.5; NaH\textsubscript{2}PO\textsubscript{4}, 1.2; HEPES, 10; glucose, 11; pH, 7.4. Intracellular solution composition (in mM) was: NaCl, 5; KCl, 126; MgCl\textsubscript{2}, 1.2; HEPES, 10; EGTA, 0.77; glucose, 11; pH, 7.2. Fluorescence experiments were performed in extracellular solution (Benham et al., 1985). Composition (in mM) was: NaCl, 145; KCl, 5; MgSO\textsubscript{4}, 0.5; CaCl\textsubscript{2}, 1; HEPES, 10; glucose, 10; pH, 7.4. Ca\textsuperscript{2+}-free conditions were accomplished by the addition of EGTA (0.77 mM) and MgCl\textsubscript{2} (3.7 mM) to calcium-free solution in the patch-clamp measurements and EGTA (0.4 mM) and MgCl\textsubscript{2} (0.7 mM) in Ca\textsuperscript{2+}-free solution used in the fluorescence measurements to remove extracellular Ca\textsuperscript{2+} and to prevent membrane leakage (Den Hertog, 1981).

**Inositol Phosphates**—To determine the effect of histamine, cells were harvested and resuspended in Dulbecco’s modified Eagle’s medium with a low inositol concentration (70 μg/liter) containing fetal calf serum (10%). Cells (2 × 10\textsuperscript{6} cells/ml) were incubated in a dish containing 1 ml of 5.10\textsuperscript{5} cells/ml. This cell suspension was labeled with myo-[\textsuperscript{3}H]inositol (7 Ci/mmol, Du Pont-New England Nuclear) for 22 h. The medium was replaced by Dulbecco’s modified Eagle’s medium (1 ml) at 22 °C 10 min prior to the experiment. Histamine was added for different periods (5–900 s). The reaction was stopped (trichloroacetic acid, 15%, 1 ml). Trichloroacetic acid was extracted (water-saturated diethyl ether), the samples were neutralized (KOH), and stored (–20 °C). Inositol phosphates were separated by HPLC as reported previously (Hoiting et al., 1990; Nelemans et al., 1990). Separation was achieved by HPLC-4000 Deltapak with a Zorbax SAX Bioseries column (Du Pont-New England Nuclear) equilibrated with H\textsubscript{2}O and kept at a flow rate of 1.5 ml/min. Standards (ATP, ADP, and AMP) were injected simultaneously with the samples and read at 259 nm. The location of D-myo-inositol 1,3,4,5-trisphosphate (D-Ins(1,3,4,5)P\textsubscript{4}) was determined by indo-1 fluorescence, as reported previously (Hoiting et al., 1990). The samples were assayed in 25 mM Tris/HC1 (pH, 9.0), 1 mM EDTA, 1 mg of bovine serum albumin, [\textsuperscript{3}H]Ins(1,4,5)P\textsubscript{3} (2,000 cpm/assay), and about 1 mg of binding protein (Challis et al., 1988). The binding protein was isolated from fresh beef liver (Donner and Reiser, 1989). Bound and free radioactivity were separated by centrifugation. The radioactivity in the pellet was determined by scintillation counting.

**RESULTS AND DISCUSSION**

The membrane current, the intracellular free Ca\textsuperscript{2+} concentration, and the formation of inositol phosphates were measured upon stimulation of DDT; MF-2 cells by histamine. After establishment of the whole-cell patch, the cells showed an outward current on exposure to histamine (Fig. 1A). The transmembrane current evoked by histamine was transient in nature and reached a maximum (0.5 ± 0.1 nA; n = 19) after about 30 s at a holding potential (–50 mV) near the resting potential (–48 ± 6 mV, n = 8) of the cells. The histamine-induced current increased linearly at positive holding potentials and was reversed at values below the potassium equilibrium potential (~76 mV; Fig. 1C). These data indicate that the outward current is carried by K\textsuperscript{+} ions since this current could not be evoked by histamine after replacing cytoplasmic K\textsuperscript{+} with Cs\textsuperscript{+} via the patch pipette (not shown). Hyperpolarization of the cells in the presence of histamine, measured by using microelectrodes, reached a maximum (~32 ± 2.4 mV; n = 6) after about the same time as the outward current (Fig. 1B) and showed comparable characteristics. The outward current was carried by K\textsuperscript{+} ions.

