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An Interaction of Oxytocin Receptors with Metabotropic Glutamate Receptors in Hypothalamic Astrocytes

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Abstract

Hypothalamic astrocytes play a critical role in the regulation and support of many different neuroendocrine events, and are affected by estradiol. Both nuclear and membrane estrogen receptors (ERs) are expressed in astrocytes. Upon estradiol activation, membrane-associated ER (mER) signals through the type 1a metabotropic glutamate receptor (mGluR1a) to induce an increase of free cytoplasmic calcium concentration ($[Ca^{2+}]_i$). Since the expression of oxytocin receptors (OTRs) is modulated by estradiol, we tested if estradiol also influences oxytocin signaling. Oxytocin at 1 nM, 10 nM, and 100 nM induced a $[Ca^{2+}]_i$ flux measured as a change in relative fluorescence ($\Delta F Ca^{2+} = 330 \pm 17$ relative fluorescent units (RFU), $\Delta F Ca^{2+} = 331 \pm 22$ RFU, and $\Delta F Ca^{2+} = 347 \pm 13$ RFU, respectively) in primary cultures of female post-pubertal hypothalamic astrocytes. Interestingly, OTRs interacted with mGluRs. The mGluR1a antagonist, LY 367385 (20 nM), blocked the oxytocin (1 nM)-induced $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 344 \pm 19$ vs. 127 ± 11 RFU, $P < 0.001$). Conversely, the mGluR1a receptor agonist, (RS)-3,5-dihydroxyphenylglycine (DHPG, 100 nM), increased the oxytocin (1 nM)-induced $[Ca^{2+}]_i$ response ($\Delta F Ca^{2+} = 670 \pm 31$ RFU) compared with either compound alone ($P < 0.001$). Since both oxytocin and estradiol rapidly signal through the mGluR1a, we treated hypothalamic astrocytes sequentially with oxytocin and estradiol to determine whether stimulation with one hormone affected the subsequent $[Ca^{2+}]_i$ response to the second hormone. Estradiol treatment did not change the subsequent $[Ca^{2+}]_i$ flux to oxytocin ($P > 0.05$) and previous oxytocin exposure did not affect the $[Ca^{2+}]_i$ response to estradiol ($P > 0.05$). Furthermore, simultaneous estradiol and oxytocin stimulation failed to yield a synergistic $[Ca^{2+}]_i$ response. These results suggest that the OTR signals through the mGluR1a to release Ca^{2+} from intracellular stores and rapid, non-genomic estradiol stimulation does not influence OTR signaling in astrocytes.

Keywords

OTR; mGluR1a; $[Ca^{2+}]_i$; rapid cell signaling; mER

INTRODUCTION

Oxytocin has important central influences on sexual, maternal, feeding, and social behaviours as well as critical peripheral roles for lactation and parturition (1–3). It is synthesized by magnocellular neurons in the SON (supraoptic nucleus) and PVN

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(paraventricular nucleus) of the hypothalamus, which project to the posterior pituitary gland and secrete oxytocin into the bloodstream. In addition to its role as a neurohormone, oxytocin is also released intra-hypothalamically. Interestingly, the dendrites of magnocellular neurons are the major sources of oxytocin in the brain (4). However, oxytocin can also be released directly from centrally-projecting parvocellular neurons in the PVN (5,6). Hypothalamic astrocytes play an important role in regulating the function of oxytocin secreting magnocellular neurons by permitting intercellular communication (7). Astrocytes can regulate their shape and retract cellular processes that separate magnocellular neurons allowing the formation of electrical coupling between magnocellular neurons and enabling the synchronous firing needed for surge releases of oxytocin (8,9). Astrocytes also regulate metabolic events (10) and synaptic function in adjacent neurons (11,12), and scavenge electrolytes and neurotransmitters from the extracellular space that accumulate during neurohypophyseal hormone secretion such as lactation and chronic dehydration. Thus, astrocytes are critical for the function of the hypothalamo-neurohypophyseal system. In fact, hypothalamic astrocytes have been implicated in many different functions, such as sexual differentiation (13), facilitation of puberty (14), regulation of releasing factor secretion (15–19), and synthesis of neurosteroids (19–24). More specifically, hypothalamic astrocytes can regulate the secretory activity of GnRH neurons through plastic rearrangements as well as signaling pathways involving growth factors and other factors, such as glutamate and prostaglandins (14,25–28). Based on the expression of receptors for numerous transmitters, peptides and steroids, our knowledge of the role of astrocytes continues to expand (29–32).

