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The effects of tetrahexyl ammonium cations (THA^+) on inositol 1,4,5-trisphosphate-induced calcium release from porcine cerebellar microsomes: THA^+ can induce calcium release selectively from the $Ins P_3$-sensitive calcium stores

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In this study we show that the potassium-channel blocker tetrahexyl ammonium chloride (THA^+) is able to inhibit inositol 1,4,5-trisphosphate ($Ins P_3$)-induced calcium release in an apparently biphasic fashion with a IC_{50} of 3 μM. This inhibition was not alleviated by valinomycin and, therefore, is not consistent with the blocking of K^+ counter-ion movement, an observation initially made by Palade et al. (Palade, P., Dettbarn, C., Volpe, P., Alderson, B. and Otero, A.S (1989) Mol. Pharmacol. 36, 664–672). THA^+ affected quantal calcium release by reducing the amount of calcium released by $Ins P_3$, but did not greatly affect the concentration of $Ins P_3$ required to cause half-maximal calcium release. THA^+ did not affect the metabolism of $Ins P_3$ or its binding to porcine cerebellar microsomes. THA^+ could also itself induce calcium release. At concentrations below 100 μM, THA^+ appears to release Ca^{2+} selectively from the $Ins P_3$-sensitive calcium stores, since prior depletion of these stores with supramaximal doses of $Ins P_3$ abolishes this response. At higher THA^+ concentrations (above 100 μM) Ca^{2+} is released non-selectively from all stores. THA^+ has no effect on the Ca^{2+}-ATPase activity at concentrations below 100 μM, indicating that selective THA^+-induced Ca^{2+} release is not due to non-specific inhibition of the microsomal Ca^{2+} pumps and does not affect Ca^{2+} leakage. A number of pharmacological modulators of intracellular calcium channels were also tested on THA^+-induced calcium release with little effect, except for spermidine which reduced this release by up to 50%. Our observations are consistent with the view that THA^+, at concentrations below 100 μM, selectively releases calcium from the $Ins P_3$-sensitive calcium stores.

Introduction

It has been shown by Palade et al. that tetrahexyl ammonium ions (THA^+) are able to inhibit both the amount and the rate of calcium release induced by inositol 1,4,5-trisphosphate from canine cerebellar microsomes with an apparent inhibition constant of 4 μM [1]. Other potassium-channel blockers studied were found to be less potent. This study also showed that although potassium was required for $Ins P_3$-induced calcium release the action of these potassium-channel blockers was not due to inhibition of K^+ counter-ion movement as suggested by Shah and Pant [2] and Muallem et al. [3] as the potasssium ionophores valinomycin and gramicidin D failed to alleviate the inhibition of $Ins P_3$-induced calcium release [1].

Since this inhibition was not due to effects on $Ins P_3$ binding, it is therefore unlikely to be similar in nature to heparin inhibition which is known to compete for the $Ins P_3$-binding site [4]. Recently caffeine and thimerosal have also been shown to inhibit $Ins P_3$-induced calcium release from cerebellar microsomes [5,6]. Inhibition by caffeine and thimerosal, like THA^+, was not due to effects on $Ins P_3$ binding, although 'quantal' calcium release was affected. Caffeine did not affect the maximum amount of calcium which could be released by $Ins P_3$ but did increase the concentration required to cause half-maximal release (IC_{50}) and also changed the apparent cooperativity of calcium release. In contrast, thimerosal affected the maximum amount of calcium which could be released by $Ins P_3$ whilst having little effect on the IC_{50}, highlighting differences in the mechanisms of inhibition of the $Ins P_3$ receptor. Here we have investigated the effects of THA^+ on $Ins P_3$-induced calcium release.

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Abbreviations: $Ins P_3$, inositol 1,4,5-trisphosphate; THA^+, tetrahexyl ammonium chloride; IC_{50}, concentration which gives half-maximal response.

