Functional Properties of the Type-3 InsP₃ Receptor in 16HBE14o—Bronchial Mucosal Cells*

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The type-3 inositol 1,4,5-trisphosphate (InsP₃) receptor is the major isoform expressed in 16HBE14o—cells from bronchial mucosa, representing 93% at the mRNA level as determined by reverse transcription-polymerase chain reaction and about 81% at the protein level as determined with isoform-specific antibodies (Sienaert, I., Huyghe, S., Parys, J. B., Malfait, M., Kunzelmann, K. De, Smiedt, H., Verleden, G. M., and Missiaen, L., Pfuegers Arch. Eur. J. Physiol., in press). The present ⁴⁵Ca²⁺ efflux experiments indicate that these InsP₃ receptors were 3 times less sensitive to InsP₃ and 11 times less sensitive to ATP than those in A7r5 cells, where the type-1 InsP₃ receptor is the main isoform. ATP did not increase the cooperativity of the InsP₃-induced Ca²⁺ release in 16HBE14o—cells, in contrast to its effect in A7r5 cells. The sulfhydryl reagent thimerosal also did not stimulate InsP₃-induced Ca²⁺ release in 16HBE14o—cells, again in contrast to its effect in A7r5 cells. Adenophostin A was more potent than InsP₃ in stimulating the release in both cell types. The biphasic activation of the InsP₃ receptor by cytosolic Ca²⁺ occurred in both cell types. We conclude that Ca²⁺ release mediated by the type-3 InsP₃ receptor mainly differs from that mediated by the type-1 InsP₃ receptor by its lack of stimulation by sulfhydryl oxidation and its lower ATP and InsP₃ sensitivity. The predominant expression of the type-3 InsP₃ receptor in the bronchial mucosa may be part of a mechanism coping with oxidative stress in that tissue.

Many cells use inositol 1,4,5-trisphosphate (InsP₃) as a second messenger to release Ca²⁺ from their internal stores (1). The InsP₃ receptors (InsP₃R) are encoded by three different genes, resulting in the existence of an InsP₃R-1, -2, and -3 (2–4), which differ in their affinity for InsP₃ (InsP₃R-2 > InsP₃R-1 > InsP₃R-3) (5). Reports on isoform-specific effects of cytosolic Ca²⁺ on InsP₃ binding are conflicting (6, 7), and nothing is known about the effect of other regulators. We recently observed that 16HBE14o—cells from bronchial mucosa express 93% type-3 InsP₃R, as judged from the relative levels of steady-state mRNA as determined by polymerase chain reaction.² Experiments using isoform-specific antibodies revealed that the type-3 InsP₃R was also the main isoform expressed at the protein level (about 81%). Two reasons may underlie the small difference in values obtained with the two techniques. First, it should be emphasized that a quantitative reverse transcription-polymerase chain reaction method was used for determining the mRNA level, whereas the determination at the protein level was semi-quantitative and based on a comparison between cell types. Second, in any cell type, the mRNA level does not necessarily reflect the protein level. Whatever the reason for the small difference, both methods are in general agreement and indicate that InsP₃R-3 is in 16HBE14o—cells the major (>81%) InsP₃R-isoform expressed. We now compared the basic properties of the InsP₃-induced Ca²⁺ release in 16HBE14o—cells with those in A7r5 cells, where InsP₃R-1 is the predominant isoform (9). The properties of the InsP₃R-3-expressing 16HBE14o—cells differed mainly from those of InsP₃R-1-expressing A7r5 cells by a lack of stimulation by sulfhydryl oxidation, an 11 times lower ATP sensitivity and a 3 times lower InsP₃ sensitivity.

EXPERIMENTAL PROCEDURES

Adenophostin A was isolated as described previously (10). ⁴⁵Ca²⁺ fluxes on monolayers of saponin-permeabilized A7r5 cells from embryonic rat aorta and 16HBE14o—cells from a bronchial surface epithelium were done at 25 °C as described (11). The stores were loaded for 1 h in 120 mM KCl, 30 mM imidazole-HCl (pH 6.8), 5 mM MgCl₂, 5 mM ATP, 0.44 mM EGTA, 10 mM NaCl, and 150 mM free Ca²⁺ (23 μM/ml) and then washed twice in an efflux medium containing 120 mM KCl, 30 mM imidazole-HCl (pH 6.8), 1 mM EGTA, and 2 μM thapsigargin. Addition of InsP₃, adenophostin A, ATP, thimerosal, or Ca²⁺ are indicated in the figures. The free [⁴⁵Ca²⁺] of the efflux medium was calculated using MaxChelator (Dr. C. Patton, Stanford University, CA). 1 ml of this medium was then added to the cells and replaced every 6 or 2 min. At the end of the experiment, the ⁴⁵Ca²⁺ remaining in the stores was released by incubation with 1 ml of 2% sodium dodecyl sulfate solution for 30 min.