One such role is in the magnocellular hypothalamo-neurohypophyseal system. Astrocytes have extensive interactions with neurons, maintaining physical separation between the magnocellular neurons. Oxytocin released from magnocellular neurons significantly reduced astrocytic coverage of oxytocin neurons (7,8,33,34). The proposed mechanism is through activation of astrocytic oxytocin receptors (OTRs), which are G protein-coupled receptors (GPCR) that signal through the Gq and activate signal transduction pathways including the rapid increase of IP₃-sensitive free cytoplasmic calcium concentration ($[Ca^{2+}]_i$) (35,36). It is the $[Ca^{2+}]_i$ that mediates astrocyte morphology by affecting cytoskeletal elements (37,38). We had noticed a similar rapid $[Ca^{2+}]_i$ flux via the PLC/IP₃ pathway in astrocytes following the activation of a membrane-associated estrogen receptor (mER) (29). Interestingly, estradiol-activated mER transactivates the type 1a metabotropic glutamate receptor (mGluR1a) to stimulate the PLC/IP₃ pathway leading to an increase in $[Ca^{2+}]_i$ (39). Based on these observations, we investigated whether estradiol and oxytocin signaling may converge at the membrane by transactivating the mGluR1a to initiate cell signaling. Hypothalamic astrocytes were cultured from post-pubertal female rats. As with estradiol signaling, blocking the mGluR1a with its antagonist, LY 367385, prevented the oxytocin-induced $[Ca^{2+}]_i$ flux in astrocytes. Conversely, use of the mGluR1a agonist, (RS)-3,5-dihydroxyphenyl-glycine (DHPG), enhanced the magnitude of the oxytocin-induced $[Ca^{2+}]_i$ flux. These results indicate that OTR signals through the mGluR1a to mediate the rapid mobilization of intracellular calcium in astrocytes. Both OTR and mER transactivate the same membrane receptor, mGluR1a, and activate the same intracellular calcium-mediated signaling pathway. Interestingly, treatment with estradiol did not alter the subsequent oxytocin response and the estradiol-induced $[Ca^{2+}]_i$ flux was not altered by oxytocin. Therefore, astrocytes do not appear to display hysteresis in their response to estradiol or oxytocin.

MATERIALS AND METHODS

Primary cell cultures

Primary hypothalamic astrocyte cultures were generated from post-pubertal (postnatal day 50) Long-Evans female rats (Charles River, Wilmington, MA). All experimental procedures

were approved by the Chancellor's Animal Research Committee at the University of California at Los Angeles. Briefly, a hypothalamic block was dissected with boundaries consisting of the rostral extent of the optic chiasm, the rostral extent of the mammillary bodies, the lateral edges of the tuber cinereum, and the top of the third ventricle. The hypothalamic tissue was enzymatically digested with trypsin and mechanically dissociated with a fire-polished glass Pasteur pipette. Cultures were grown for 7 to 10 days in an incubator at 37 degrees Celsius (°C) in DMEM/F12 (Mediatech, Herndon, VA) with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and 1% penicillin (10,000 IU/ml)-streptomycin (10,000 µg/ml) solution (PS; Mediatech, Herndon, VA). Once confluent, astrocyte cultures were purified from other glial cells using a technique (22,23,39) modified from McCarthy and de Vellis (40). Flasks were shaken on an orbital shaker at 200 rpm and 37° C for at least 24 hours to eliminate oligodendrocytes. All astrocyte cultures were replaced with fresh medium after shaking.

For Ca^{2+} imaging, the medium was removed from flasks, astrocytes were washed with Hanks' balanced salt solution (HBSS; Mediatech, Herndon, VA), dissociated with a 2.5% trypsin solution, and resuspended in 5 ml of DMEM/F12 medium with 10% FBS. Astrocytes were centrifuged for 3 min at $80 \times g$, then plated and grown on Poly-D lysine (0.1 mg/ml; Sigma-Aldrich, St. Louis, MO) coated 15 mm glass coverslips in 12 well culture plates. Cells were incubated for 48–72 hours in DMEM/F12 medium with 10% FBS and 1% PS before experimentation. Cultures were routinely checked for purity using immunocytochemistry for glial fibrillary acidic protein (GFAP; Chemicon, Temecula, CA) with a Hoechst 3342 (Sigma-Aldrich, St. Louis, MO) nuclear stain. Cultures were determined to be greater than 95% pure astrocytes as previously reported (22,23,39).