Palade et al. [1] also showed that at concentrations higher than those required to inhibit $Ins P_3$-induced Ca^{2+} release, THA^+ elicited calcium release from microsomes actively loaded with calcium. It was suggested that this release was non-specific, possibly due to disruption in the organisation of the membrane. Here we provide evidence to suggest that THA^+-induced calcium release, at concentrations below 100 μM, comes selectively from the $Ins P_3$-sensitive calcium stores.

Materials and Methods

Fluo-3 was obtained from Molecular Probes, $Ins P_3$ and ryanodine from Calbiochem, [^{3}H]$Ins P_3$ from Amersham International and THA^+ from Aldrich. Heparin, spermidine, tetracaine and ruthenium red were obtained from Sigma. All other reagents were of analytical grade.

Porcine cerebella were obtained from freshly slaughtered pigs at a local abattoir. Porcine cerebellar microsomes were prepared as previously described in Ref. 7. The cerebella were minced and homogenised in 10 vols. of cold buffer (0.32 M sucrose, 5 mM Hepes, 0.1 mM PMSF (pH 7.4)) with a Teflon Potter-Elvehjem homogeniser and centrifuged at $500 \times g$ for 10 min. The resulting pellet was homogenised in 5 vols. of buffer and again centrifuged at $500 \times g$ for 10 min. The supernatants were pooled and the mitochondria removed by centrifugation at $10\,000 \times g$ for 15 min. The microsomal pellet was obtained by centrifugation for 1 h at $100\,000 \times g$. The pellet was resuspended in Hepes-sucrose buffer and stored at -70°C.

Calcium uptake and release were measured using fluo-3 as described in Ref. 5. Cerebellar microsomes were suspended in a buffer containing 10 mM potassium phosphate, 3.5 mM potassium pyrophosphate, 100 mM KCl, 250 nM fluo-3, 10 μg/ml creatine kinase and 10 mM creatine phosphate (pH 7.2) and measurements were carried out at 37°C. Typically, 0.25 mg/ml cerebellar microsomes were added to a continuously stirred cuvette and calcium uptake was initiated by the addition of 1.5 mM Mg-ATP. The fluorescence change was monitored in a Perkin-Elmer LS-50 spectrofluorimeter exciting at 506 nM and detecting the emission at 526 nM. Following calcium accumulation, further uptake was inhibited by the addition of 0.5 mM orthovanadate which substantially inhibited the Ca^{2+} pumps and $Ins P_3$ was then added. Total calcium accumulation within the microsomes was measured by permeabilization with 12.5 μg/ml A23187 and fluorescence intensity was related to calcium concentration as described in Ref. 5. The amount of $Ins P_3$-induced Ca^{2+} release was expressed as the% of calcium release by $Ins P_3$ compared to that released by A23187.

The effect of THA^+ on $Ins P_3$ binding was determined as described in Ref. 8. Since the K_d for $Ins P_3$ binding under these conditions (50 mM Tris-HCl (pH 8.3), 1 mM EDTA, 100 mM KCl) was determined to be 60 ± 8 nM with a B_{max} of 2.0 ± 0.2 pmol/mg, the effect of THA^+ on $Ins P_3$ binding was determined at 60 nM $Ins P_3$.

The effects of THA^+ on the metabolism of $Ins P_3$ in porcine cerebellar microsomes was determined as described in Refs. 5 and 6. 0.25 mg of porcine cerebellar microsomes were preincubated with up to 100 μM THA^+ under identical conditions to those used for calcium uptake and release. 10 μM $Ins P_3$ doped with 0.003 μCi [^{3}H]$Ins P_3$ was added and the reaction quenched after 30 s with 200 μl cold 10% perchloric acid, neutralised with KOH and the products separated on gravity-fed anion exchange columns as described in Ref. 9. The effects of THA^+ on $Ins P_3$ metabolism in porcine cerebellar microsomes under simulated 'intracellular' conditions was assessed as previously described in Refs. 5 and 6.

Calcium-dependent ATPase activities were measured as described by Brown et al. [10].

