Spontaneous and Beta-Adrenergic Receptor-Mediated Taurine Release from Astroglial Cells Are Independent of Manipulations of Intracellular Calcium

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Stimulation of beta-adrenergic receptors on LRM55 astroglial cells results in cAMP-dependent release of taurine. We have previously demonstrated that extracellular Ca^{2+} is not required for either spontaneous or receptor-mediated taurine release (Martin et al., 1988b). In the present series of experiments we investigated the relationship between changes in intracellular free Ca^{2+} ($[\text{Ca}^{2+}]_i$) and taurine release. $[\text{Ca}^{2+}]_i$ was measured using the fluorescent probe fura-2 and was manipulated by changing the concentration of Ca^{2+} in the incubation medium and by using the Ca^{2+} ionophore ionomycin. $[\text{Ca}^{2+}]_i$ was reduced from 150 ± 95 nM ($n = 46$) in control medium (containing 1.1 mM CaCl_2) to 46 ± 10 nM ($n = 43$) in saline containing no CaCl_2 and 10 μM EGTA. $[\text{Ca}^{2+}]_i$ was rapidly elevated to ≥ 1 μM in medium containing 100 μM CaCl_2 and 10 μM ionomycin. Taurine release, either spontaneous or stimulated by isoproterenol, was not significantly affected by these manipulations of $[\text{Ca}^{2+}]_i$. $[\text{Ca}^{2+}]_i$ did not change when cells were stimulated with 100 nM isoproterenol in either control saline containing 1.1 mM CaCl_2 or in CaCl_2 -free saline containing 10 μM EGTA. Other secretagogues (serotonin and ethanol) did not cause changes in $[\text{Ca}^{2+}]_i$. These data indicate that neither spontaneous or receptor-mediated taurine release from astroglial cells is Ca^{2+} dependent. However, when cells were preloaded with Ca^{2+} , allowed to recover briefly, and then stimulated with isoproterenol, it was possible to demonstrate transient increases in Ca^{2+} . Since these observations were made in CaCl_2 -free medium containing 10 μM EGTA, the Ca^{2+} released was probably from internal stores. When taurine release was examined using these same conditions responses were similar to controls, indicating that Ca^{2+} , released from intracellular stores, does not function to facilitate taurine release.

Receptor-mediated release of the neuroactive inhibitory amino acid taurine may be an important function of astrocytes (Martin et al., 1988). Direct evidence for this glial cell function has

been obtained using primary cell cultures and LRM55 astroglial cells (Shain and Martin, 1984; Perrone et al., 1986; Shain et al., 1986; Madelian and Shain, 1987; Shain et al., 1987b). Indirect evidence, consistent with this hypothesis, has been obtained by measuring taurine release from kainate-lesioned hippocampus (Butcher et al., 1987).

Receptor-mediated release of most hormones and neurotransmitters occurs by exocytosis. We have begun to characterize the mechanism of receptor-mediated taurine release from astroglial cells by determining if the process has properties similar to exocytotic release. One property of exocytotic release is that it is Ca^{2+} dependent. We have demonstrated that receptor-mediated taurine release from astroglia is not associated with a change in Ca^{2+} permeability through known types of Ca^{2+} channels since release occurs in the presence of the Ca^{2+} channel blockers diltiazem, nifedipine, and verapamil and when Ca^{2+} in the extracellular medium is replaced by Mn^{2+} , Cd^{2+} , or Co^{2+} (Martin et al., 1989). Furthermore, taurine release from astroglia may not be dependent on any increase in plasma-membrane Ca^{2+} permeability since neither spontaneous nor receptor-mediated release is affected by removing Ca^{2+} from the incubation medium (Martin et al., 1989).