Intracellular Ca^{2+} measurements

Hypothalamic astrocyte cultures were steroid starved by incubating in DMEM/F12 medium with 5% charcoal-stripped FBS for 18 hours at 37 °C. The cells were then incubated for 45 min at 37 °C with HBSS and the calcium indicator Fluo-4 AM (4.5 µM; Invitrogen, Eugene, OR), dissolved in dimethyl sulfoxide (DMSO) and methanol, before imaging. Excess Fluo-4 AM was removed with three washes using HBSS. Glass coverslips were mounted into a 50 mm RC-61T-01 chamber insert (Warner Instruments, Hamden, CT) secured inside a 60 × 15 mm cell culture dish (Corning, Corning, NY) and placed into a QE-2 quick exchange platform (Warner Instruments, Hamden, CT). Gravity perfusion with PE160 tubing and MP series perfusion manifolds (Warner Instruments, Hamden, CT) was used to constantly irrigate the astrocytes with HBSS and to deliver the drugs. Media and drugs were removed from the apparatus through vacuum suction. Fluo-4 AM imaging was performed using the Axioplan2-LSM 510 Meta confocal microscope (Zeiss, Thornwood, New York, NY) with an IR-Achroplan 40X/0.80 water immersion objective (Zeiss, Jena, Germany), 488 nm laser excitation, and emission monitored through a low-pass filter with a cutoff at 505 nm.

For our experiments, drugs were used in following concentrations: 1 nM to 100 nM oxytocin (Bachem, Torrance, CA); 1 nM cyclodextrin-encapsulated 17β -estradiol (Sigma-Aldrich, St. Louis, MO); 20 nM LY367385 (Tocris, Ellisville, MO); and 100 nM DHPG (Tocris, Ellisville, MO). Stock solutions and final working concentrations were prepared in HBSS. Controls were treated with HBSS only.

Statistics

Data are presented as mean $\pm$ standard error of the mean (SEM) in relative fluorescent units (RFU). The $\Delta F \text{Ca}^{2+}$ was calculated as the difference between baseline fluorescent intensity and peak response to drug stimulation. Statistical comparisons were made using the paired Student's *t*-test when comparing means across 2 groups and one-way analysis of variance

(ANOVA) with Student-Newman-Keuls post hoc test when comparing means across 3 or more groups. Statistical calculations were carried out using SigmaStat 3.5 (Systat Software, San Jose, CA). Differences at the $P < 0.05$ level were considered significant.

RESULTS

Effects of oxytocin on $[Ca^{2+}]_i$ flux in hypothalamic astrocytes

Approximately 90% of the tested hypothalamic astrocytes responded rapidly to oxytocin with an increase in $[Ca^{2+}]_i$ flux. Oxytocin at 1 nM, 10 nM, and 100 nM induced a similar increase in $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 330 \pm 17$ RFU, $n=28$; $\Delta F Ca^{2+} = 331 \pm 22$ RFU, $n=18$; and $\Delta F Ca^{2+} = 347 \pm 13$ RFU, $n=27$, respectively; Figure 1). The $[Ca^{2+}]_i$ response to all of these oxytocin concentrations were significantly greater compared to control astrocytes ($\Delta F Ca^{2+} = 137 \pm 5$ RFU, $n=14$, $p<0.001$). A double stimulation paradigm with oxytocin was used. Oxytocin (1 nM), followed by an intervening 2-minute washout, and then restimulation with oxytocin produced similar levels of $[Ca^{2+}]_i$ flux (first stimulation: $\Delta F Ca^{2+} = 330 \pm 17$ RFU; second stimulation: $\Delta F Ca^{2+} = 339 \pm 13$ RFU; $n = 28$, $p>0.05$; Figure 2). Similarly, astrocytes responded equally to both applications of oxytocin at 10 nM (first stimulation: $\Delta F Ca^{2+} = 331 \pm 22$ RFU; second stimulation: $\Delta F Ca^{2+} = 329 \pm 30$ RFU; $n = 18$, $p>0.05$; Figure 2). Therefore, a washout period as brief as 2 minutes was sufficient for refilling of intracellular Ca^{2+} stores and allowing the astrocytes to respond maximally to a subsequent oxytocin stimulation.

