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Studies on the role of calcium in the 5-HT-stimulated release of glutamate from C6 glioma cells

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Abstract

We recently reported that 5-hydroxytryptamine$_{2A}$ (5-HT$_{2A}$) receptor activation on cultured glial cells induces glutamate release [J. Neurosci. Res. 67 (2002) 399]. Here we use C6 glioma cells to examine the role of calcium in this response. 5-Hydroxytryptamine (5-HT) increases glutamate release from C6 glioma cells, an effect blocked by low calcium conditions. The calcium ionophores ionomycin and calcimycin also released glutamate from C6 glioma cells in a Ca^{2+}-dependent manner. The effect of 5-HT was reduced by the phospholipase C inhibitor U73122 (1-[6[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione), but not its inactive enantomer U73343(1-[6[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-2,5-pyrrolidinedione). The protein kinase C inhibitors staurosporine and calphostin C had no effect on the response to 5-HT, whereas the response was blocked by thapsigargin and caffeine. Neither the L-type calcium channel blockers, nifedipine and verapamil, nor the N-type calcium channel blocker ω-conotoxin GVIA inhibited the effect of 5-HT, whereas $NiCl_2$ and KCl blocked the response to 5-HT. We conclude that the 5-HT-induced efflux of glutamate from C6 glioma cells is Ca^{2+}-dependent and involves, at least in part, the mobilisation of Ca^{2+} from inositol (1,4,5) tris phosphate (IP_3) sensitive intracellular stores.

Keywords: 5-Hydroxytryptamine; Glial; Calcium; IP_3; Glutamate

1. Introduction

It is speculated that transmitter-evoked release of glutamate from glial cells could play an important role in neuron-glia interactions in both physiological and pathological conditions (Araque et al., 1999; Bezzi et al., 1998; Parpura et al., 1994). In support of this idea, we have recently reported that activation of 5-hydroxytryptamine$_{2A}$ (5-HT$_{2A}$) receptors on cultured glia stimulates glutamate efflux (Meller et al., 2002). The ability of 5-hydroxytryptamine (5-HT) to increase glutamate release was associated with its ability to raise intracellular levels of calcium (Meller et al., 2002). In particular, neuroligands that have been shown to raise intracellular calcium levels in C6 glioma cells (5-HT, bradykinin and carbachol) (Reiser et al., 1989) also stimulated glutamate efflux from these cells. Furthermore, unlike differentiated C6 glioma cells, undifferentiated C6 glioma cells do not show either a calcium response or a glutamate response to 5-HT (Cholewinski and Leslie, 1996). The effect of 5-HT was reduced by EGTA, hence it is unclear whether the increase in cytosolic Ca^{2+} is due to either influx of extracellular Ca^{2+} or mobilisation of Ca^{2+} from intracellular stores.

Receptor-mediated increases in intracellular Ca^{2+} may occur due to the release of Ca^{2+} from either inositol (1,4,5) tris phosphate (IP_3)-sensitive or ryanodine-sensitive/IP_3-insensitive calcium stores (Berridge, 1993; Furuichi and Mikoshiba, 1995; Sheppard et al., 1997; Ullmer et al., 1996). In C6 glioma cells, 5-HT acting via the 5-HT$_{2A}$ receptor increases phosphoinositide hydrolysis, to produce IP_3, resulting in the liberation of Ca^{2+} from the IP_3-sensitive Ca^{2+} store (Elliott et al., 1995; Hoyer et al., 1994; Murphy and Pearce, 1987). However, increased phosphoinositide hydrolysis would also produce diacylglycerol, which activates protein kinase C. Activation of protein kinase C enhances the release of neurotransmitters from both in vitro brain preparations (Browning and Dudek, 1992; Dekker

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et al., 1991; Zimmermann, 1990) and cultured neurones (Goodall et al., 1997; Murphy et al., 1992) via an effect independent of IP_3. Therefore, the ability of 5-HT to increase glutamate release from the C6 glioma cell line may also involve a protein kinase C-dependent mechanism.