Results

Maximum $Ins P_3$-induced calcium release occurred at around 10–20 μM $Ins P_3$ giving between 12–16.5% calcium release compared to that releasable by A23187, dependent upon the microsomal preparation. Fig. 1 shows the effects of THA^+ upon $Ins P_3$-induced by 10 μM $Ins P_3$. The inhibition profile was biphasic in appearance with half-maximal inhibition (IC_{50}) of 3 μM. Addition of 5 μM valinomycin did not alleviate this inhibition confirming the findings of Palade et al. [1] that the blocking of K^+ counter-ion movement was not responsible for $Ins P_3$-induced calcium release inhibition.

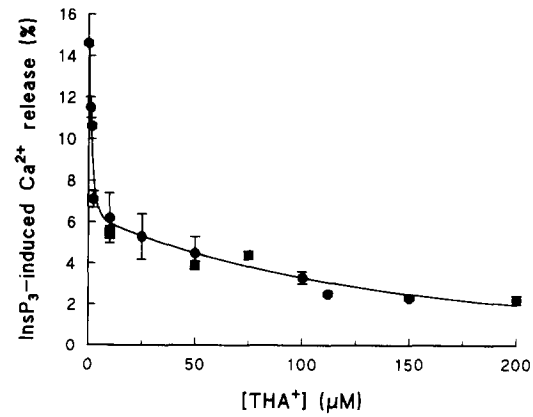

Fig. 1. The effects of THA^+ on $Ins P_3$-induced Ca^{2+} release. The amount of calcium released by 10 μM $Ins P_3$ was expressed as a % of that released by A23187. (●), The effects of THA^+ alone or (■) THA^+ plus 5 μM valinomycin, on $Ins P_3$-induced Ca^{2+} release. Data are presented as the mean ± S.E. of three determinations.

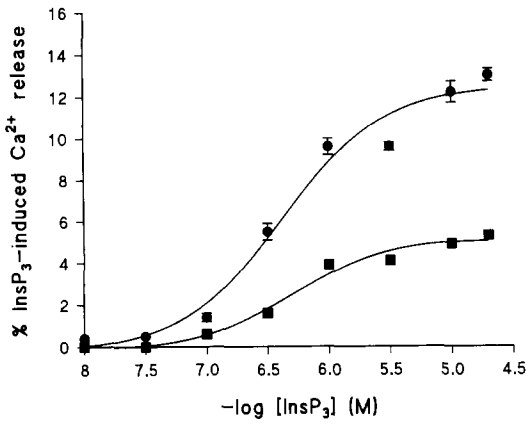

Fig. 2. The effects of THA^+ on quantal $Ins P_3$-induced Ca^{2+} release. Effects of $Ins P_3$ on the amount of Ca^{2+} release in the absence (●) and presence (■) of 3 μM THA^+. Data are presented as the mean ± S.E. of three determinations and is expressed as the % calcium released by $Ins P_3$ compared to that released by A23187.

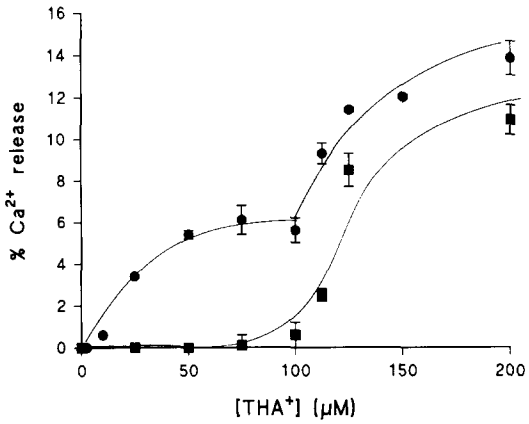

Fig. 4. THA^+-induced Ca^{2+} release before or after $Ins P_3$-induced Ca^{2+} release. (●), The effects of [THA^+] on Ca^{2+}-release from cerebellar microsomes, expressed as a % of that released by 12.5 μg/ml A23187; (■), the effects of [THA^+] on Ca^{2+}-release after the addition of 20 μM $Ins P_3$ again expressed as a % of that released by A23187. Data represent the mean ± S.E. of three determinations.