RESULTS AND DISCUSSION

InsP₃- and Adenophostin A-induced Ca²⁺ Release in Permeabilized A7r5 and 16HBE14o—Cells—The non-mitochondrial Ca²⁺ stores were loaded with ⁴⁵Ca²⁺ and then challenged with a progressively increasing [InsP₃] or [adenophostin A] in efflux medium. The closed symbols in Fig. 1 illustrate the Ca²⁺ release as a function of the [InsP₃] for the two cell types. The EC₅₀ was 0.7 μM InsP₃ in A7r5 cells (closed circles) and 1.9 μM

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InsP₃ in 16HBE14o− cells (closed squares). This difference in EC₅₀ value probably represents the different affinity of the major InsP₃R isoform expressed, which is InsP₃R-1 in A7r5 cells (9) and InsP₃R-3 in 16HBE14o− cells. InsP₃R-3 had a much lower affinity than InsP₃R-1 if bacterial recombinant ligand binding domains of these two isoforms were compared under identical conditions (5).

Adenophostin A is much more potent than InsP₃ in stimulating InsP₃R-1 (10, 12). These data were confirmed for A7r5 cells, i.e. a cell type where InsP₃R-1 is the main isoform (open circles in Fig. 1). Also, 16HBE14o− cells responded to adenophostin A (open squares in Fig. 1), indicating that adenophostin A activated InsP₃R-3 as well. The EC₅₀ was again lower than for InsP₃.

### Table I

| Nucleotide | Stimulation of the release |
|------------|--------------------------|
|            | A7r5 cells               | 16HBE14o− cells |
| ATP        | 100                      | 100            |
| ADP        | 101 ± 5                  | 95 ± 6         |
| AMP        | 67 ± 6                   | 53 ± 4         |
| GTP        | 52 ± 5                   | 60 ± 2         |
| ITP        | 40 ± 7                   | 48 ± 3         |

The stimulation of the Ca²⁺ release induced by 3 μM InsP₃ by the indicated compounds (1 mM) was determined as in Fig. 2. The stimulation was normalized to that observed in the presence of 1 mM ATP. The values are expressed as mean ± S.E. for three independent experiments.
steady state with 45Ca2+

increasing [InsP3] is affected by 1 mM ATP. We confirmed that

A

are the means of five (plotted as fractional loss, steps, each lasting 6 s, as indicated below the tracings. The results are

conserved in all isoforms (18). InsP3R-1 in addition has, de-

pendently because of the higher [InsP 3] used in the present study.

InsP3R-1 and/or its effect on the cooperativity of the Ca 2

nucleotide binding sites (18, 19). The higher affinity for ATP of

InsP3R-1 whereas AMP, GTP, and ITP were less
effective in both cell types (Table I). The inhibition in A7r5 cells
was added. The [InsP 3] was then increased from 3 n M to 3 M in 60
sensitizing effect of thimerosal did not occur in 16HBE14o

cells. (22). This is most likely due to the absence of ATP in the

A7r5 cells occurred at a lower [thimerosal] than reported before

This inhibition occurred in both cell types. The inhibition in

A7r5 cells reached its maximum at 0.1 M Ca2+

is expressed as a percentage of the Ca2+ release at 5 nM free Ca2+. Mean ± S.E. is shown for five independent experiments.

release may therefore represent the activity of one of the latter

sites.

Effect of Thimerosal on InsP3-induced Ca2+ Release in Permeabilized A7r5 and 16HBE14o— Cells—The sulfhydryl reagent thimerosal can, depending on its concentration, both stimulate and inhibit the InsP3R in many cell types (20–26). In contrast, thimerosal does not stimulate the (as yet unidenti-

fied) InsP3R isoform in mouse lacrimal cells (24). Since it is not

known which of the three InsP3R isoforms are stimulated by

thimerosal, we have compared its effect on the response of

permeabilized A7r5 and 16HBE14o— cells to a progressively

increasing [InsP3] (Fig. 4). In A7r5 cells, thimerosal dose-de-

pendently shifted the threshold for initiating Ca2+ release to-

ward lower InsP3 concentrations (closed circles in Fig. 4A). The

sensitizing effect of thimerosal did not occur in 16HBE14o—
cells (closed circles in Fig. 4B). Thimerosal also lowered the

maximum of the curves, which represents the inhibitory effect.

This inhibition occurred in both cell types. The inhibition in

A7r5 cells occurred at a lower [thimerosal] than reported before

(22). This is most likely due to the absence of ATP in the

present experiments (data not shown).