The 5-HT-induced release of glutamate from C6 glioma cells requires the presence of extracellular Ca^{2+} (Meller et al., 2002), which implies that Ca^{2+} influx from extracellular sources plays a role in the glutamate response. Neurotransmitter release from neurones is mediated by the opening of voltage-sensitive Ca^{2+} channels (Borst and Sakmann, 1996; Sabria et al., 1995; Sudhof, 1995). Glial cells and astrocytes express voltage-gated calcium channels and activation of certain metabotropic receptors can affect Ca^{2+} channel conductance (Barres, 1991). Hence, Ca^{2+} influx into C6 glioma cells via calcium channels may also be involved in the glutamate response to 5-HT.

The aim of this study was to examine which source of Ca^{2+} is responsible for the 5-HT-induced release of glutamate from C6 glioma cells. The glutamate response to 5-HT was examined in the presence of drugs that inhibit phospholipase C or protein kinase C (U73122, staurosporine and calphostin C), Ca^{2+} release from intracellular calcium stores (thapsigargin, ryanodine and caffeine) and transmembrane Ca^{2+} influx (calcium channel blockers).

2. Materials and methods

2.1. Cell culture

Rat C6 glioma cells (passage number 73-110) were obtained from the European Collection of Animal Cell Cultures, Porton Down (Wiltshire, UK). Cells were grown in Dulbecco's Modified Eagle's Media (DMEM) containing 10% (vol/vol) foetal calf serum, at 37 °C in a humidified atmosphere of 5% CO_2. Cells were subcultured onto 24-well plates (Nunc) at a density of ~50,000 cells/well for 4 days in 10% (vol/vol) foetal calf serum supplemented DMEM. In most experiments, the media was then changed to DMEM plus 1% (vol/vol) dialysed foetal calf serum and dibutyryl cyclic AMP (1 mM) for a further 3 days to induce differentiation of the C6 glioma cell into an astrocytic phenotype (Segovia et al., 1994).

2.2. Glutamate efflux experiments

Measurement of glutamate efflux from incubated C6 glioma cells was carried out as described previously (Meller et al., 2002). C6 glioma cells were washed three times for 10 min with 1 ml of warmed (37 °C) Krebs (in mM, NaCl 11 8, $NaHCO_3$ 24, KCl 3, $MgSO_4$: 7 H_2O 1, KH_2PO_4 1.2, D-glucose 11 and $CaCl_2$ 2.5) gassed with 95% O_2/5% CO_2. Cells were then incubated in 0.5 ml Krebs containing the glutamate uptake blocker pyrollidine 2,4-dicarboxylic acid (50 μM) for 10 min at 37 °C in a shaking water bath.

The effect of U73122, U73343, caffeine, thapsigargin, ryanodine, verapamil, nifedipine, ω-conotoxin GIVA, staurosporine and calphostin C on the glutamate response to 5-HT was examined. Concentrations of drug were used which have previously been shown to specifically block their targets, especially in C6 glioma or glial preparations. These drugs were added to the final 10-min wash and were present throughout the 10-min incubation of the C6 glioma cells with 5-HT (10 μM). $NiCl_2$ (5 mM) and KCl (56 mM) were co-administered with 5-HT (10 μM). Each set of experiments included a vehicle only control (Krebs), and a control 5-HT (10 μM) challenge. Experiments used 4–6 wells of the 24-well plate per treatment condition. Following incubation, 200 μl supernatant samples were removed centrifuged (20,000×*g*, 5 min) and frozen at −20 °C until glutamate levels were determined by high-performance liquid chromatography with electrochemical detection (HPLC-EC).

2.3. Glutamate analysis

Supernatants of incubated cells were analysed for endogenous glutamate using HPLC-EC combined with a pre-column derivatisation method, as previously described (Meller et al., 2002). Samples were derivatised using an O-phthaldialdehyde sulphite reagent, and separated on a Rainin Dynamax reversed-phase column (Microsorb 5 μm C_{18} particles, 250×4.6 mm I.D., Anachem). The mobile phase consisted of 0.1 M di-sodium hydrogen phosphate, 0.5 mM EDTA and 10% v/v HPLC grade methanol, adjusted to pH 5.8 using 1 M orthophosphoric acid. Isocratic separation was achieved at room temperature, using a flow rate of 1.0 ml/min. Separated sample constituents were detected using a Bioanalytical Systems LC-4 amperometric detector with a glassy carbon working electrode (+0.85 V vs. Ag/AgCl reference electrode). Detector output current was monitored using a Thermo Separation Products SP4400 chrome jet integrator (Thermo Separation Products, UK).