Fig. 2 shows the effects of 3 μM THA^+ on the quantal calcium release induced by 0.01–20 μM $Ins P_3$. In this preparation of porcine cerebellar microsomes maximal $Ins P_3$-induced calcium release was determined to be 12.2 ± 0.5% with a concentration of $Ins P_3$ causing half-maximal release of 420 ± 135 nM. Addition of 3 μM THA^+ reduced the maximum amount of $Ins P_3$-induced release to 4.9 ± 0.1% (60% inhibition), but did not substantially affect the IC_{50} which was determined to be 660 ± 260 nM $Ins P_3$. The apparent cooperativity of quantal $Ins P_3$-induced calcium release for porcine cerebellar microsomes was less than that observed for rat cerebellar microsomes, with a Hill coefficient (n) of 1.3 ± 0.2 for pig (determined from three separate preparations) compared to 2.0 ± 0.1 (5 separate preparations) for rat. The cooperativity in the presence of THA^+ was only slightly affected, decreasing the n to 1.0 ± 0.1.

Fig. 3A shows that THA^+ causes some of the accumulated calcium to be released. This calcium release was preparation dependent with 75 μM THA^+ causing between 4.3–6.0% calcium release compared to that amount of calcium released by A23187 ($Ins P_3$ released 12–16.5%). However, if the $Ins P_3$-sensitive calcium stores were first depleted by the addition of supramaximal concentrations of $Ins P_3$ (20 μM), 75 μM THA^+ induced little or no calcium release (Fig. 3B). Fig. 4 shows the effects of THA^+-induced calcium release in microsomes which had been actively loaded with calcium. THA^+-induced calcium release was also complex occurring in at least two distinct phases. In the first

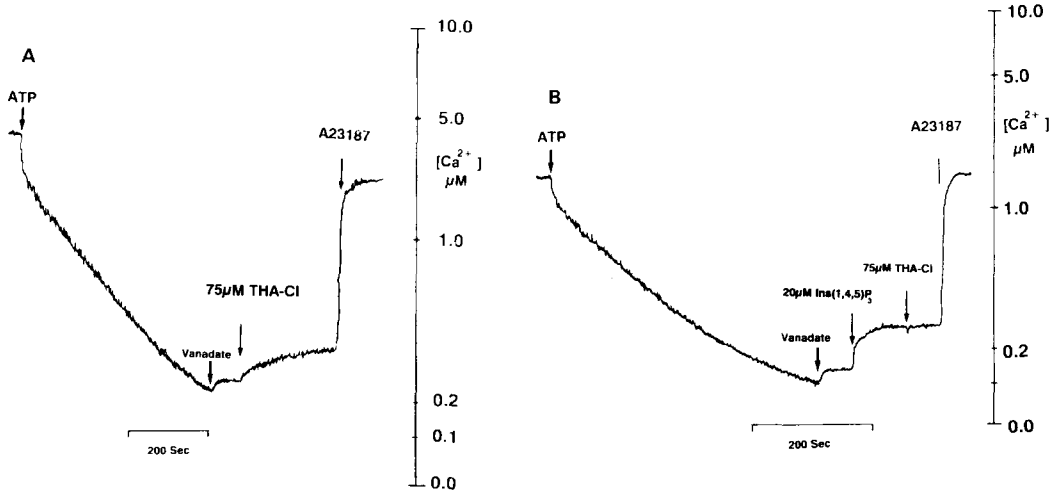

Fig. 3. The effects of THA^+ and $Ins P_3$ on Ca^{2+} release. Calcium uptake was initiated by the addition of 1.5 mM Mg-ATP (ATP) and inhibited with 0.5 mM oligovanadate (vanadate). (A), The effects of 75 μM THA^+ on calcium release followed by 12.5 μg/ml A23187; (B), the effects of 75 μM THA^+ on calcium release after the $Ins P_3$ sensitive stores were depleted by the addition of 20 μM $Ins P_3$.