![Fig. 1. Electrophysiological responses evoked by histamine (10\textsuperscript{-4} M) in DDT1 MF-2 cells. A, the membrane current at a holding potential of ~50 mV measured using the whole-cell patch-clamp method. An outward current is represented by upward deflection. B, the membrane potential as measured by a microelectrode. Downward deflection represents hyperpolarization of the cell. C, the maximal outward current at different holding potentials in the presence of histamine (10\textsuperscript{-4} M; n = 4).](image-url)
potassium current can readily explain hyperpolarization of the cells in the presence of histamine. The similarity of both responses also indicates that dialysis of the cells with ICS in the whole-cell patch-clamp configuration does not interfere with the cellular response to histamine. Therefore, the EGTA concentration (7.7 × 10⁻⁴ M) used in this study to avoid spontaneously developing current fluctuation in the absence of this chelator is considered acceptable. It is noticed that the histamine-induced current was abolished by dialyzing the cell with a 4-fold higher concentration of EGTA (3 × 10⁻³ M; see Fig. 6B). In addition to the voltage insensitivity of the histamine-evoked K⁺ current, it was not affected by apamin (3 × 10⁻⁷ M), known to block certain receptor-activated K⁺ channels (Den Hertog, 1982). It was also unaffected by 3,4-diaminopyridine (10⁻⁴ M) inactivating slow rectifying K⁺ conductances (Okae et al., 1987) or by gliptide (10⁻⁴ M) blocking ATP-mediated K⁺ channels (Hescheler et al., 1987; not shown). Thus, the outward current evoked by histamine in DDT; MF-2 cells presumably passed novel K⁺ channels, which are voltage independent (Fig. 1C) and insensitive to established channel blocking agents.

The amplitude of the outward current increased at higher agonist concentrations to reach a maximum at about 10⁻⁴ M histamine (Fig. 2A). The exclusive expression of Hzial histaminergic receptors on these cells was demonstrated by binding experiments (Mitsuhashi and Payan, 1989). In agreement, blocking H₂ histaminergic receptors by cimetidine (10⁻⁴ M) did not affect the outward current elicited by histamine (10⁻⁴ M), but mepyramine (10⁻⁶ M), acting as a H₁ histaminergic receptor antagonist, blocked this response completely (not shown). The experiments described below are performed at a holding potential of −10 mV in case of the whole-cell patch-clamp and are showing cellular responses to a maximal effective histamine concentration (10⁻⁴ M), if not stated otherwise.

It was found in several cell types that changes in membrane current are linked to the internal Ca²⁺ concentration (Morris et al., 1987; Molleman et al., 1989; Hoiting et al., 1990). To examine whether such a coupling also exists with respect to the histamine-induced K⁺ current in DDT; MF-2 cells, internal Ca²⁺ was measured. Stimulation of the H₁ histaminergic receptors caused a concentration-dependent rise in internal Ca²⁺ (Fig. 2B) followed by a low sustained phase (Fig. 3B). The sustained component (12 ± 4 nM; n = 8) amounted to less than 10% of the maximum value (Table I) in agreement with previous reports (Mitsuhashi and Payan, 1988). This increase in internal Ca²⁺ with histamine is not affected by blocking voltage-dependent Ca²⁺ channels with diltiazem (10⁻⁵ M; n = 4; not shown). The sustained phase, however, was not observed in the absence of external Ca²⁺ (15 min; Fig. 3B) and accordingly is most likely due to Ca²⁺ entry from the extracellular space via a voltage-insensitive pathway. The remaining transient increase in Ca²⁺ caused by histamine under Ca²⁺-free conditions could be evoked only once (Fig. 3B). The transient rise in cytosolic Ca²⁺ is obviously due to release from internal stores, a process observed in many cell types (Den Hertog, 1981; Takemura et al., 1989; Irvine, 1990). Refilling of the stores with Ca²⁺ from the extracellular space is required to evoke a second Ca²⁺ response with histamine under Ca²⁺-free conditions (not shown), indicating that reuptake from the cytoplasm, as in parotid acinar cells, does not play an important role (Takemura et al., 1989). Comparable properties of internal Ca²⁺ stores were observed in taenia

![Figure 2](image_url) **Fig. 2.** The relationship between the histamine concentration and the evoked cellular response. A, the maximum current measured by using the whole-cell patch-clamp method (holding potential, −10 mV). The response to 10⁻⁴ M histamine was taken as 100% (n = 4). B, the internal Ca²⁺ concentration determined by using indo-1 fluorescence. C, the Ins (1,3,4,5)P₄ formation expressed as dps/10⁶ cells and analyzed using HPLC. All data represent increases above basal values as given in Table I.

