Carboxyl-terminal Peptide of β-Amyloid Precursor Protein Blocks Inositol 1,4,5-Trisphosphate-sensitive Ca\(^{2+}\) Release in Xenopus laevis Oocytes*  

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The effects of Alzheimer’s disease-related amyloidogenic peptides on inositol 1,4,5-trisphosphate receptor-mediated Ca\(^{2+}\) mobilization were examined in Xenopus laevis oocytes. Intracellular Ca\(^{2+}\) was monitored by electrophysiological measurement of the endogenous Ca\(^{2+}\)-activated Cl\(^{-}\) current. Application of a hyperpolarizing pulse released intracellular Ca\(^{2+}\) in oocytes primed by pre-injection of a non-metabolizable inositol 1,4,5-trisphosphate analogue. The carboxyl terminus of the amyloid precursor protein inhibited inositol 1,4,5-trisphosphate receptor-mediated intracellular Ca\(^{2+}\) release in a dose-dependent manner. Equimolar β-amyloid peptides Aβ1–40 or Aβ1–42 had no effect, and whereas a truncated carboxyl terminus lacking the Aβ domain was equipotent to the full-length one, a carboxyl terminus fragment lacking the NPTY sequence was less effective than the full-length fragment. The inhibition induced by the carboxyl terminus was not associated with the block of the Ca\(^{2+}\)-dependent Cl\(^{-}\) channel itself or compromised Ca\(^{2+}\) influx. We conclude that the carboxyl terminus of the amyloid precursor protein inhibits inositol 1,4,5-trisphosphate-sensitive Ca\(^{2+}\) release and could thus disrupt Ca\(^{2+}\) homeostasis and that the carboxyl terminus is much more effective than the β-amyloid fragments used. By perturbing the coupling of inositol 1,4,5-trisphosphate and Ca\(^{2+}\) release, the carboxyl terminus of the amyloid precursor protein can potentially be involved in inducing the neural toxicity characteristic of Alzheimer's disease.  

Alzheimer’s disease (AD) is a progressive and fatal neurodegenerative disease characterized by amyloid plaques and neurofibrillary tangles (for a review, see Ref. 1). These plaques are associated with degenerating neuronal processes and consist primarily of fibrillar aggregates of β-amyloid protein (Aβ). Although Aβ fragments are the main component of amyloid plaques and have been shown to damage or kill cultured neurons (for a review, see Ref. 2), Aβ may not be the sole neurotoxic component of the amyloid precursor protein (APP). For example, Aβ peptide deposition has been observed without accompanying neurodegeneration (3–5). Another amyloidogenic fragment, the carboxyl terminus (CT) of APP, which is composed of 99–105 amino acid residues containing the complete Aβ sequence, also appears to be toxic to neurons (6, 7). CT has been found not only in presynaptic terminals of rodent entorhinal neurons (8) but also in paired helical filaments (9), in senile plaques (10), in microvessels (11–13), in choroid plexus from human brain in AD, in the white matter of Down’s syndrome brains (14), and in both cytosol and media of lymphoblastoid cells obtained from patients with early- or late-onset familial AD (15) and Down’s syndrome (16). A transgenic mouse model expressing CT exhibited neuropathology very similar in many respects to that of AD, especially as regards age-dependent neuronal and synaptic degeneration (17) and spatial-learning deficits and electrophysiological alterations that suggested impairment of synaptic plasticity (18). A 31-amino acid carboxyl-terminal fragment of APP (CT31) generated from CT by caspase-9 cleavage was reported to have a pro-apoptotic effect and to mainly contribute to the CT toxicity in neuronal cells. Both CT31 and activated caspase-9 were present in the brains of AD patients but not in control brains (19). These findings together indicate that C Ts themselves may also contribute to the neurodegenerative processes in vivo not only as a precursor to make Aβ. Earlier studies showed that Aβ-bearing C Ts were released from several different cells and/or were more easily released from the damaged neurons into the media or extracellular fluid (20–22). Recent data has shown the presence of CT in the nuclei (23) and the transcriptionally active complex of CT with Fe65 and histone acetyltransferase, Tip60 (24). These findings suggest that CT plays important roles in delayed neurodegeneration via transcriptional regulation. Our previous study also demonstrated that CT fragments without Aβ and the transmembrane domain may also exert toxicity in nerve growth factor-differentiated PC12 cells (25). CT can also contribute to stimulation of inflammatory processes linked to delayed neurodegeneration in AD (26–28). Aβ and CT peptides have been shown to damage cultured neurons by a mechanism involving disruption of Ca\(^{2+}\) homeostasis (29, 30). However, the potential role of amyloidogenic  