2.4. Data presentation and statistical analysis

The absolute amount of glutamate in each supernatant (pmol/50 μl) was expressed as either a percentage of mean control levels (Krebs only) or a percentage of the response to 5-HT (10 μM). The raw data were analysed statistically by unpaired Student's *t*-test and *P* values of 0.05 or less were considered statistically significant.

2.5. Drugs and materials

The principal drugs and chemicals used (with suppliers) were as follows: ethylene glycol-bis (β-amino-ethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) (disodium salt), dibutyryl cyclic adenosine mono phosphate (dbcAMP), 5-HT (creatinine sulphate), ryanodine (from Ryania speciosa), caffeine, thapsigargin, verapamil (HCl), ionomycin (Ca^{2+} salt) and

nifedipine (Sigma, Dorset, UK), ω-conotoxin GVIA, U73122 (1-[6[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione) and U73343 (1-[6[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]-hexyl]-2,5-pyrrolidinedione) (RBI, Herts, UK) and 2,4 pyrollidine di-carboxylic acid (Tocris Cookson, Bristol Avon, UK). All other chemicals and reagents were of analytical grade from BDH (Dagenham, Essex, UK).

Fresh solutions of drugs were made up daily, by dissolving a known amount of drug into Krebs and diluting to the required concentration. Ryanodine, caffeine, thapsigargin, nifedipine, U73122 and U73343 were dissolved in dimethyl sulfoxide (DMSO) prior to dilution in Krebs (final DMSO concentration <1% v/v). DMSO had no effect on basal or 5-HT-stimulated glutamate release from C6 glioma cells (data not shown). Nifedipine was stored in the dark until use to prevent breakdown.

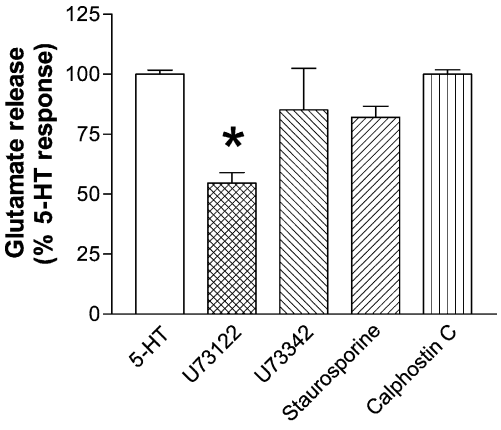

Fig. 2. Effect of phospholipase C inhibitor U73122 and its inactive enantiomer U73343 on the glutamate response to 5-HT. C6 glioma cells were pre-treated with U73122 (1.0 μM) or U73343 (1.0 μM) for 10 min prior to incubation with 5-HT (10 μM). Data shown is a mean±S.E.M. of 4–5 determinations, *P<0.05 vs. effect of 5-HT.

3. Results

Glutamate was detected in supernatant samples, following incubation of C6 glioma cells at 37 °C for 10 min (basal levels approx. 20 pmol/50 μl sample). Addition of 5-HT (0.01–100 μM) to the incubation medium caused an increase in glutamate efflux from C6 glioma cells, with a steep concentration response curve (estimated EC_{50}=75 nM) (Fig. 1). The glutamate response to 5-HT (100 μM) was eliminated in low Ca^{2+} medium (1 mM EGTA/5 mM $MgCl_2$) (164±5.9% of control vs. 95.6±9.5% of control, P<0.01, n=4), but not by replacing the Ca^{2+} in the medium with $MgCl_2$ alone (5 mM) (data not shown). There was no change in basal glutamate efflux from C6 glioma cells in low Ca^{2+} medium (n=6).

3.1. Effect of ionomycin and calcimycin

The Ca^{2+} selective ionophore calcimycin (A23187) (10 μM) increased glutamate efflux about six-fold (629±21% of control levels, n=4), an effect completely reversed in low Ca^{2+} medium ($MgCl_2$ 5 mM/EGTA 1 mM) (94±1.8% of control, n=4, P<0.01). Another calcium ionophore, ionomycin (1 μM) caused a larger increase in glutamate efflux (2550±480% of control levels, n=4) that was reduced in low Ca^{2+} medium (375±75% of control, n=4, P<0.01).