![Figure 3](image_url) **Fig. 3.** The outward current and internal Ca²⁺ evoked by histamine (10⁻⁴ M) measured under different experimental conditions. A, the outward current and B, the internal Ca²⁺ concentration in the absence of external Ca²⁺ (15 min) followed by second application of histamine under Ca²⁺-free conditions (30 min) not resulting in a response (middle recording) followed by the histamine response elicited in the presence of Ca²⁺ (15 min; right recording).

| TABLE I | The effect of histamine and noradrenaline stimulation on the outward current (Iₒ), internal Ca²⁺ ([Ca²⁺]ᵢ) and the formation of Ins (1,4,5)P₃ and Ins (1,3,4,5)P₄. |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
| Iₒ (pA) | Histamine (10⁻⁴ M) | Noradrenaline (10⁻⁵ M) |
|---------|------------------|----------------------|
| Control |                  |                      |
| 950 ± 80 (29) | 990 ± 226 (6)* | ND (6) |
| [Ca²⁺]ᵢ (nM) | 149 ± 5 (53) | 163 ± 17 (8) | 50 ± 9 (35) |
| 83 ± 8 (10)* | 48 ± 7 (10)* |                      |
| Ins (1,4,5)P₃ (dps/10⁶ cells) | 36.6 ± 6.9 (22) | −2.1 ± 7.0 (4) | 11.3 ± 1.5 (4) |
| Ins (1,3,4,5)P₄ (dps/10⁶ cells) | ND (16) | 2.7 ± 0.5 (4) | ND (4) |

* A. Den Hertog, personal observation.
caeci smooth muscle cells (Den Hertog, 1981; Okabe et al., 1987) and DDT; MF-2 cells (Hoiting et al., 1990; Nelemans et al., 1990) on stimulation of P2 purinoceptors or α1-adrenoceptors. The changes in internal Ca2+ under different experimental conditions described here are accompanied by an outward current following similar time-related characteristics (Fig. 3A; Table I). It is noticed that the inability to evoke a second histamine-induced response under Ca2+-free conditions is also reflected by the absence of a change in transmembrane current (Fig. 3A).