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¶‡ The abbreviations used are: AD, Alzheimer’s disease; IP\(_{3}\), inositol 1,4,5-trisphosphate; 3-F-IP\(_{3}\), D-3-deoxy-3-fluoro-myio-inositol 1,4,5-trisphosphate; Aβ, β-amyloid protein; APP, amyloid precursor protein; CT, carboxyl-terminal; GST, glutathione S-transferase; TM, transmembrane.  

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Inhibition of IP₃-sensitive Ca²⁺ Release by CT Peptide of APP

Inhibitory effects of Aβ or CT peptides on IP₃ signaling have been hampered by the intracellular location of IP₃ receptors. Furthermore, limitations concerning specific inhibitors or activators of the IP₃ receptors, not affecting ryanodine receptors, have also made it difficult to investigate Aβ effects on the IP₃ pathway (31). To circumvent these difficulties, we have used Xenopus laevis oocytes as a model system. The large size of Xenopus oocytes facilitated precise intracellular application of IP₃ and APP fragments. Moreover, the Ca²⁺ response of Xenopus oocytes was not complicated by ryanodine receptors, which are absent in the oocytes (32). Another advantage of this model system is that injection of IP₃ into Xenopus oocytes elevates the cytosolic Ca²⁺ level and activates Ca²⁺-dependent Cl⁻ currents (33), which can be used as a real-time indicator of cytosolic Ca²⁺ concentration (34, 35). In addition, Yao and Parker (36) showed that application of a hyperpolarizing pulse to Xenopus oocytes pre-injected with an enzyme-resistant IP₃ analogue, n-3-deoxy-3-fluoro-myo-inositol 1,4,5-triphosphate (3-F-IP₃), induced a current that represented Ca²⁺ release from IP₃-sensitive stores. We have adopted this protocol to study the effect of amyloidogenic peptides on the IP₃ pathway.

EXPERIMENTAL PROCEDURES

Preparation of Recombinant CT105 and Aβ—Recombinant CT105 was prepared on the basis of human APP770 cDNA as described previously (37). Briefly, the expression plasmid pCS-CT105 was constructed by ligating the 704-bp BglII-ClaI fragment excised from pSP65-APP770 into ptrpSF9, digested with BglII and SmaI, treated with calf intestinal alkaline phosphatase, and transformed into Escherichia coli XL1-Blue. CT105 peptide (M, 14,242) was purified by a combination of urea solubilization and ion exchange chromatography and then subjected to dialysis against 10 mM Tris-HCl (pH 7.4) followed by lyophilization. To rule out the effect of possible co-purified contaminants of this model system is that injection of IP₃ into Xenopus oocytes pre-injected with an enzyme-resistant IP₃ analogue, n-3-deoxy-3-fluoro-myo-inositol 1,4,5-triphosphate (3-F-IP₃), induced a current that represented Ca²⁺ release from IP₃-sensitive stores. We have adopted this protocol to study the effect of amyloidogenic peptides on the IP₃ pathway.