Oxytocin-induced $[Ca^{2+}]_i$ flux acts through the mGluR1a

To determine whether the oxytocin-induced $[Ca^{2+}]_i$ flux was dependent on the mGluR1a, astrocytes were stimulated twice, so each astrocyte was its own control. Astrocytes responded maximally to the initial 1 nM of oxytocin ($\Delta F Ca^{2+} = 344 \pm 19$ RFU, $n = 13$; Figure 3). Following a 2-minute washout and 7-minute perfusion with the mGluR1a antagonist, LY 367385 (20 nM), re-stimulating the same astrocytes with oxytocin at 1 nM resulted in a significantly attenuated $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 127 \pm 11$ RFU, $n = 13$, $p<0.05$; Figure 3). Therefore, antagonism of the mGluR1a lead to a 63.1% decrease in oxytocin-induced $[Ca^{2+}]_i$ response.

mGluR1a activation enhances the oxytocin-induced $[Ca^{2+}]_i$ flux

Since oxytocin signals through the mGluR1a to induced a $[Ca^{2+}]_i$ flux, we investigated whether simultaneous activation of the mGluR1a with DHPG would have an additive effect to oxytocin stimulation. As previously reported, DHPG at 100 nM ($\Delta F Ca^{2+} = 346 \pm 25$ RFU, $n=19$) induced a significantly greater $[Ca^{2+}]_i$ flux than controls and DHPG at 1 nM or 10 nM (39). There was no significant difference in $[Ca^{2+}]_i$ response between oxytocin at 1 nM and DHPG at 100 nM ($P > 0.05$) (Figure 4). However, DHPG augmented the oxytocin response in astrocytes, such that oxytocin (1 nM) in combination with DHPG (100 nM) produced a significantly greater $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 670 \pm 31$ RFU, $n=20$) compared with 1 nM of oxytocin ($p<0.001$) or 100 nM of DHPG ($p<0.001$) (Figure 4).

Oxytocin- and estradiol-induced $[Ca^{2+}]_i$ flux is rapidly reset and non-synergistic

We have previously demonstrated that 17β -estradiol rapidly stimulates a robust $[Ca^{2+}]_i$ flux in hypothalamic astrocytes (39). Since both the OTR and the mER signal through the mGluR1a, we treated astrocytes sequentially with oxytocin then estradiol, or the reverse, to determine whether the $[Ca^{2+}]_i$ response would show hysteresis. As with oxytocin, estradiol can also be used in a double-stimulation paradigm: two statistically similar $[Ca^{2+}]_i$ spikes can be induced in hypothalamic astrocytes when they are separated by a 2-minute washout interval (39). Astrocytes perfused with 1 nM of oxytocin followed by 1 nM of estradiol, with

an intervening 2-minute washout, resulted in a $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 328 \pm 14$ RFU for oxytocin and $\Delta F Ca^{2+} = 608 \pm 25$ RFU for estradiol, $n=19$; Figure 5). The order of treatment was then reversed: 1 nM of estradiol, 2-minute washout, and stimulation with 1 nM of oxytocin resulted in a similar $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 586 \pm 24$ RFU for estradiol and $\Delta F Ca^{2+} = 339 \pm 20$ RFU for oxytocin, $n=24$; Figure 5). Both $[Ca^{2+}]_i$ responses to oxytocin were similar whether or not the astrocytes were previously treated with estradiol ($P>0.05$). Similarly, the application of oxytocin before estradiol treatment did not influence the estradiol-induced $[Ca^{2+}]_i$ flux ($P>0.05$). Therefore, astrocyte response to oxytocin or estradiol treatment is rapidly reset and an order of application effect was not observed. Since both the rapid estradiol and oxytocin membrane-initiated signaling pathways involve the mGluR1a, simultaneous application of oxytocin and estradiol was performed. Oxytocin (1 nM) in combination with estradiol (1 nM) did not produce a different $[Ca^{2+}]_i$ response compared with estradiol (1 nM) alone ($P>0.05$, data not shown). Therefore, simultaneous estradiol treatment failed to potentiate the rapid oxytocin-induced $[Ca^{2+}]_i$ flux in hypothalamic astrocytes.

DISCUSSION

OTRs have been identified in astrocyte cultures and our current demonstration of a rapid oxytocin-induced $[Ca^{2+}]_i$ flux in hypothalamic astrocytes confirms earlier studies showing OTR mediated release of IP_3 -sensitive intracellular Ca^{2+} stores (35,36). Similarly, estradiol acting through mER activates the PLC/ IP_3 pathway leading to a dramatic rise in $[Ca^{2+}]_i$ flux, which was more robust than the oxytocin-induced increase (29). The major findings of these experiments were that both the OTR and the mER transactivate the mGluR1a to stimulate a $[Ca^{2+}]_i$ flux in hypothalamic astrocytes. Moreover, neither estradiol nor oxytocin induced a long-lasting alteration in membrane signaling. The effects of both hormones were rapidly washed out from the astrocytes.