The results obtained so far are consistent with the concept that internal Ca2+ is involved in activation of K+ channels. Inositol phosphates may also play a role in the Ca2+-releasing process and generation of the K+ current upon histamine stimulation. In particular Ins (1,4,5)P3 and Ins (1,3,4,5)P4 are considered to function as second messengers to enhance internal Ca2+ (Morris et al., 1987; Takemura et al., 1989; Changya et al., 1989). It was reported that phosphatidylinositol metabolites are formed upon H1 histaminergic receptor stimulation in rabbit aorta (Villalobos-Molina and Garcia-Sainz, 1983) and in guinea pig ileum (Bielkiewicz-Vollrath et al., 1987). Analysis of the inositol phosphate formation in DDT; MF-2 cells showed a considerable basal value of Ins (1,4,5)P3 obtained via incubation of the cells with myo-[3H]inositol (Taber et al., 1990). Addition of histamine to the cells caused only a small increase in the formation of Ins (1,4,5)P3 after stimulation for 2 min (Fig. 4A). To obtain more accurate data on the Ins (1,4,5)P3 formation, especially shortly after stimulation by histamine, we determined the cellular content by mass measurement. The basal cellular level of Ins (1,4,5)P3 was 49 ± 3 pmol/106 cells (n = 8). With an estimated cell volume of 1.0 μl/106 cells this equals a concentration of about 3 × 10−6 M. These mass measurements revealed that the delayed increased after 2 min also a transient rise could be detected with histamine (Fig. 4B; significantly different at t = 15). A short lasting increase in Ins (1,4,5)P3 mass was also observed in airway smooth muscle (Chilvers et al., 1989; Murray et al., 1989) upon receptor stimulation. The initial transient change in Ins (1,4,5)P3 caused by histamine in DDT; MF-2 cells was followed by a concentration-dependent rise in Ins (1,3,4,5)P4, reaching a plateau after about 60 s (Figs. 2C and 4C). The formation of Ins (1,3,4,5)P4 is thought to originate from Ins (1,4,5)P3 by activation of Ins (1,4,5)P3 3-kinase (Hansen et al., 1989; Shears, 1989). This kinase possesses a central position in the inositol phosphate metabolism, in particular in regulating the Ca2+-modulating compounds Ins (1,4,5)P3 and Ins (1,3,4,5)P4. The kinase is activated by Ins (1,4,5)P3-released Ca2+ (Johanson et al., 1988; Mualem et al., 1989; Berridge, 1990) explaining the formation of Ins (1,3,4,5)P4, and the associated decay of Ins (1,4,5)P3 in DDT; MF-2 cells (Fig. 4). The sustained formation of Ins (1,3,4,5)P4 in response to histamine might be due to regulation of the Ins (1,4,5)P3 3-kinase activity by protein kinase C and/or cAMP-dependent protein kinase similar to that in other cells (Imboden and Pattison, 1987; Biden et al., 1988). Saturation of the Ins (1,4,5)P3 3-kinase activity and the low activity of Ins (1,3,4,5)P4 5-phosphatase in DDT; MF-2 cells (Hoiting et al., 1990) can explain the second slow phase in Ins (1,4,5)P3 formation (Fig. 4). Such a delayed and small increase in Ins (1,4,5)P3 was also found in BC3H-1 muscle cells upon α1-adrenoceptor stimulation, showing a normal rise in internal Ca2+ (Ambler and Taylor, 1989).

Several models are presented to describe the inositol phosphate-regulated Ca2+ release from internal stores (Putney et al., 1989; Takemura et al., 1989; Berridge and Irvine, 1989). Stimulation of Ins (1,4,5)P3-sensitive receptors on the endoplasmic reticulum or related structures (Volpe et al., 1989) is thought to cause Ca2+ release. In view of the inositol phosphates formed throughout stimulation of the cells with histamine, receptor desensitization or lack of inositol phosphates cannot account for the transient characteristics of the rise in internal Ca2+. The transient nature of the Ca2+ response is explained in terms of a quantal release process (Mualem et al., 1989) or a feedback mechanism (Irvine, 1990). In the quantal release process the Ca2+ stores are supposed to display different sensitivities to Ins (1,4,5)P3 and in the feedback process regulation of the Ins (1,4,5)P3 receptor affinity is considered to occur by stored Ca2+ itself. The short lasting transient increase in Ins (1,4,5)P3 upon H1 histaminergic stimulation is also accompanied by an outward current following similar time-related characteristics (Fig. 3A; Table I). It is noticed that the inability to evoke a second histamine-induced response under Ca2+-free conditions is also reflected by the absence of a change in transmembrane current (Fig. 3A).

FIG. 4. Changes in Ins (1,4,5)P3 and Ins (1,3,4,5)P4 formation after histamine (10−7 M) stimulation. A, the [3H]Ins (1,4,5)P3 formation. B, the mass measurement of Ins (1,4,5)P3. C, the [3H]Ins (1,3,4,5)P4 formation. Basal values of incorporated mg of [3H]inositol for both Ins (1,4,5)P3 and Ins (1,3,4,5)P4 are given in Table I. Basal level of Ins (1,4,5)P3 mass is 49 ± 3 pmol/106 cells (n = 8).

FIG. 5. The outward current (holding potential, −10 mV) evoked in the same cell by noradrenaline (10−6 M) and histamine (10−7 M), respectively. After obtaining the whole-cell configuration (dot, left recording), noradrenaline (middle recording), and after washout (15 min) histamine (right recording) were added extracellulary. A, with intracellular solution in the patch pipette. Noradrenaline did not affect the membrane current whereas histamine caused an outward current (upward deflection). B, after the addition of Ins (1,4,5)P3 (10−6 M) to the cytoplasm via the patch pipette. Irregular occurring oscillations are introduced by Ins (1,4,5)P3, and also on application of noradrenaline. Histamine caused an outward current.
The only component responsible for the Ca\(^{2+}\) response referring to the time course. The function of Ins (1,3,4,5)P\(_4\) in the washout Ca\(^{2+}\) to the cytoplasm via the patch pipette showed an initial outward current. Shortlasting fluctuations in membrane current were introduced by noradrenaline and caused a pronounced current (note the scale). After the addition of EGTA (3 mM) to the cytoplasm via the patch pipette, histamine only caused a shortlasting oscillating increase in outward current.