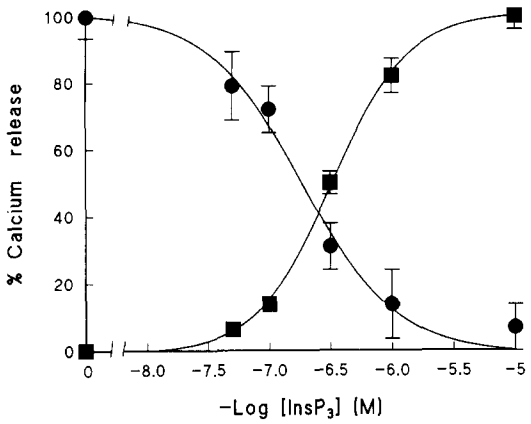

Fig. 5. The dependence of THA^+-induced Ca^{2+}-release on the fullness of the $Ins P_3$-sensitive Ca^{2+} stores. (■), $Ins P_3$-induced Ca^{2+} release. Maximum $Ins P_3$-induced calcium release was caused by 10 μM $Ins P_3$ and corresponded to 16.5% of that releasable by A23187. (●), THA^+-induced Ca^{2+} release following depletion of $Ins P_3$-sensitive Ca^{2+} stores by 0–10 μM $Ins P_3$. Maximum Ca^{2+} release following addition of 75 μM THA^+ corresponded to 4.3% of the calcium releasable by A23187. Data represent the mean ± S.E. of 3–6 determinations.

phase, THA^+-induced calcium release reached a plateau between 50–100 μM THA^+. The maximum amount of calcium released by THA^+ during this first phase was 6.0% of the calcium releasable by A23187. This corresponded to about 40% of the calcium in the $Ins P_3$-sensitive calcium stores. However, concentrations of THA^+ up to 10 μM which inhibited $Ins P_3$-induced calcium release by about 60% (see Fig. 1) caused little calcium release. Thus, THA^+-induced inhibition of $Ins P_3$-induced calcium release cannot be explained solely in terms of depletion of the calcium stores. The second phase of THA^+-induced calcium release occurred at higher THA^+ concencentrations. If the $Ins P_3$-sensitive calcium stores were initially depleted of calcium by the addition of 20 μM $Ins P_3$, the first phase of calcium release was abolished. However, with THA^+ concentrations above 100 μM the second phase of calcium release was still observed.

As THA^+-induced calcium release during the first phase of calcium release was likely to come selectively from the $Ins P_3$-sensitive calcium stores, this was further investigated using 75 μM THA^+ in cerebellar microsomes which had their $Ins P_3$-sensitive calcium stores partially depleted of calcium. Fig. 5 shows that as the $Ins P_3$-sensitive calcium stores become depleted of calcium by increasing the $Ins P_3$ concentration, THA^+-induced calcium release decreased in a concomitant fashion. The IC_{50} for $Ins P_3$-induced and THA^+-induced Ca^{2+} release were determined to be 0.3 ± 0.05 and 0.2 ± 0.1 μM, respectively. As there is a direct correlation between the fullness of the $Ins P_3$-sensitive stores and THA^+-induced calcium release, this observation is consistent with THA^+-induced calcium release coming selectively from the $Ins P_3$-sensitive store at THA^+ concentrations up to 100 μM, as no THA^+-induced calcium release occurred once these stores were empty, even though the $Ins P_3$-insensitive calcium stores which constitute approx. 85% of the total calcium stores were unaffected. Higher THA^+ concentrations, however, release calcium from both $Ins P_3$-sensitive and insensitive calcium stores (Fig. 4).

TABLE I

The effects of THA^+ on [^{3}H] $InsP_3$ binding and $InsP_3$ metabolism

N.D., not determined. Data presented as the mean ± S.E. of 3–6 determinations.

[THA^+] (μM)	$Ins P_3$ bound (pmol/mg)	$Ins P_3$ metabolized (%)
0.0	1.2 ± 0.1	10.7 ± 2.7, 93.0 ± 10.0 [a]
2.5	1.4 ± 0.1	8.0 ± 0.3
5.0	1.4 ± 0.3	n.d.
10.0	n.d.	7.0 ± 1.2
25.0	1.3 ± 0.1	8.3 ± 0.6
50.0	1.2 ± 0.3	7.2 ± 0.7
100.0	1.2 ± 0.1	6.5 ± 0.5

[a] Measured in 'intracellular' buffer (see Materials and Methods).