While these data suggest that taurine release from astroglia is distinctly different from exocytotic synaptic release from neurons, they do not indicate whether taurine release from astroglial cells is Ca^{2+} independent or whether release is dependent on Ca^{2+} mobilized from intracellular stores. In order to investigate whether receptor-mediated taurine release depends on the concentration of free cytoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_i$), we studied cytoplasmic Ca^{2+} levels in LMR55 astroglial cells using the fluorescent Ca^{2+} indicator fura-2 (Grynkiewicz et al., 1985). Our results indicated that (1) $[\text{Ca}^{2+}]_i$ can be decreased by removing Ca^{2+} from the extracellular medium or greatly increased by exposing cells to media containing Ca^{2+} and the Ca^{2+} -ionophore ionomycin (Liu and Hermann, 1978), (2) neither increasing nor decreasing $[\text{Ca}^{2+}]_i$ affected the rates of spontaneous or receptor-mediated taurine release, (3) stimulation of beta-adrenergic receptors, which induces taurine release, does not result in an increase in $[\text{Ca}^{2+}]_i$ under conventional incubation conditions. Thus, we conclude that receptor-mediated taurine release from astroglia occurs by a process distinct from exocytosis and transmitter release from neurons since it does not depend on changes in $[\text{Ca}^{2+}]_i$. Under specifically defined conditions, e.g., when cells are extensively preloaded with Ca^{2+} , we found that beta-adrenergic stimulation can mediate an increase in $[\text{Ca}^{2+}]_i$ by releasing

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Ca^{2+} from intracellular stores. Similar treatment of cells did not result in changes of either spontaneous or receptor-mediated taurine release.

Materials and Methods

Materials. (–)-Isoproterenol was purchased from Sigma (St. Louis, MO). Ionomycin was purchased from Calbiochem (San Diego, CA). Fura-2 acetoxy-methyl ester (fura-2/AM) was purchased from Molecular Probes (Junction City, OR). ^3H -taurine and scintillation counting solution (ACS) were purchased from Amersham (Chicago, IL). All other chemicals were purchased at the highest possible purity.

Cell cultures and media. LRM55 astroglial cells were used for all experiments. This continuous cell line exhibits a number of astrocytic phenotypes including $\text{HCO}_3^-/\text{Cl}^-$ exchange (Wolpaw and Martin, 1984), glutamine synthesis activity (Waniewski and Martin, 1984), glial fibrillary acidic protein (W. Shain, unpublished observations) receptor-mediated shape-change (Shain et al., 1987a), and receptor-mediated taurine release (Shain and Martin, 1984). Cells were maintained as mass cultures in 100 mm dishes with modified F12 medium (Vogel et al., 1972) supplemented with 5% fetal bovine serum. For release experiments, cells were transferred to pieces of cell support film (Bellco, Vineland, NJ) and grown to confluency. For intracellular Ca^{2+} measurements, cells were transferred to glass cover slips (#1, 18 mm diameter) and allowed to grow until approximately 50% confluent.

Three different saline solutions were used to manipulate $[\text{Ca}^{2+}]_i$. All 3 were buffered with HEPES adjusted to pH 7.3 with NaOH and supplemented with 0.1 gm/liter sodium ascorbate. Control saline was HEPES-buffered Hanks saline (HHA; Martin and Shain, 1979) and contained 1.1 mM CaCl_2 as the only added calcium salt. The saline used to lower $[\text{Ca}^{2+}]_i$ was made by omitting all of the CaCl_2 from HHA, adding 3.3 mM sucrose to maintain osmolality, and adding 10 μM EGTA to buffer any contaminating Ca^{2+} from other constituents. This medium was designated low- Ca^{2+} HHA (LCHHA). $[\text{Ca}^{2+}]_i$ was increased by using the Ca^{2+} ionophore ionomycin (Calbiochem) in a reduced- Ca^{2+} HHA (RCHHA). This medium was prepared by reducing the concentration CaCl_2 to 100 μM and adding 3.2 mM sucrose. Ionomycin was added at 1–100 μM .