Recently, we determined that estradiol signals through a mER α that transactivates the mGluR1a to induce an increase in $[Ca^{2+}]_i$ in post-pubertal hypothalamic astrocytes (39). A similar mER α -mGluR1a signaling mechanism has also been reported in hypothalamic and hippocampal neurons, indicating the general nature of this mechanism that accounts for membrane-initiated estradiol signaling (41,42). Interestingly, the OTR, which is a *bona fide* GPCR with a seven-pass membrane structure, appears to also use the mGluR1a to initiate rapid intracellular signaling. In hypothalamic astrocytes, the oxytocin-induced $[Ca^{2+}]_i$ flux was blocked by the mGluR1a antagonist, LY 367385 (Figure 3). Conversely, the oxytocin-induced $[Ca^{2+}]_i$ flux was enhanced by the concurrent use of the mGluR1a agonist, DHPG (Figure 4). Our present results are consistent with findings that show inhibition of oxytocin-dependent changes in hypothalamic astrocyte morphology when glutamatergic receptors are antagonized (34). Together, these results indicate that an interaction with the mGluR1a may be a common signaling mechanism for a number of membrane receptors.

Expression of astrocytic OTR is influenced by estrogen exposure, and it appears to be spatially restricted (43,44). Effects of estradiol on oxytocin actions in astrocytes can range from genomic to non-genomic regulations. Recent studies have shown that the regulation of OTR expression is due to the activation of intracellular ERs, which increase promoter activity due to GC-rich SP1 binding sites, thereby directly regulating gene transcription of the OTR (45). However, the present studies indicate that other actions of estradiol may be due to interaction with mERs, which lead to the activation of cell signaling pathways that can result in the phosphorylation of the cyclic AMP response element binding (CREB) protein and transcriptional regulation. Since both OTR and mER activate the mGluR1a to initiate rapid activation of G proteins and the PLC/ IP_3 pathway leading to the increase in $[Ca^{2+}]_i$ release, hypothalamic astrocytes were serially stimulated with oxytocin then

estradiol or estradiol then oxytocin to determine if the OTR and mER signaling pathways compete or synergize with each other. Our results demonstrate that the $[Ca^{2+}]_i$ response to oxytocin was independent of prior astrocyte exposure to estradiol. Similarly, previous exposure to oxytocin did not change the subsequent $[Ca^{2+}]_i$ response to estradiol stimulation. Although oxytocin and estradiol may share a common intracellular signaling pathway through the mGluR1a, rapid membrane signaling with oxytocin does not affect subsequent membrane signaling with estradiol, nor does estradiol alter the response to oxytocin. Furthermore, simultaneous estradiol and oxytocin treatment failed to yield a synergistic $[Ca^{2+}]_i$ response compared with estradiol alone. Estradiol has been reported to potentiate the OTR response, but estradiol does not seem to regulate this response through rapid, non-genomic, membrane-initiated effects on $[Ca^{2+}]_i$ flux, rather this effect may be due to up regulation of OTR gene transcription by estradiol (34,45). A genomic response to estradiol stimulation was not tested in this experiment since it would likely required a longer exposure to estradiol than 8 seconds and the 2 minute washout between stimulations would be insufficient for transcription, translation, and shuttling of the OTR to the plasma membrane.

The present results demonstrate that OTR interaction with mGluR1a is required to initiate rapid cell signaling resulting in a rapid elevation of $[Ca^{2+}]_i$ flux in hypothalamic astrocytes. Intracellular calcium regulation and homeostasis is critical for the regulation of gene expression, development, and survival in astrocytes (46). Furthermore, astrocytic elevation in $[Ca^{2+}]_i$ flux has been associated with the modulation of microvascular tone and neuronal transmission (47–50). These experiments provide a mechanism to understand how oxytocin-induced $[Ca^{2+}]_i$ flux is modified in astrocytes, which may ultimately influence brain function. DHPG alone and oxytocin alone are not as effective at stimulating $[Ca^{2+}]_i$ flux as they are together, indicating that for maximal signaling in astrocytes both glutamate and oxytocin need to be present. These results suggest that the OTR signals through the mGluR1a, therefore, astrocyte response to oxytocin may be greatest near active glutamatergic nerve terminals. Although beyond the scope of these experiments, these findings suggest that astrocytes will have an enhanced response to oxytocin released from magnocellular dendrites of the supraoptic and paraventricular nuclei when this brain region is simultaneously activated by glutamatergic stimulation during the milk ejection neuroendocrine reflex.