Construction of Truncated CT Peptides—To determine which region is the active domain responsible for the effect on IP₃ signaling of CT, we have generated truncated carboxy-terminal fragments of APP. Because Aβ, the transmembrane portion, and the NPTY sequence in the cytoplasmic tail are believed to be important in mediating toxic effects of CT, we have prepared truncated CT peptides. All data have been presented as means ± S.E. of the means (S.E.). The number of measurements given (n) represents different oocytes; the number of batches, i.e. toads used (N) for a given experiment, is also indicated. Data were analyzed using a statistics package, SAS/6.03 (SAS Institute, Cary, NC). Effect of a given peptide on current amplitude was analyzed by one-way ANOVA, after all the percentage data were transformed to logarithmic values. A Tukey test was used for multiple comparisons. Wilcoxon test (non-parametric) was used for comparison of two groups. Correlation coefficients were determined by non-parametric Spearman test. Statistical significance was considered at the following levels: p < 0.05 (**), p < 0.01 (**), p < 0.001 (**), and p < 0.0001 (**).
was not due to 150 nM 3-F-IP3 saturating the Ca$^{2+}$ stores. This confirmed, under our experimental conditions, $T_{in}$ was not simply generated by Ca$^{2+}$ entering from outside.

These characteristics taken together are consistent with the $T_{in}$ current representing a Cl$^{-}$-current activated by Ca$^{2+}$ released from IP$_3$-sensitive stores by the triggering action of hyperpolarization-induced Ca$^{2+}$ influx, as in the study of Yao and Parker (36).

Inhibitory Effect of CT on $T_{in}$

Following a control (pre-injection) recording of $T_{in}$ in oocytes primed with 3-F-IP$_3$, water (as vehicle control) or an amyloidogenic fragment of APP (A$\beta_{1-40}$, A$\beta_{1-42}$, or CT) was applied intracellularly. Experiments in which the oocytes were injected with distilled water alone, showed that $T_{in}$ was stable and that the post-injection amplitudes were not much different from those of pre-injection controls (residual current = 96.5 $\pm$ 4.8%; n = 18/n = 4; p > 0.05; Figs. 2A and 3A). Application of either A$\beta_{1-40}$ or A$\beta_{1-42}$ (0.22–3.0 $\mu$M for both) had no significant effect on $T_{in}$ (n = 8 for each; Figs. 2B, 2C, and 3A). In contrast, injection of CT peptide was very effective in suppressing $T_{in}$ (Figs. 2D and 3A). The inhibitory effect of CT peptide on $T_{in}$ was dose-dependent (Fig. 4). The concentration of CT peptide that induced half-maximal inhibition of $T_{in}$ was about 0.4 $\mu$M.

We also investigated if CT would inhibit $I_{in}$, induced directly by 3-F-IP$_3$, injection. 3-F-IP$_3$ (1 $\mu$M) typically evoked peak inward currents of 50–350 nA. There was no statistically significant effect on the peak inward current when 1.3 $\mu$M CT (a concentration that consistently inhibited $T_{in}$) was co-injected with 3-F-IP$_3$ (n = 13/n = 3; p > 0.1). In addition, the reduction of $T_{in}$ was not overcome by 10- or 100-fold higher concentration of 3-F-IP$_3$ (i.e. 1.5 and 15 $\mu$M, respectively; data not shown). In addition, we tested whether IP$_3$ re-injection could evoke the current following the initial block by CT. We could not find any recovery of $T_{in}$ in this condition (data not shown).

Comparison of Inhibitory Effect of CT and the Truncated CT Peptides on $T_{in}$—To explore the active part of the CT domain responsible for its inhibitory effects on $T_{in}$, we made truncated CT peptides, CTdNPTY and CTFdA/ATM. Water, 1.2 $\mu$M GST, CT, or each GST-conjugated, truncated CT peptide was applied intracellularly, after checking $T_{in}$ in oocytes primed with 3-F-IP$_3$. CTFdNPTY suppressed $T_{in}$ less effectively than full-length CT injection (residual current = 57.2 $\pm$ 10.3%; n = 7; p < 0.05; Fig. 3B), whereas CTFdA/ATM showed no significant difference (residual current = 19.3 $\pm$ 10.3%; n = 7; Fig. 3B) at the same concentration. The control oocytes injected with GST or water showed stable $T_{in}$ (residual current of GST = 101.0 $\pm$ 15.5%; n = 7; p > 0.05; residual current of water = 96.5 $\pm$ 4.8%; n = 18; p > 0.05; Fig. 3A). These results show that the YENPTY domain in the cytoplasmic tail seems to have a more crucial role in inhibiting IP$_3$ signaling rather than the A$\beta$ or TM domains.