Table I shows the effects of THA^+ on both [^{3}H]$Ins P_3$ binding to cerebellar microsomes and $Ins P_3$ metabolism. THA^+ had little effect on binding or metabolism at concentrations up to 100 μM and therefore any effects upon inhibition of $Ins P_3$-induced calcium release were not due to the effects of THA^+ on ligand binding nor on the rapid breakdown of $Ins P_3$. It was also shown that the amount of THA^+-induced calcium release was independent of the initial amount of calcium actively loaded into the microsomes, since there was no dependence between calcium accumulated and calcium released by 75 μM THA^+ (data not shown). Table II shows the effects of THA^+ on micro-

TABLE II

The effects of THA^+ and orthovanadate on Ca^{2+} uptake and Ca^{2+}-dependent ATPase activity

100% Ca^{2+} uptake is equivalent to 0.25 nmol Ca^{2+}/min per mg microsomal protein. 100% Ca^{2+} ATPase activity is equivalent to 117 nmol P_i/min per mg microsomal protein, measured at 1 μM free Ca^{2+}. The data represent the mean ± S.E. of 3–6 determinations.

[THA^+] (μM)	Ca^{2+} uptake (%)	Ca^{2+}-ATPase activity (%)
0.0	100 ± 5	100 ± 10
25	n.d.	95 ± 11
50	105 ± 5	94 ± 8
75	99 ± 5	102 ± 8
100	91 ± 4	81 ± 9
200	n.d.	62 ± 5
Orthovanadate (0.5 mM)	13 ± 7	7 ± 3

TABLE III

The effects of pharmacological modulators of intracellular calcium channels on InsP_3-induced and THA$^+$-induced calcium release

Compound	InsP_3-induced Ca^{2+} release (10 μM)	THA^+-induced Ca^{2+} release (75 μM)
Control [a]	14.6±0.2	5.0±0.5
Control	15.7±0.4	4.3±0.2
Ag^+ (5 μM) [a]	1.0±0.2	4.9±0.2
Heparin [a] (0.25 mg/ml)	0.5±0.4	4.6±0.1
Ryanodine (10 μM)	n.d.	4.1±0.6
Ruthenium red (10 μM)	n.d.	4.4±0.2
Tetracaine (100 μM)	n.d.	3.9±0.2
Spermidine (25 mM)	10.1±0.3	2.5±0.2

[a] Microsomal preparation used caused 14.6% Ca^{2+} release with 10 μM InsP_3 and 5.0% with 75 μM THA^+.

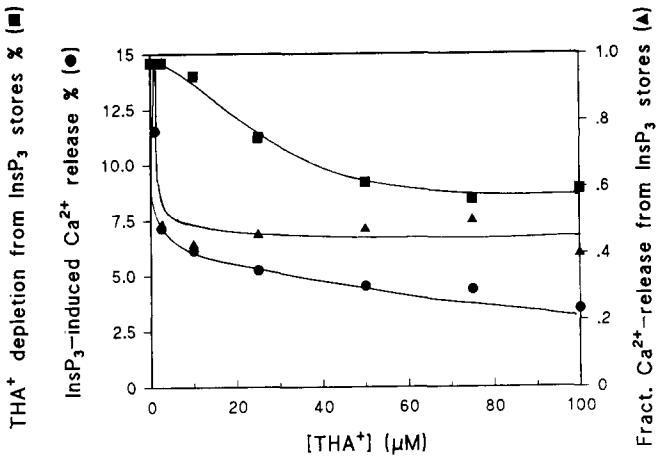

Fig. 7. Fractional inhibition of InsP_3-induced Ca^{2+} release by THA^+. (●), The biphasic inhibition curve shown in Fig. 1. THA^+-induced depletion of the InsP_3-sensitive calcium stores, calculated from the data in Fig. 4 (■) and the fractional inhibition of InsP_3-induced calcium release by THA^+, taken as the ratio of the two curves, which corresponds to the actual inhibition of InsP_3-induced Ca^{2+} release by THA^+ irrespective of depletion of Ca^{2+} stores (▲).