Taurine release. Taurine release was measured by using a perfusion method that we have described previously (Shain and Martin, 1984). Briefly, cells were loaded with tracer quantities of ^3H -taurine (10 $\mu\text{Ci}/\text{ml}$; 300 nM), washed free of radiolabeled substrate, loaded into a glass perfusion column (final volume, 250 or 500 μl), and continuously perfused with HHA at 0.5 ml/min. The eluent was collected as 1 min fractions. Changes in perfusion salines or drug applications were made by means of a series of in-line valves. Taurine release was monitored as ^3H -taurine by scintillation counting. The data were analyzed by calculating the fractional rate of release for each 1 min sample during the entire course of the experiment.

Measurement of intracellular Ca^{2+} . Intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) were measured by determining the ratio of the fluorescence of fura-2 using excitation at 340 and 380 nm as described previously (Connor et al., 1987a, b, 1988). Cells were loaded with the fluorescence indicator by incubation with fura-2/AM (final concentration, 5 μM) in growth medium for 30 min at 37°C. Intracellular fura-2 concentrations were estimated to be in the range of 100–300 μM by methods described elsewhere (Tsien et al., 1985; Connor et al., 1987a, b). Two coverslips were incubated at one time. One of them was washed with HHA and directly mounted into the recording chamber. The second was rinsed 3 times with HHA and incubated at room temperature until used (1.5–2 hr). These incubation times and the maintenance of the cells at room temperature after loading with fura-2/AM were used to reduce compartmentalization and extrusion of the indicator. Excitation light was passed through narrow-band interference filters centered at 340 and 380 nm to a UV-F 40 $\times$ objective (Nikon) in an epiillumination configuration. The emission signal was filtered with a 480 nm long-pass filter. Exposure time for individual frames was 0.5 sec with a frame pair (340, 380 nm), separated by approximately 1 sec. A cooled, charge-coupled-device camera (Photometrics Ltd., Tucson, AZ, model 81-A) was used as the photodetector. The 140 $\times$ 240 pixel images were stored and analyzed by using a DEC LSI 11-73-based computer and photographed from a video monitor. Intracellular Ca^{2+} concentrations were calculated from the fluorescence ratios (340/380) using the following equation:

$$[\text{Ca}^{2+}]_i = K_D[(R - R_{\min})/(R_{\max} - R)](F_o/F_s), \quad (1)$$

Figure 1. Changes in $[\text{Ca}^{2+}]_i$ observed when cells were exposed to Ca^{2+} -free media. At the time indicated the medium in the recording chamber was changed by removing HHA, washing 3 times with LCHHA, and then continuing incubation in LCHHA. Each symbol represents the data from an individual cell. In other experiments, measurements were made at intermediate times, resulting in values similar to those indicated by the extrapolated lines.

where $R_{\min} = 0.45$, $R_{\max} = 11.7$, $F_o/F_s = 7.5$, and $K_D = 225$. R is the observed ratio of the fluorescence intensity excited by 340 and 380 nm illumination, respectively.

Results

The experiments reported here were designed to investigate the relationship between taurine release and intracellular Ca^{2+} levels and to test the possibility that release is facilitated by Ca^{2+} released from intracellular stores.

The first series of experiments was designed to determine if the $[\text{Ca}^{2+}]_i$ of LRM55 astroglial cells could be manipulated by either changing the extracellular Ca^{2+} concentration or increasing membrane permeability to Ca^{2+} by using the selective ionophore ionomycin (Kauffman et al., 1980). $[\text{Ca}^{2+}]_i$ levels were quite variable in cells bathed in control saline (HHA); the mean $[\text{Ca}^{2+}]_i$ was 150 ± 95 nM ($n = 46$). When the saline was changed from HHA to LCHHA (see Materials and Methods), $[\text{Ca}^{2+}]_i$ decreased to approximately 50 nM and was maintained at that level for as long as cells were observed (60 min; Fig. 1). The mean $[\text{Ca}^{2+}]_i$ for cells incubated in LCHHA for >3 min was 47.0 ± 10.1 ($n = 43$). When the saline was changed from HHA to RCHHA (see Materials and Methods) with ionomycin, $[\text{Ca}^{2+}]_i$ increased rapidly and dramatically to concentrations >1 μM (Fig. 2). At these high $[\text{Ca}^{2+}]_i$ values it became difficult to calculate $[\text{Ca}^{2+}]_i$ because the fluorescence at 380 nm excitation became extremely weak.