![Fig. 6. The outward current (holding potential, -10 mV) evoked in the same cell by noradrenaline (10\(^{-5}\) M) and histamine (10\(^{-4}\) M), respectively. After obtaining the whole-cell configuration (dot, left recording), noradrenaline (middle tracing), and after washout (15 min) histamine (right recording) were added extracellularly. A, the addition of Ca\(^{2+}\) (10\(^{-4}\) M) and EGTA to the cytoplasm via the patch pipette caused an initial outward current. Shortlasting fluctuations in membrane current were introduced by noradrenaline and caused a pronounced current (note the scale). B, after the addition of EGTA (3 mM) to the cytoplasm via the patch pipette, relative high concentrations compared with basal values were applied to prevent an essential increase in these components during receptor stimulation. Internal application of Ins (1,4,5)P\(_3\) initially introduced transient outward currents occurring in about one out of three experiments (Fig. 5B), called base-line spiking (Ryu et al., 1987). Such irregular outward current fluctuations were also induced by noradrenaline (Fig. 5B), having characteristics different from the histamine-induced current, which response was not affected by Ins (1,4,5)P\(_3\) (Fig. 5, A and B). Internal Ca\(^{2+}\) fluctuations can be triggered by Ins (1,4,5)P\(_3\)-induced Ca\(^{2+}\) release and Ins (1,3,4,5)P\(_4\)-mediated Ca\(^{2+}\) translocation (Ryu et al., 1987) and apparently also by applying internal Ca\(^{2+}\), reflected by current fluctuations as shown (Figs. 5B, 6A, 7A). The histamine-induced K\(^+\) current was increased in amplitude (204 ± 31% of control value; n = 9) and duration (5 min) in the presence of high cytosolic Ca\(^{2+}\) (Fig. 6A). With sufficient internal Ca\(^{2+}\), activation of K\(^+\) channels is thought to be determined by the availability of Ins (1,3,4,5)P\(_3\). In agreement with these results the addition of EGTA (9 × 10\(^{-3}\) M) intracellularly decreased the basal outward current and reduced the histamine-induced current substantially (Fig. 6B) by lowering the basal internal Ca\(^{2+}\) level and binding Ca\(^{2+}\) released upon receptor stimulation. As expected a change in transmembrane current was not recorded with noradrenaline under high internal Ca\(^{2+}\) conditions (Fig. 6A), with the combination of internal Ca\(^{2+}\) and Ins (1,4,5)P\(_3\) (not shown) or with EGTA (Fig. 6B).

Fig. 8. A simplified model. The formation of Ins (1,4,5)P\(_3\) resulted in Ca\(^{2+}\) release from internal stores without affecting the transmembrane current as caused by stimulation of \(\alpha_1\)-adrenoceptors. The formation of Ins (1,3,4,5)P\(_4\) promoted Ca\(^{2+}\) release from internal stores and opened potassium channels, resulting in a transmembrane outward current, which events were observed by stimulation of \(\alpha_1\)-histaminergic receptors.

Ca\(^{2+}\) release process is attributed to translocation of Ca\(^{2+}\) toward the Ins (1,4,5)P\(_3\)-sensitive store (Berridge and Irvine, 1989; Irvine, 1990). Thus, Ins (1,4,5)P\(_3\) and Ins (1,3,4,5)P\(_4\) are apparently both involved in the generation of the Ca\(^{2+}\) release, in particular in the onset and in the development of the Ca\(^{2+}\) response. Based on the histamine concentration-related action and the time-related characteristics it is concluded that the inositol phosphate formation, enhancement of internal Ca\(^{2+}\), and the potassium current are related phenomena in DDT\(_1\) MF-2 cells.