somal ATP-dependent calcium uptake and Ca^{2+}-dependent ATPase activity. THA^+ at concentrations below 100 μM, had no effect on the Ca^{2+}-pump, and therefore the Ca^{2+} released by THA^+ (< 100 μM) could not be due to a net increase in Ca^{2+} efflux by inhibition of the residual orthovanadate-insensitive Ca^{2+} uptake via the Ca^{2+} pump. In fact, at the orthovanadate concentration used in these experiments (0.5 mM) approx. 90% of the Ca^{2+} pump activity had been inhibited (Table II). At higher THA^+ concentrations (> 100 μM) some inhibition of the Ca^{2+} pump was observed and may well, in part, account for the non-specific THA^+-induced Ca^{2+} release observed.

As THA^+-induced calcium release (at low THA^+ concentrations) selectively came from the InsP_3-sensitive calcium stores, one possible mechanism for calcium release was via intracellular calcium channels associated with these stores. The effects of a number of pharmacological modulators of intracellular calcium channels on THA^+-induced calcium release were assessed. Table III lists the effects of a variety of agents on THA^+-induced calcium release. Ruthenium red, ryanodine, silver ions, tetracaine and heparin all had little or no effect on THA^+-induced calcium release. Only 25 mM spermidine reduced THA^+-induced calcium release by about 45%. 25 mM spermidine also reduced the InsP_3-induced calcium release by 35%, indicating that it also inhibits the InsP_3 receptor, as well as the ryanodine receptor [11]. It was unlikely that spermidine decreased both InsP_3-induced and THA^+-induced calcium release by partially emptying the InsP_3-sensitive calcium stores, since no calcium release was observed when spermidine was added to microsomes actively loaded with calcium. Fig. 6 shows the concentration dependence of spermidine on THA^+-induced calcium release. Spermidine was only partly effective in reducing the THA^+-induced calcium release (75 μM THA^+), decreasing it by up to 50%.

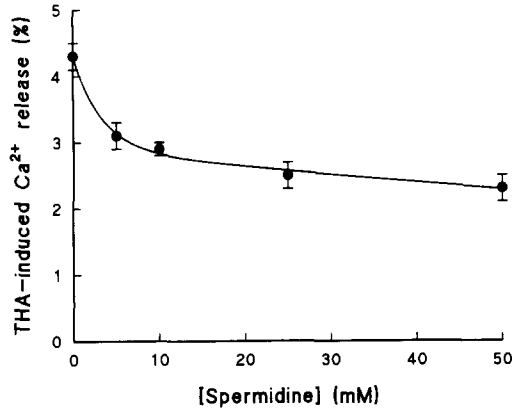

Fig. 6. The effects of spermidine on THA^+-induced Ca^{2+} release. The effects of spermidine (0–50 mM) on Ca^{2+} release induced by 75 μM THA^+ and expressed as a % of the A23187 releasable Ca^{2+} stores. Data represent the mean±S.E. of three determinations.

Discussion

In this study we confirm that the potassium-channel blocker THA^+ is a potent inhibitor of InsP_3-induced calcium release from porcine cerebellar microsomes. We have also observed this inhibition in rat cerebellar microsomes and permeabilized HL 60 cells (Sayers, L. and Michelangeli, F., unpublished observations). In this study we have shown this inhibition to be complex, occurring in an apparently biphasic fashion. From Fig. 4 it can be seen that THA^+ itself, at concentrations up