Release experiments were designed to parallel those used to measure $[\text{Ca}^{2+}]_i$. In a typical experiment, cells were first perfused with HHA for 30 min (Fig. 3). During this time there was an initial washout of ^3H -taurine followed by a relatively constant baseline rate of release. All measurements of taurine release were made after the spontaneous release rate had reached the constant baseline rate. A control response to 100 nM IPR was elicited before the perfusion medium was changed to manipulate intracellular Ca^{2+} .

The effect of increasing $[\text{Ca}^{2+}]_i$ on taurine release was determined by incubating cells with ionomycin (10 μM). In these experiments, cells were exposed to RCHHA for 30 min, after which a control response to IPR was obtained (Fig. 3). Cells

Figure 2. Changes in $[Ca^{2+}]_i$ observed when cells were exposed to RCHHA containing $10 \mu M$ ionomycin. The change in medium was made as described for Figure 1. Each symbol represents the data from an individual cell.

were then exposed to ionomycin alone or to ionomycin and IPR. Ionomycin did not stimulate release when applied alone, nor did it potentiate IPR-stimulated release. These data indicate that directly increasing $[Ca^{2+}]_i$ does not stimulate taurine release. We have also demonstrated that both spontaneous and receptor-mediated taurine release were not affected by removing extracellular Ca^{2+} (Martin et al., 1988b).

In a third series of experiments, $[Ca^{2+}]_i$ was measured while the cells were stimulated with 100 nM IPR. Cells were exposed to IPR in medium containing 1.1 mM $CaCl_2$ (HHA; Fig. 4A) or in LCHHA after $[Ca^{2+}]_i$ had dropped to lower concentrations (Fig. 4B). No changes in $[Ca^{2+}]_i$ were observed. Several other taurine secretogogs were tested for their abilities to affect $[Ca^{2+}]_i$. Stimulation with serotonin ($10 \mu M$) or ethanol (100 mM) did not cause observable changes in $[Ca^{2+}]_i$.

Figure 4. Effect of 100 nM isoproterenol on $[Ca^{2+}]_i$ when cells were incubated in HHA (A) or LCHHA (B). Each symbol represents an individual cell.

From the preceding experiments we conclude that neither spontaneous nor receptor-mediated taurine release is affected by changing $[Ca^{2+}]_i$ and that stimulation of beta-adrenergic receptors under normal conditions does not result in changes in $[Ca^{2+}]_i$.

Under specialized conditions, however, we did observe beta-adrenergic receptor-mediated release of Ca^{2+} from what must be intracellular stores. If cells were first exposed to LCHHA for a prolonged period (15–60 min), returned to normal saline, and then returned to LCHHA before drug application, an increase in $[Ca^{2+}]_i$ was observed. A representative experiment is illustrated in Figure 5. When cells were changed from HHA to LCHHA, a reduction in $[Ca^{2+}]_i$ was observed. Panels are designated by the total time in minutes that the cells had been mounted on the microscope. Cells were changed from (6.30-min panel) HHA to LCHHA at 9 min. The 39.53-min panel shows $[Ca^{2+}]_i$ approximately 30 min after the exchange for LCHHA at 9 min. The shape and relative position of the 8 cells

Figure 3. Effects of increasing $[Ca^{2+}]_i$ on spontaneous and receptor-mediated release. Isoproterenol, 100 nM , and ionomycin, $10 \mu M$, were added during the times indicated by the bars. The medium was changed from HHA to RCHHA as indicated.

in the field are shown in the simple fluorescence picture at the bottom right of the series.