Lack of Effect of CT on Capacitative Ca$^{2+}$ Entry or Ca$^{2+}$-dependent Cl$^{-}$ Channels—To eliminate the possibility that CT

Figure 1. Left panel, A, membrane current recorded from an oocyte (voltage-clamped at −40 mV) in response to injection of 1 $\mu$M 3-F-IP$_3$. The injection of 3-F-IP$_3$ activates a large transient inward current $(I_{in})$ followed by a long-lasting secondary inward current with some oscillations. The arrowhead indicates the injection point of 3-F-IP$_3$. B, hump current $(I_{hump})$ evoked by a hyperpolarizing pulse applied to oocytes previously injected with 3-F-IP$_3$ (150 nM). The oocyte was loaded with 3-F-IP$_3$ about 30 min before applying the voltage pulse (−40 to −110 mV; 4.5-s duration). C, similar protocol (as in B) applied to control, non-injected oocyte. No hump current is generated. Currents were not leak-subtracted. Right panel, effect of extracellular Ca$^{2+}$ on $T_{in}$. D, the control current response of a 3-F-IP$_3$-injected oocyte bathed in normal Ringer’s solution. E, oocyte bathed in Ca$^{2+}$-free Ringer’s solution. F, recovery on returning to normal Ringer’s solution. The recordings were obtained from the same oocyte. The scale bar in A applies to part A, whereas the scale bar in part B refers to all other parts of the figure.
Fig. 2. Effects of amyloidogenic fragments on $T_{in}$. Traces in the left panel show control recordings of $T_{in}$ before injection. Corresponding traces on the right show the current responses after injection in each case. A, water injection as a vehicle control. B, $3 \mu M A\beta_{1-40}$; C, $3 \mu M A\beta_{1-42}$; D, $3 \mu M CT$. The same volume (46 nl) of sterile distilled water or peptide solution was injected into each oocyte. Other experimental conditions were as in Fig. 1B.
could compromise capacitative $\text{Ca}^{2+}$ entry, oocytes that had been pre-treated with thapsigargin overnight (in the absence of extracellular $\text{Ca}^{2+}$), to set up capacitative $\text{Ca}^{2+}$ entry (44), were injected with $1.3 \mu M$ CT. Current responses to hyperpolarizing pulses were $646 \pm 142$ nA before, compared with $590 \pm 157$ nA following CT injection ($p = 0.18$, $n = 11/3$; Fig. 5). In addition, thapsigargin treatment is known to lengthen the time-to-peak of $T_{in}$ (41). We therefore measured the time-to-peak of $T_{in}$ before and following $1.3 \mu M$ CT injection ($1245 \pm 196$ ms versus $1134 \pm 150$ ms, respectively). The values were not significantly different ($p = 0.19$; $n = 12$). Together, these results are consistent with CT having no significant effect on capacitative $\text{Ca}^{2+}$ entry.

To elucidate whether the inhibitory effect of CT involved directly the $\text{Ca}^{2+}$-activated Cl$^-$ channels, we compared the decay time courses of $T_{in}$ before and after CT injection in oocytes in which CT reduced, but did not abolish, the peak $T_{in}$ current (see later). Current decay could be fitted with a single exponential of which the time constant ($\tau$) did not change significantly following CT injection ($964 \pm 156$ ms before and after CT, respectively, $n = 16/n = 4$; $p > 0.4$; Fig. 6).