Stimulation of \(\alpha_1\)-adrenoceptors by noradrenaline (10\(^{-5}\) M) caused an increase in the cellular level of Ins (1,4,5)P\(_3\) and Ca\(^{2+}\) not associated with the formation of Ins (1,3,4,5)P\(_4\) or a change in transmembrane current (Nelemans et al., 1990; Table I; Fig. 5A). Comparison of the results obtained with histamine and noradrenaline suggest a role of Ins (1,3,4,5)P\(_4\) in the generation of the K\(^+\) current. This was examined by applying the inositol phosphates (10\(^{-5}\) M), Ca\(^{2+}\) (10\(^{-4}\) M), or EGTA (3 × 10\(^{-3}\) M) directly to the cytoplasm via the patch pipette. Relative high concentrations compared with basal values were applied to prevent an essential increase in these components during receptor stimulation. Internal application of Ins (1,4,5)P\(_3\) initially introduced transient outward shortlasting currents occurring in about one out of three experiments (Fig. 5B), called base-line spiking (Ryu et al., 1987). Such irregular outward current fluctuations were also induced by noradrenaline (Fig. 5B), having characteristics different from the histamine-induced current, which response was not affected by Ins (1,4,5)P\(_3\) (Fig. 5, A and B). Internal Ca\(^{2+}\) fluctuations can be triggered by Ins (1,4,5)P\(_3\)-induced Ca\(^{2+}\) release and Ins (1,3,4,5)P\(_4\)-mediated Ca\(^{2+}\) translocation (Ryu et al., 1987) and apparently also by applying internal Ca\(^{2+}\), reflected by current fluctuations as shown (Figs. 5B, 6A, 7A). The histamine-induced K\(^+\) current was increased in amplitude (204 ± 31% of control value; n = 9) and duration (5 min) in the presence of high cytosolic Ca\(^{2+}\) (Fig. 6A). With sufficient internal Ca\(^{2+}\), activation of K\(^+\) channels is thought to be determined by the availability of Ins (1,3,4,5)P\(_3\). In agreement with these results the addition of EGTA (9 × 10\(^{-3}\) M) intracellularly decreased the basal outward current and reduced the histamine-induced current substantially (Fig. 6B) by lowering the basal internal Ca\(^{2+}\) level and binding Ca\(^{2+}\) released upon receptor stimulation. As expected a change in transmembrane current was not recorded with noradrenaline under high internal Ca\(^{2+}\) conditions (Fig. 6A), with the combination of internal Ca\(^{2+}\) and Ins (1,4,5)P\(_3\) (not shown) or with EGTA (Fig. 6B).

The addition of Ins (1,3,4,5)P\(_4\) to the cytoplasm caused a marked outward current (Fig. 7A) in contrast with its action
in lacrimal acinar cells (Morris et al., 1987; Changya et al., 1989). The duration of this response in DDT; MF-2 cells is most likely limited by the Ca" release, facilitated by the applied amount of Ins(1,3,4,5)P_4 (Berridge and Irvine, 1989) in the presence of a basal level of Ins(1,4,5)P_3. Thus, formation of an additional amount of Ins(1,3,4,5)P_4 by histamine does not initiate a second rise in internal Ca" sufficient to generate the typical histamine-induced outward current but only a shortlasting reduced current (Fig. 7A). Accordingly, continuing the experiment (25 min) showed that a histamine-induced current could not be evoked anymore (not shown). However, the rise in internal Ca" evoked by Ins(1,4,5)P_3 formed upon stimulation of a_2-adrenoceptors (Nelemans et al., 1990; Table I) was accompanied by an outward current if internal Ins(1,3,4,5)P_4 was present (Fig. 7A), implying that both internal Ca" and Ins(1,3,4,5)P_4 are required to activate K" channels. In agreement, the combination of Ins(1,3,4,5)P_4 and Ca" applied to the cytoplasm caused a sustained outward K" current, achieved via receptor stimulation or by direct addition.

A simplified model, depicting the action of internal Ins(1,4,5)P_3, Ins(1,3,4,5)P_4, and Ca" on the transmembrane current, achieved via receptor stimulation or by direct addition, is presented (Fig. 8). These results show for the first time that the combined action of internal Ca" and Ins(1,3,4,5)P_4 is required to activate K" channels responsible for the histamine-evoked transmembrane current in DDT; MF-2 cells.

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