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Figure 1.

Oxytocin induces a change in $[Ca^{2+}]_i$ flux in post-pubertal hypothalamic astrocytes. Calcium imaging of astrocytes stimulated with oxytocin at 1 nM, 10 nM, and 100 nM all induced a statistically greater $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 330 \pm 17$ RFU, $n=28$; $\Delta F Ca^{2+} = 331 \pm 22$ RFU, $n=18$; and $\Delta F Ca^{2+} = 347 \pm 13$ RFU, $n=27$, respectively) than control ($\Delta F Ca^{2+} = 137 \pm 5$ RFU, $n=14$, $P < 0.001$). Increasing the oxytocin exposure from 1 nM to 100 nM did not significantly increase the $[Ca^{2+}]_i$ response. * Significantly different ($P < 0.001$, one-way ANOVA with Student-Newman-Keuls post hoc test).

Figure 2.

Effect of double oxytocin stimulation on $[Ca^{2+}]_i$ flux in post-pubertal hypothalamic astrocytes. Oxytocin produced an increase in $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 330 \pm 17$ RFU, $n=28$ for oxytocin at 1 nM and $\Delta F Ca^{2+} = 331 \pm 22$ RFU, $n=18$ for oxytocin at 10 nM). Following the 2-minute washout, the second oxytocin stimulation produced a similar $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 339 \pm 13$ RFU, $n=28$ for oxytocin at 1 nM and $\Delta F Ca^{2+} = 329 \pm 30$ RFU, $n=18$ for oxytocin at 10 nM). Statistically, both the initial and the second oxytocin stimulations were the same for 1 nM and 10 nM concentrations of oxytocin ($P > 0.05$). No significant difference ($P > 0.05$, paired Student's t -test).

Figure 3.

Effect of LY 367385, mGluR1a antagonist, on the change in $[Ca^{2+}]_i$ flux upon exposure to oxytocin in post-pubertal hypothalamic astrocytes. Astrocytes stimulated with 1 nM of oxytocin responded with a $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 344 \pm 19$ RFU, $n=13$, $P < 0.001$ vs. control) that was significantly attenuated with LY 367385 ($\Delta F Ca^{2+} = 127 \pm 11$ RFU, $n=13$, $P < 0.001$). * Significantly different ($P < 0.001$, paired Student's t -test).

Figure 4.

The mGluR1a agonist, DHPG, augmented the oxytocin-induced $[Ca^{2+}]_i$ flux in post-pubertal hypothalamic astrocytes. Astrocytes stimulated with 1 nM of oxytocin induced a similar $[Ca^{2+}]_i$ response ($\Delta F Ca^{2+} = 330 \pm 17$ RFU, $n=28$) compared with 100 nM of DHPG ($\Delta F Ca^{2+} = 346 \pm 25$ RFU, $n=19$, $P > 0.05$). Oxytocin at 1 nM in combination with DHPG at 100 nM stimulated a significantly greater $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 670 \pm 31$ RFU, $n=20$) than with oxytocin or DHPG alone ($P < 0.001$ vs. oxytocin at 1 nM and $P < 0.001$ vs. DHPG at 100 nM). * Significantly different ($P < 0.001$, one-way ANOVA with Student-Newman-Keuls post hoc test).

Figure 5.

Order of treatment did not effect the $[Ca^{2+}]_i$ flux in post-pubertal hypothalamic astrocytes. Astrocytes stimulated with 1 nM of oxytocin induced a $[Ca^{2+}]_i$ flux ($\Delta F Ca^{2+} = 328 \pm 14$ RFU, $n=19$) similar to astrocytes initially treated with 1 nM of estradiol followed by 1 nM of oxytocin ($\Delta F Ca^{2+} = 339 \pm 20$ RFU, $n=24$, $P > 0.05$). Estradiol at 1 nM induced a similar $[Ca^{2+}]_i$ response regardless of whether astrocytes were previously stimulated with 1 nM of oxytocin ($\Delta F Ca^{2+} = 586 \pm 24$ RFU, $n=24$ without prior oxytocin and $\Delta F Ca^{2+} = 608 \pm 25$ RFU, $n=19$ with prior oxytocin, $P > 0.05$). White and black columns represent sequential drug stimulation with an intervening 2 minute washout period. No significant difference between the oxytocin groups and between the estradiol groups ($P > 0.05$, one-way ANOVA with Student-Newman-Keuls post hoc test).