In conclusion, the inhibitory effect of CT on $\text{Ca}^{2+}$ release was most likely due to suppression of $\text{IP}_3$-sensitive $\text{Ca}^{2+}$ release from internal stores, rather than any effect on capacitative $\text{Ca}^{2+}$ entry or $\text{Ca}^{2+}$-activated Cl$^-$ current.

**Relation of Time to Peak of $T_{in}$ and Inhibitory Effect of CT**—We explored the relationship between the time-to-peak of $T_{in}$
Inhibition of IP$_3$-sensitive Ca$^{2+}$ Release by CT Peptide of APP

**DISCUSSION**

We investigated the effects of amyloidogenic fragments of APP on the IP$_3$ pathway in *X. laevis* oocytes using the Ca$^{2+}$-activated Cl$^-$ current as an indicator of [Ca$^{2+}$]$_i$. The main conclusion is that CT peptide, but not the A$\beta$ fragments, inhibits Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores when activated by an IP$_3$ analogue and triggered by hyperpolarization-induced Ca$^{2+}$ influx.

$T_{in}$ required the presence of extracellular Ca$^{2+}$ to be triggered, because it was abolished in Ca$^{2+}$-free medium (Fig. 1, E and F). If $T_{in}$ were to be mediated exclusively by Ca$^{2+}$ entry, however, it should have persisted in oocytes when capacitative Ca$^{2+}$ entry was induced by use of thapsigargin (43). The absence of $T_{in}$ in thapsigargin-treated oocytes suggested that the current was not dependent upon Ca$^{2+}$ influx alone. It was concluded, therefore, that entry of Ca$^{2+}$ induced by membrane hyperpolarization triggered $T_{in}$, as a consequence of Ca$^{2+}$ release from IP$_3$-sensitive Ca$^{2+}$ stores in continuous presence of IP$_3$. This is consistent with the results of Yao and Parker (36). It should be noted, however, that under conditions associated with more “complex” second-messenger associated events (e.g. 5-hydroxytryptamine receptor activation (36)), there is evidence that $T_{in}$ can be activated by interaction of Ca$^{2+}$ influx and Cl$^-$ channels, in a pathway that bypasses the need for intracellular Ca$^{2+}$ release. However, under our experimental conditions, this was clearly not the case.

Enhanced depletion of the Ca$^{2+}$ store obtained by high (1.5 $\mu$m) concentration of 3-F-IP$_3$ did not result in any significant increase of $T_{in}$ amplitude. This ruled out the possibility that $T_{in}$ was a simple delayed release of Ca$^{2+}$ from incompletely depleted Ca$^{2+}$ stores. However, it is possible that the IP$_3$-dependent Ca$^{2+}$ release could involve a refilling mechanism occurring after Ca$^{2+}$ store depletion.

Control experiments, in which oocytes were injected with distilled water alone, showed that $T_{in}$ was stable and that the post-injection amplitude was not much different from the pre-injection amplitude (Figs. 3A and 4). Intracellular application of the CT peptide strongly attenuated $T_{in}$, whereas A$\beta_{1–40}$ and A$\beta_{1–42}$ had no significant effect under identical experimental conditions (Figs. 3, B–D, and 4A). In addition, the reduction of $T_{in}$ was not overcome by 10- or 100-fold higher concentration of 3-F-IP$_3$ (i.e. 1.5 and 15 $\mu$m, respectively, data not shown). In addition, we tested whether IP$_3$ re-injection could evoke the current following the initial block by CT. We could not find any recovery of $T_{in}$ in this condition (data not shown). These experiments indicate that CT inhibits $T_{in}$ by altering the IP$_3$-induced receptor-signaling pathway rather than simply by blocking IP$_3$ receptor directly.

The deletion studies showed that YENPTY sequence, which is known to have a role in intracellular sorting (24), rather than the A$\beta$ or TM domains, may at least in part, be responsible for the attenuation of IP3 induced signal (Fig. 3B).