The cells were returned to normal saline at 42.5 min, and the next 5 ratio measurements were taken in rapid succession (42.68–43.25 min). These panels show that $[Ca^{2+}]_i$ increases dramatically, but with different time courses in all the cells. Over the next 3 min $[Ca^{2+}]_i$ dropped spontaneously to levels below control (46.10 min). These data have been quantified and presented in Figure 6A. When the HHA was again exchanged for LCHHA the levels dropped further and remained low (53.67 and 55.87 min). In these 2 panels, there are quite noticeable areas of higher $[Ca^{2+}]_i$ around the nucleus that are especially evident in the lower left-hand cell. This type of pattern is a common observation in astroglial cells, as well as in other types of cells (Benjamin et al., 1988). Isoproterenol (100 μ M) was added to the LCHHA during the interval between 55.87 and 56.01 min and the time course of $[Ca^{2+}]_i$ changes followed at 15 sec intervals over the next 2 min (56.01–58.5 min). There was a transient, nonsynchronized rise in $[Ca^{2+}]_i$ in 6 of the 8 cells. These data were quantified and are presented in Figure 6B. The apparent peak of the responses varied greatly from cell to cell, but this may be because we missed the maxima of these relatively short, transient changes. $[Ca^{2+}]_i$ distribution during the peak response of the large flat cell in the lower left of panel for 56.29 min is interesting because many of the regions of highest $[Ca^{2+}]_i$ before stimulation (55.87 min) are among the lowest during the response. Since these cells were in LCHHA at the time of the response and had been in this saline for the previous 3 min, the Ca^{2+} responsible for the indicator signal, in all probability, came from intracellular stores that were loaded as a result of the cycle through normal (HHA) and Ca^{2+} -free saline (LCHHA). Since these were the only conditions under which we observed clear increases in $[Ca^{2+}]_i$ from intracellular stores, we examined taurine release under similar conditions (Fig. 7). No demonstrable effect on taurine release was observed. When Ca^{2+} is released from intracellular stores, taurine release is not affected.

Discussion

The data presented in this report indicate that receptor-mediated release of taurine from astroglia is not dependent on Ca^{2+} . This conclusion is based on 4 lines of evidence. First, receptor-mediated taurine release is unaffected when Ca^{2+} is eliminated from the perfusion medium, is not blocked by substituting Cd^{2+} , Co^{2+} , and Mn^{2+} for extracellular Ca^{2+} , and is not inhibited by the organic Ca^{2+} channel blockers nifedipine, diltiazem, and veratradine (Martin et al., 1989). Thus, extracellular Ca^{2+} is not involved. Second, stimulation of beta-adrenergic receptors by protocols that cause the release of taurine from astrocytes does not in ordinary circumstances result in changes of $[Ca^{2+}]_i$. Third, conditions that decrease or increase $[Ca^{2+}]_i$ over a range from <50 nM to >1 μ M have no effect on receptor-mediated taurine release. And finally, taurine release is not stimulated by procedures that increase $[Ca^{2+}]_i$ to >1 μ M. Therefore, receptor-mediated taurine release from astroglia is distinctly different from the Ca^{2+} -dependent exocytotic release of neurotransmitters from neurons. This conclusion is, perhaps, not surprising, as astrocytes are not known to have specialized cell–cell contacts, i.e., concentrations of exocytotic vesicles and presynaptic specializations.