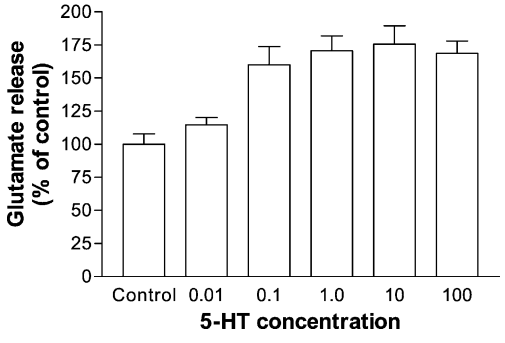

Fig. 1. Effect of 5-HT on glutamate release from C6 glioma cells. Cells were incubated for 10 min with various concentrations of 5-HT (0.01–100 μM). All points are mean±S.E.M. of 4–6 determinations.

3.2. Effect of phospholipase C inhibitors

The glutamate response to 5-HT (10 μM) was inhibited following 10 min pre-treatment with the phospholipase C inhibitor U73122 (1.0 μM) (Fig. 2), whereas the inactive enantiomer of U73122, U73343 (1.0 μM), had no effect on the glutamate response to 5-HT (Fig. 2). Neither U73122 nor U73343 had any effect on the basal release of glutamate (115% and 96% of control levels; n=4–5).

3.3. Effect of protein kinase C inhibitors

The non-selective protein kinase inhibitor staurosporine (0.5 μM) had no effect on the 5-HT-induced glutamate release from C6 glioma cells (Fig. 2). The protein kinase C selective inhibitor calphostin C (1 μM) was also without effect on the 5-HT-induced release of glutamate (Fig. 2). Staurosporine (0.5 μM) and calphostin C (1 μM) did not affect basal glutamate efflux (data not shown, n=5–6).

3.4. Role of intracellular calcium stores in 5-HT-induced glutamate efflux

C6 glioma cells were pre-treated with the Ca^{2+} ATPase inhibitor thapsigargin for 10 min prior to exposure to 5-HT (10 μM). Thapsigargin (1.0 μM) inhibited the effect of 5-HT on glutamate release (Fig. 3). Incubation of the C6 glioma

cells with (0.1 and 1.0 μM) thapsigargin alone did not increase basal glutamate release per se (n=4), although at higher concentrations (>10 μM) basal glutamate efflux was increased (223% of basal levels; n=4).

Pre-treating the C6 glioma cells with ryanodine (10 μM, 10 min) did not inhibit the glutamate response to 5-HT (10 μM) (Fig. 3). However, treatment of the C6 glioma cells with caffeine (20 mM, 10 min) significantly reduced the effect of 5-HT (Fig. 3). Ryanodine (10 μM) and caffeine (20 mM) had no effect on basal glutamate efflux when administered alone (data not shown, n=5–6).

3.5. Role of extracellular Ca^{2+} in the 5-HT-induced glutamate release

The glutamate response to 5-HT (10 μM) was not inhibited by the L-type calcium channel blocker verapamil (50 μM, 10 min) (Fig. 4). Similarly, the L-type calcium channel blocker nifedipine (50 μM, 10 min) did not inhibit the glutamate response to 5-HT (10 μM) (Fig. 4). Neither verapamil (50 μM) nor nifedipine (50 μM) changed the basal efflux of glutamate from C6 glioma cells (data not shown, n=6).

The glutamate response to 5-HT (10 μM) was not decreased by the N-type voltage gated calcium channel blocker ω-conotoxin GVIA (1 μM, 10 min) (175±11.1% of control, n=6, P<0.07). However, the glutamate response to 5-HT (10 μM) was abolished following co-administration with the non-selective calcium channel blocker Ni^{2+} (5 mM) (Fig. 4). Neither ω-conotoxin GVIA (1 μM) nor Ni^{2+} (5 mM) affected basal glutamate efflux (data not shown, n=5). The glutamate response to 5-HT (10 μM) was inhibited in the presence of 56 mM KCl (Fig. 4). However, high K^+ (56 mM) had no effect on the basal glutamate efflux from C6 glioma cells compared to control (n=6).