Interestingly, injection of the CT peptide had no effect on the size of $I_t$. At present, the reasons for this are unclear, because both $I_t$ and $T_{in}$ are dependent on IP$_3$. However, the two events are distinct in that (i) $I_t$ is an immediate event following IP$_3$ injection, whereas $T_{in}$ takes many minutes to develop and (ii) $I_t$, unlike $T_{in}$, is not dependent upon extracellular Ca$^{2+}$ (45). Thus, possible differences in the intracellular events controlling these two discrete events may explain their distinct sensitivity to CT.

The inhibitory effect of CT on $T_{in}$ was concentration-dependent, with the half-maximal effect occurring at about 0.4 $\mu$m (Fig. 4). This is in the range of CT concentrations found previ...

**FIG. 6.** Decay time courses of Ca$^{2+}$-activated Cl$^-$ channels. These were not altered by CT injection. Representative traces of $T_{in}$ evoked by hyperpolarization from 0 mV to −110 mV in 3-F-IP$_3$-injected oocytes, recorded before (A) and following (B) CT injection. The traces are from different oocytes. The straight lines represent the exponential best fits to the current decays, with time constants (τ) as indicated.

**FIG. 7.** Correlation of residual current amplitudes (normalized; % as described in the legend to Fig. 3) and time-to-peak (log scale). The inhibitory effect of CT on $T_{in}$ was enhanced in oocytes displaying longer time-to-peak before CT peptide injection. A non-parametric Spearman test showed that there is a strong inverse correlation between the two data sets (correlation coefficient $r = −0.78; p < 0.001$).
Inhibition of IP₃-sensitive Ca²⁺ release by CT Peptide of APP

ously to be toxic to oocytes (46) and to a variety of mammalian cells, including neurons (7).

Several possible mechanisms were tested to elucidate the one or more steps involved in the inhibitory effect of CT on Tₘ. In particular, the possible effects of CT on 1) capacitative Ca²⁺ entry, 2) the Ca²⁺-activated Cl⁻ channel, or 3) the cytoplasmic Ca²⁺ level were evaluated.

Compromised Ca²⁺ entry, preventing Ca²⁺ release from intracellular stores, was unlikely to be involved, because capacitative Ca²⁺ entry induced by pre-treatment of thapsigargin did not change significantly after CT injection (Fig. 5) and CT had no effect on the length of the time-to-peak of Tₘ. This is in agreement with recent work by Yoo et al. (47), who found that neither Aβ42 nor full-length bAPP had any effect on capacitative Ca²⁺ entry in Chinese hamster ovary cells.

It was possible that CT affected the Ca²⁺-dependent Cl⁻ channels directly. However, the decay time constant (τ) of Tₘ measured before and after CT injection did not change (Fig. 6). Furthermore, oscillatory currents due to the Ca²⁺-activated Cl⁻ current were observed in oocytes after CT injection, consistent with lack of effect of CT on the Cl⁻ channel (46). Taken together, the possible involvement of the Ca²⁺-activated Cl⁻ channel was ruled out as a significant component of the inhibitory effect of CT on Tₘ.

Another explanation for the inhibition of Ca²⁺ release was that CT lowered cytosolic Ca²⁺ concentration to low levels by chelating Ca²⁺. Chelation of Ca²⁺ would prevent the Cl⁻ current evoked by Ca²⁺ release from its stores. However, Ca²⁺ chelation was unlikely to occur, because, in fact, an increase of [Ca²⁺] has been observed when CT was applied to Purkinje cells of rat cerebellum (48).

Ca²⁺ influx, enhanced by CT, could lead to levels of [Ca²⁺], high enough to block IP₃ receptors, because very high [Ca²⁺] has been reported to block IP₃ receptors completely (49, 50). If this mechanism occurred under our experimental conditions, however, the capacitative Ca²⁺ entry induced by 3-F-IP₃ or thapsigargin should have increased after CT injection. Such an increase was not observed (Figs. 2D and 5). Therefore, increased Ca²⁺ influx cannot account for the inhibitory effect of CT on IP₃-sensitive Ca²⁺ release. However, we cannot rule out the possibility that the Ca²⁺ influx enhanced by CT was large enough to block Ca²⁺ release through IP₃ receptors, but the change was too small to be detected by electrophysiological recording. This possibility remains to be investigated further.