Ca^{2+} dependency of taurine release has been studied in several *in vitro* preparations, including primary cultures of astrocytes and retina. Philibert et al. (1988) studied the Ca^{2+} dependency

of K^+ -stimulated taurine release from primary cultures of astrocytes. They observed that K^+ -stimulated taurine release was inhibited by 10 mM Mg^{2+} and that no additional K^+ -stimulated release was observed after treating cells with 1 mM EGTA. Treatment with 1 mM EGTA resulted in a large increase in spontaneous activity. They concluded that K^+ -stimulated taurine release was Ca^{2+} -independent. We found that IPR-stimulated taurine release was similarly inhibited by 10 mM Mg^{2+} but concluded that this effect was due to the elevated osmotic pressure of the medium since these effects could be mimicked by equiosmolar changes using sucrose or NaCl (Martin et al., 1989). We also found that perfusion with medium containing 1 mM EGTA caused a large increase in spontaneous taurine release, resulting in a lack of response to IPR (Martin et al., 1989). Our observations are, therefore, very similar to those of Philibert et al. (1988). However, the results presented here and from other experiments, including those with Ca^{2+} channel blockers (Martin et al., 1989), have led us to a different conclusion—taurine release from astroglial cells is Ca^{2+} independent. Taurine efflux from a neuroblastoma $\times$ glioma hybrid was Ca^{2+} independent but also could not be stimulated by high external K^+ (Kruzinger and Hamprecht, 1981). Lombardini (1988) reported that taurine release from retinal homogenates is Ca^{2+} independent and is stimulated by high K^+ but not depolarizing agents, and he concluded that this release occurs from glial components.

Taurine release has been observed with neural tissues *in vitro* (Bernardi et al., 1984; Lehmann et al., 1985; Tossman et al., 1985; Girault et al., 1986) and *in vivo* by using indwelling perfusion techniques (Collins and Topiwala, 1974; Korpi and Oja, 1983; Kontro and Oja, 1987). The cellular origin of this release was left unclear by these studies; however, Butcher et al. (1987), who used kainate to lesion neurons selectively, still demonstrated taurine release. The Ca^{2+} independency of astrocytic taurine release provides a tool that may help to distinguish astrocytic release from exocytotic neuronal release in studies with more intact tissue preparations. However, recent reports indicate that Ca^{2+} dependency, by itself, will not be sufficient to distinguish astrocytic and neuronal release, as Ca^{2+} -independent release has been observed in platelets (Rink et al., 1982) and neurons (Schwartz, 1987). The mechanism of this release has not been described.

There have been relatively few studies of Ca^{2+} entry or cytoplasmic free Ca^{2+} in astrocytes. Ca^{2+} can enter cells through Ca^{2+} -selective ion channels that may be voltage dependent or receptor activated (Miller, 1987). Voltage-dependent Ca^{2+} channels have been observed in astroglial cells, and ion influx is particularly prominent when Ba^{2+} is substituted for Ca^{2+} (MacVicar, 1984). Under other conditions, e.g., normal Ca^{2+} concentrations, different authors using different preparations have reported that Ca^{2+} fluxes are (Lazarewicz et al., 1977; Newman, 1985) or are not (Barnes and Mandel, 1981; Walz and Wilson, 1986) voltage dependent. LRM55 astroglial cells appear not to have voltage-sensitive Ca^{2+} channels since depolarization of cells in HHA containing 1.1 mM $CaCl_2$ and 50 mM KCl does not cause changes in cytoplasmic free Ca^{2+} (W. Shain and J. A. Connor, unpublished observations).

Receptor-mediated changes in $[Ca^{2+}]_i$ have been reported in C6 glioma cells after stimulation with serotonin (Sugino et al., 1984). However, serotonin did not cause increases in Ca^{2+} in LRM55 astroglial cells, nor have we observed changes in cytoplasmic free Ca^{2+} following stimulation by isoproterenol, the kappa-opiate U-50,488, or ethanol when cells were continuously

Figure 6. Quantification of changes in $[Ca^{2+}]_i$ following changes from LCHHA to HHA beginning at 42.68 min (A) and after isoproterenol stimulation upon return to LCHHA beginning at 55.87 min (B). The data are presented for individual cells.

incubated in HHA. However, we did find that intracellular Ca^{2+} increased upon stimulation with IPR if the cells were preloaded with Ca^{2+} . This increase in $[Ca^{2+}]_i$ appears to be due to release from intracellular stores and not to Ca^{2+} from the extracellular medium since the response was observed in medium devoid of extracellular Ca^{2+} (LCHHA). Because no response was observed in normal HHA or LCHHA if the cells were not preloaded, intracellular storage sites may usually be depleted. It is possible that the differences between the results with C6 and LRM55 cells may be related to differences in the experimental handling of the cells or to differences in basal Ca^{2+} storage between the cell lines.