to 100 μM, induced calcium release selectively from the InsP_3-sensitive calcium stores (see later). This would therefore have the effect of reducing the amount of calcium releasable by InsP_3, after THA^+ treatment, leading to artifactually higher levels of inhibition than would otherwise be observed. An estimate of the fractional inhibition of InsP_3-induced Ca^{2+} release by THA^+ can, however, be determined, irrespective of Ca^{2+} depletion of the InsP_3-sensitive Ca^{2+} stores, by taking ratios of the experimentally determined inhibition of InsP_3-induced Ca^{2+} release at individual THA^+ concentrations (data from Fig. 1) and Ca^{2+} remaining in the InsP_3-sensitive Ca^{2+} stores. This is obtained by subtracting the THA^+-induced Ca^{2+} release (data from Fig. 4) from the maximal InsP_3-induced Ca^{2+} release. In order to simplify this analysis only data up to 100 μM THA^+ was used since below this concentration the release induced by THA^+ was shown to come specifically from the InsP_3-sensitive Ca^{2+} stores. Fig. 7 shows the data representing (i) Ca^{2+} remaining within the InsP_3-releasable stores after THA^+ treatment and expressed as a percentage of the total accumulated Ca^{2+}; (ii) the biphasic inhibition of InsP_3-induced calcium release by THA^+ again expressed as a percentage of the total accumulated Ca^{2+} and (iii) the fractional inhibition of the InsP_3-sensitive Ca^{2+} stores by THA^+ calculated from the ratio of the two values. The fractional inhibition curve had an IC_{50} of approx. 3 μM and reached a plateau when around 60% of the InsP_3-sensitive calcium stores had been depleted. One possible explanation for partial inhibition may be that since the InsP_3 receptor has been shown to have multiple conductance states [12], THA^+ may only block some of these states. Electrophysiological studies have recently shown that related tetraalkyl ammonium cations also partially block the calcium conductance of the purified cardiac ryanodine receptor [13]. It is already well established that this intracellular calcium channel has a high degree of similarity with the InsP_3 receptor [14] and partial blocking by tetraalkyl ammonium cations may well be another common characteristic of both channels. Alternatively, as serveral isoforms of the InsP_3 receptor are known to exist within the cerebellum [18], these isoforms may have different sensitivities to THA^+. The inhibition of quantal calcium release by THA^+ is more like that observed for inhibitory concentrations of thimerosal than caffeine, since the maximum amount of calcium release is reduced yet the IC_{50} remains little affected.

THA^+ also causes calcium release to occur in the absence of InsP_3, in at least two distinct phases as shown in Fig. 4. The first phase of THA^+-induced calcium release reaches a plateau after 50 μM THA^+ releasing up to 6.0% of calcium which is releasable by A23187. Above 100 μM THA^+ the amount of calcium release again increases. If the InsP_3-sensitive calcium stores are initially depleted by the addition of 20 μM InsP_3 the first phase of THA^+-induced calcium release is abolished, indicating that in this phase calcium release specifically originates from the InsP_3-sensitive calcium stores. The direct dependence between the amount of THA^+-induced calcium release and the fullness of the InsP_3-sensitive calcium stores also confirms this observation.

As THA^+-induced calcium release below 100 μM arises selectively from the InsP_3-sensitive calcium stores, and does not arise from an increase in the Ca^{2+} efflux due to Ca^{2+}-pump inhibition, the possiblity that it occurs via an intracellular calcium channel was explored. A number of pharmacological modulators of intracellular calcium channels were assessed in order to investigate its site of action. Inhibitors of InsP_3-induced calcium release such as heparin [4] and silver ions [15] had no effect on THA^+-induced calcium release. Inhibitors of the ryanodine receptor, e.g., ruthenium red [16] and local anaesthetics [17] also had no effect. However, spermidine which is known to inhibit both the ryanodine receptor [11] and from this study the InsP_3 receptor (Table III), partially blocks THA^+-induced calcium release. From this pharmacological data it remains unclear whether THA^+-induced calcium release from the InsP_3-sensitive calcium stores occurs through the InsP_3-receptor or via some other protein unique to the InsP_3-sensitive calcium stores. Irrespective of the exact mechanism of THA^+-induced calcium release, we have demonstrated that THA^+, at concentrations below 100 μM, is able to selectively release calcium from the InsP_3-sensitive calcium stores.

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