Effect of Cytosolic Ca2+ on InsP3-induced Ca2+ Release in Permeabilized A7r5 and 16HBE14o— Cells—Fig. 5 illustrates how cytosolic Ca2+ modified the Ca2+ release induced by 3 μM InsP3. The biphasic activation of the InsP3R by Ca2+ (13, 27, 28, 30) occurred both in A7r5 cells (circles) and in 16HBE14o—
cells (squares). The almost complete inhibition of the release at

10 μM free Ca2+ in 16HBE14o— cells indicates that all the

InsP3Rs expressed in this cell type were inhibited. As a conse-

quence, the predominantly expressed isoform (InsP3R-3) was

also inhibited by cytosolic Ca2+. The curve for 16HBE14o—
cells reached its maximum at 0.1 μM Ca2+, whereas A7r5
reached its maximum at 0.3 μM Ca2+. This finding indicates

that the activation by Ca2+ of the type-1 InsP3R, which is a

quantitatively unimportant isoform in 16HBE14o— cells, could

not be responsible for the stimulatory part of the Ca2+ response

curve in 16HBE14o— cells.

Conclusions—We observed major differences in the regula-
tion of the InsP3R-induced Ca2+ release between a cell type that

predominantly expresses InsP3R-3 and a cell type that mainly

expresses InsP3R-1. We confirmed at the functional level the

16HBE14o— cells (341 μM) than in A7r5 cells (32 μM). ADP was

as effective as ATP, whereas AMP, GTP, and ITP were less
effective in both cell types (Table I). The inhibition in A7r5 cells
by high ATP concentrations (16), which is due to a competition
with InsP3 (17), was not observed in the present study, proba-

bly because of the higher [InsP3] used in the present study.

ATP increases the cooperativity of the InsP3-induced Ca2+
release in A7r5 cells (16). Fig. 3 compares how the response of

the stores of A7r5 and 16HBE14o— cells to a progressively

increasing [InsP3] is affected by 1 mM ATP. We confirmed that

ATP increased the steepness of the response to InsP3 in A7r5

cells, as judged by the clear shift of the maximum of the curve

toward lower InsP3 concentrations (arrowheads in Fig. 3A). This

effect was, however, much less pronounced in 16HBE14o—
cells (arrowheads in Fig. 3B).

A comparison of the sequences of the three InsP3R isoforms
reveals that one predicted adenine-nucleotide binding site is

conserved in all isoforms (18). InsP3R-1 in addition has, de-

pending on the splice isoform, 1 or 2 other potential adenine-
nucleotide binding sites (18, 19). The higher affinity for ATP of

InsP3R-1 and/or its effect on the cooperativity of the Ca2+

FIG. 4. Effect of thimerosal on InsP3-induced Ca2+ release in permeabilized A7r5 and 16HBE14o— cells. The non-mitochondrial Ca2+ stores of A7r5 cells (A) and 16HBE14o— cells (B) were loaded to steady state with 45Ca2+. After a 7-min incubation in efflux medium without (C) or with the indicated thimerosal (Thim. ●, 3 mM InsP3 was added. The [InsP3] was then increased from 3 mM to 3 μM in 60

steps, each lasting 6 s, as indicated below the tracings. The results are plotted as fractional loss, i.e. the rate of Ca2+ release over a 6-s period divided by the Ca2+ content of the stores at that time. The data points are the means of five (A) and four (B) independent experiments.

FIG. 5. Biphasic activation of the InsP3Rs in permeabilized A7r5 and 16HBE14o— cells by cytosolic Ca2+. The non-mitochon-
drial Ca2+ stores of A7r5 cells (●) and 16HBE14o— cells (□) were loaded to steady state with 45Ca2+ and incubated in non-labeled efflux medium containing 2 μM thapsigargin and 5 mM free 45Ca2+ for 10 min. The stores were then challenged for 2 min with 3 μM InsP3 in the presence of the indicated free [45Ca2+]i, which was added at the time of InsP3 addition. The Ca2+ release at the indicated [Ca2+]i is expressed as a percentage of the Ca2+ release at 5 nM free Ca2+. Mean ± S.E. is shown for five independent experiments.
known difference in InsP$_3$ affinity (5) and in addition observed that InsP$_3$R-3 was 11 times less sensitive to ATP and was not activated by sulphydryl oxidation. The predominant expression in the respiratory mucosa of InsP$_3$R-3 and the predominant expression of the SERCA3 Ca$^{2+}$ pump isoform (29), which is very resistant to reactive oxygen species (8), may be part of a mechanism coping with oxidative stress in that tissue.

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