not only reveal how global Ca^{2+} signals are composed (Bootman et al. 1997), but also provide information about the functioning of $I(1,4,5)P_3$ receptors at a microscopic level in vivo. In this regard, the influence of feedback regulation by Ca^{2+} and the kinetics of $I(1,4,5)P_3$ binding in determining the time course of Ca^{2+} puffs are unknown. If the time course of Ca^{2+} puffs is determined by the time for which agonist remains bound to the IP_3 receptor, one might expect agonist affinity, which reflects in part the ligand dissociation rate from the receptor, to influence the duration of these events. Therefore, we compared puffs evoked by $I(1,4,5)P_3$ with those elicited by $I(2,4,5)P_3$ and adenophostin A [AdA]. $I(2,4,5)P_3$ has an ~100-fold lower affinity than $I(1,4,5)P_3$ for the *Xenopus* IP_3 receptor (Callamaras and Parker. 1994. *Cell Calcium.* 15:66–76), whereas AdA is the most potent agonist yet identified, with an affinity 10–100-fold greater than $I(1,4,5)P_3$ (Takahashi et al. 1994. *J. Biol. Chem.* 269:369–372). Taking the dissociation constants (K_d) of AdA, $I(1,4,5)P_3$, and $I(2,4,5)P_3$ under physiological conditions to be ~45 nM, ~450 nM, and ~50 μM, respectively, and assuming a diffusion-limited association rate of $\sim 10^8\ M^{-1}\ s^{-1}$, the half-times for dissociation of these agonists from the $I(1,4,5)P_3$ receptor would be 150 ms, 15 ms, and 140 μs, respectively.

Xenopus oocytes were microinjected with caged $I(1,4,5)P_3$ and the fluorescent Ca^{2+} indicator Oregon-green 488 BAPTA-1 (final concentrations ~5 and 40 μM, respectively) 1 h before recording confocal linescan images at 22°C. The duration (at half-maximal amplitude) of Ca^{2+} puffs evoked by intracellular ionophoresis of analogs were compared with those evoked by photorelease of $I(1,4,5)P_3$ within the same oocyte. Both $I(2,4,5)P_3$ and adenophostin A evoked discrete puffs before global increases in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$). Despite the lower affinity of $I(2,4,5)P_3$, the duration of $I(2,4,5)P_3$-evoked Ca^{2+} puffs was similar to those evoked by $I(1,4,5)P_3$ (151 vs. 130 ms, respectively, n = 110 events). Maximally effective concentrations of AdA caused a slower increase in global $[Ca^{2+}]_{cyt}$ than saturating concentrations of $I(1,4,5)P_3$, suggesting that AdA diffused more slowly than $I(1,4,5)P_3$. However, Ca^{2+} puffs triggered by AdA were briefer than $I(1,4,5)P_3$-evoked Ca^{2+} puffs (80 vs. 150 ms, respectively, n = 23 events): opposite to the prediction that the duration of elementary events may lengthen with agonist affinity. We conclude that the duration of Ca^{2+} puffs cannot be determined by agonist dissociation alone, but rather other regulatory mechanisms must delimit the period of Ca^{2+} flux through the IP_3 receptor. (Supported by NIH grant GM 48071 and a Wellcome Trust Prize Fellowship to J. Marchant.)

87. Clustering of "Hot Spots" of $InsP_3$-evoked Calcium Release Determines the Patterns of Calcium Signals in Pancreatic Acinar Cells K.E. FOGARTY,‡ R. TUFT,‡ and P. THORN,* **Department of Pharmacology, Cambridge University, Cambridge, United Kingdom; and ‡Department of Physiology, University of Massachusetts Medical School, Worcester, Massachusetts* (Sponsor: John V. Walsh)

Pancreatic acinar cells have two modes of $InsP_3$-induced calcium signaling: local, within the secretory pole, and global. We used high temporal resolution calcium imaging methods, coupled with spot (4-μm diameter) photolytic release of caged $InsP_3$, to map out in detail the regional response of the cell to $InsP_3$. Furthermore, we used three-dimensional imaging and EGTA buffering to examine the cellular location of the discrete events that underlie the "hot spots" of calcium release.

Our results show hot spots of $InsP_3$ responsiveness, with similar $InsP_3$ thresholds and calcium release kinetics, in all regions of the acinar cell. However, the probability of observing hot spots was much less in the basal pole than in the secretory pole. Within the secretory pole, the apparent density of hot spots and the speed of calcium release was too great to allow us to resolve discrete events. We therefore turned to techniques of high speed three-dimensional calcium imaging coupled with the use of EGTA to buffer calcium diffusion. EGTA (200 μM) slowed the rising phase and the spread of the global calcium response to global release of caged $InsP_3$. In the three-dimensional images, the presence of EGTA revealed evidence for a matrix of clustered, discrete, calcium release events.

We conclude that the clustering of hot spots of $InsP_3$-evoked calcium release is a fundamental parameter in defining the cellular calcium signal. At low $InsP_3$ concentrations, the calcium signal is restricted to the regions containing a high density of calcium release sites. At higher $InsP_3$ concentrations, the calcium signal can spread across the cell, travelling in a saltatory manner from release site to release site.

88. Calcium Diffusion Coordinates Discrete $InsP_3$-evoked "Hot Spots" of Calcium Release in Pancreatic Acinar Cells J.F. KIDD,* K.E. FOGARTY,‡ R. TUFT,* and P. THORN,* **Department of Pharmacology, Cambridge University, Cambridge, United Kingdom; and ‡Department of Physiology, University of Massachusetts Medical School, Worcester, Massachusetts*

$InsP_3$-evoked calcium responses are proposed to result from the summation of discrete release events, but how these relate to downstream cell functions is still unclear. Pancreatic acinar cells show a local calcium signal that regulates fluid secretion and exocytosis and is thought to represent the coordinated activity of a number of discrete "hot spots" of calcium release (Thorn et al. 1996. *EMBO* (*Eur. Mol. Biol. Organ.*) *J.* 15:999) Our hypothesis is that calcium acts to coordinate this hot spot activity by a process of calcium-induced calcium release at the $InsP_3$ receptor. We therefore predict that EGTA, a slow calcium buffer, would act to limit calcium diffusion and give rise to asynchronous and autonomous hot spot activity. We report here on the use of the $Cl_{(Ca)}$ whole cell current as a high temporal resolution (2 kHz) monitor of the repetitive calcium spikes induced by the injection of (2,4,5)$InsP_3$.

Under conditions of low intracellular calcium buffering (50 μM EGTA), the majority (92%) of spikes had a monotonic rising phase. However, with increasing EGTA concentrations, from 100 to 300 μM, we observed a dose-dependent increase in the occurrence of discrete steps in the rising phase of the spikes (from 25 to 61%) and also the appearance of smaller amplitude spikes. Control experiments indicated that the EGTA effects were due to a primary action on the mechanisms of calcium release rather than the detection of the calcium signal by the $Cl_{(Ca)}$.

We conclude that EGTA is acting to reduce the coordination between adjacent hot spots of calcium release that then release calcium in an autonomous manner and give rise to discrete steps and smaller events in the $Cl_{(Ca)}$ current records. High speed, 3-D imaging experiments confirmed that EGTA acts to restrict the spread of the hot spots that, under high buffer conditions, become recognizable as discrete units. (Supported by the Medical Research Council, The Wellcome Trust, NSF.)

89. Local Calcium Signaling by Inositol Trisphosphate in Purkinje Cell Dendrites ELIZABETH A. FINCH and