Finally, the observation of regional differences in Ca^{2+} levels within cells as shown in Figure 5 is common when cells have been loaded by using the /AM form of fura-2 (Williams et al., 1985; Benjamin et al., 1988; Connor and Tseng, 1988). Such differences are not seen where the ionic form of the indicator has been injected into either Purkinje neurons or fibroblasts (Connor and Tseng, 1988; C. W. Benjamin, J. A. Connor, and R. R. Gorman, unpublished observations). In these studies the injected indicator demonstrates uniform Ca^{2+} levels, even in fibroblasts where the fura-2/AM-loaded indicator demonstrates distributions almost identical to those shown here in astrocytes. By comparing data from injected and fura-2/AM-loaded fibroblasts, it appears that the signal measured in the portion of the cell occupied by the nucleus most accurately reflects cytoplasmic Ca^{2+} levels, while areas surrounding the nucleus, where the indicator often demonstrates significantly higher levels, are heavily

Figure 7. Effects of stimulating Ca^{2+} from intracellular stores on taurine release. Conditions were similar to those used to measure $[Ca^{2+}]_i$ in Figure 5. A control response to 100 nM isoproterenol was obtained in HHA. The medium was changed to LCHHA for 30 min with no observed decrease in taurine release. The medium was then changed back to HHA, again with no change in taurine release. This treatment produced a transient increase in $[Ca^{2+}]_i$ (see Figs. 5, 6A). Finally, cells were stimulated with 100 nM isoproterenol. Under these conditions this should produce a transient increase in $[Ca^{2+}]_i$ (see Figs. 5, 6B). The response to isoproterenol at this time was similar to that observed under control conditions.

ly influenced by the contents of intracellular stores (see middle records, Fig. 5). These areas correspond to the general location of the endoplasmic reticulum in astroglial cells (Shain et al., 1987a). We do not see any need to argue that the nucleus itself partitions Ca^{2+} differentially from the cytoplasm, as has been argued previously (Williams et al., 1985).

This mixing of signals, which at first glance might appear to be a disadvantage, is actually a benefit, as long as one does not attach too much importance to the absolute levels of Ca^{2+} reported, because the data give a simultaneous look at both cytoplasmic and compartmentalized Ca^{2+} levels. Our measurements clearly show that under standard conditions IPR does not change Ca^{2+} levels in any compartment accessible to fura-2/AM during taurine release.

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Figure 5. Stimulation of Ca^{2+} from intracellular stores. In order to observe release of Ca^{2+} from intracellular stores, the following protocol was followed. (Each panel is designated by the time in minutes after the fura-2 measurements were initiated.) Cells in HHA (6.30 min) were washed with LCHHA and incubated for approximately 30 min (39.53 min) to deplete intracellular Ca^{2+} . The medium was changed back to HHA at 42.48 min and a transient increase in $[Ca^{2+}]_i$ was observed (42.68–46.10 min). HHA was replaced with LCHHA and cells incubated for approximately 7.5 min (53.67 min). At 55.67 min, isoproterenol was added to the incubation medium (final concentration, 100 nM). A transient increase in $[Ca^{2+}]_i$ was observed in all cells (55.87–57.85 min). The final micrograph illustrates the fluorescence image (excitation wave length = 380 nm) of cells at the end of the experiment. The values of $[Ca^{2+}]_i$ for the false colors are indicated by the bar.

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