We tested whether CT would affect directly Ca²⁺ release from IP₃-sensitive Ca²⁺ stores. The analysis shown in Fig. 7 revealed a strong, inverse relationship between time-to-peak of Tₘ measured before CT injection and residual current amplitude after CT injection. The response to IP₃ varied greatly from oocyte to oocyte, possibly due to the differences in the number and spatial distribution of IP₃ receptors (45, 51). Oocytes, which were highly sensitive to 3-F-IP₃, would deplete their Ca²⁺ stores more in response to a given concentration of 3-F-IP₃ compared with relatively insensitive oocytes. CT more potently blocked Tₘ (Ca²⁺ release from IP₃-sensitive stores) in oocytes displaying longer time-to-peak.

Two existing hypothesis can be adopted to explain the relationship between time-to-peak and sensitivity of each oocyte to IP₃ as follows: First, there is the steady-state model. It has been suggested that Ca²⁺ release is controlled by the intraluminal Ca²⁺ level within Ca²⁺ stores (52). The steady-state model considers that decreasing the Ca²⁺ content of the stores would slow down further the Ca²⁺ release until it stopped when the level of luminal Ca²⁺ fell to a low concentration (53). Consequently, “sensitive” oocytes would have less full Ca²⁺ stores after injection of 3-F-IP₃ and would need more Ca²⁺ refilling to reach a certain threshold for Ca²⁺ release, which may result in delayed Tₘ (i.e. longer time-to-peak), as found (Fig. 7). Second, there is the feedback model. Feedback inhibition of the IP₃ receptor by cytosolic Ca²⁺ would limit further release of Ca²⁺, and recovery of sensitivity to IP₃ would follow the subsequent decline of cytosolic Ca²⁺ as the latter was re-sequestered (54, 55). Accordingly, oocytes highly sensitive to 3-F-IP₃ would have more Ca²⁺ liberated by injection of 3-F-IP₃. Such oocytes could take longer time to sequester Ca²⁺ to a non-blocking level of [Ca²⁺] and this may result in delayed Tₘ.

Thus, the time-to-peak can be considered as a measure of the IP₃ sensitivity of each oocyte. Ca²⁺ release in highly sensitive oocytes (where a large portion of Ca²⁺ store was exhausted by the 3-F-IP₃) was inhibited more by CT. Such a correlation would arise as a result of CT directly suppressing IP₃-sensitive Ca²⁺ release rather than one or more other auxiliary mechanisms. Hence, the inverse relationship found is consistent with CT inhibition being specific to the Ca²⁺ release mechanism from IP₃-sensitive Ca²⁺ stores. However, CT did not appear to inhibit directly the binding of 3-F-IP₃ to IP₃ receptors or compete with 3-F-IP₃ for IP₃ binding sites, because there was no significant effect of CT on Iᵢ when co-injected with 3-F-IP₃, and high concentrations of 3-F-IP₃ could not overcome the CT-induced block. CT was most likely to uncouple the triggering action of Ca²⁺ influx from induction of the IP₃-sensitive Ca²⁺ release. It remains to be determined whether such an uncoupling effect occurs in vivo and whether it could lead to any long-term effect.

In overall conclusion, we have shown that the CT fragment of bAPP, but not the Aβ fragments, suppresses Ca²⁺ release from IP₃-sensitive Ca²⁺ stores triggered by extracellular Ca²⁺ entry in the Xenopus oocyte model. Although the effect of CT peptide on Ca²⁺ release from IP₃-sensitive Ca²⁺ stores must be confirmed in the neuronal system, our results support further the view that the CT peptide plays an important role in pathogenesis of AD (56, 57). The inhibitory effect of CT on IP₃-sensitive Ca²⁺ release could disrupt intracellular Ca²⁺ homeostasis and thus contribute to the cellular toxicity in AD.

REFERENCES

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