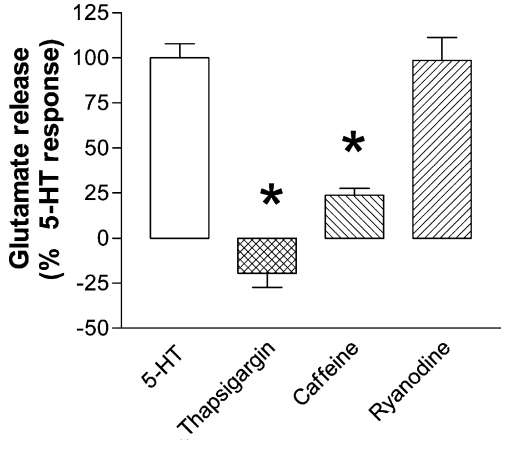

Fig. 3. Effect of thapsigargin, ryanodine and caffeine pre-treatment on the glutamate response to 5-HT. Cells were pre-treated with thapsigargin (1.0 μM) ryanodine (10 μM), or caffeine (20 mM) for 10 min prior to incubation with 5-HT (10 μM). Each point is a mean±S.E.M. of 5–6 determinations, *P<0.001 vs. effect of 5-HT.

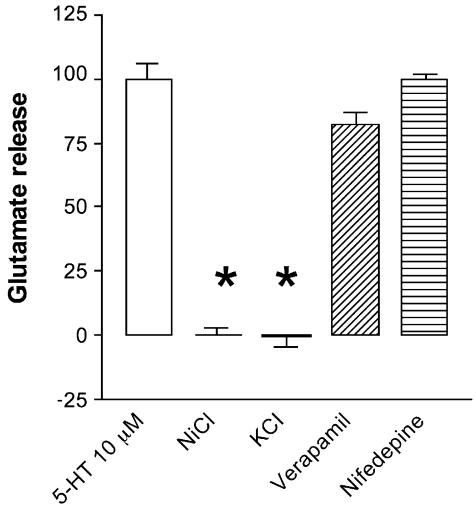

Fig. 4. Effect of L-type Ca^{2+} channel inhibitors nifedipine and verapamil, Ni^{2+} and high K^+ on the glutamate response to 5-HT. C6 glioma cells were pre-treated with either nifedipine (50 μM) or verapamil (50 μM) for 10 min prior to incubation with 5-HT (10 μM), or incubated for 10 min with 5-HT (10 μM) in the presence of either NiCl (5 mM) or KCl (56 mM). Data shown is a mean±S.E.M. of 5–6 determinations, *P<0.001 vs. effect of 5-HT.

4. Discussion

Experiments in this study were carried out to examine the source of calcium in the 5-HT-induced release of glutamate from C6 glioma cells. The glutamate response to 5-HT was decreased by the phospholipase C inhibitor U73122, but not by its inactive enantiomer U73343. The glutamate response to 5-HT was inhibited by the Ca^{2+} ATPase inhibitor thapsigargin, caffeine, but was ryanodine-insensitive. The response to 5-HT was staurosporine- and calphostin C-insensitive, which would imply that the response is not mediated by protein kinase C. These data suggest that the 5-HT-evoked release of glutamate from C6 glioma cells may involve Ca^{2+} release from an IP_3-sensitive intracellular calcium store. Neither the L-type calcium channel inhibitors verapamil and nifedipine nor the N-type Ca^{2+} channel inhibitor ω-conotoxin GVIA (1 μM) inhibited the 5-HT-induced release of glutamate. However, in the presence of the non-selective calcium channel blocker Ni^{2+} and high concentrations of K^+ ions, the glutamate response to 5-HT was abolished. Together these data suggest that the 5-HT-evoked glutamate response requires influx of Ca^{2+} from an extracellular source the mechanism of which remains currently unknown, but may be associated with the refilling of intracellular calcium stores (see below).

4.1. Role of phospholipase C and protein kinase C

The glutamate response to 5-HT was inhibited by the phospholipase C inhibitor U73122, but not by its inactive enantiomer U73343 (Fig. 2). The inhibition of the 5-HT effect by U73122 was predicted on the basis that the 5-HT_{2A} receptor is positively coupled to phospholipase C and at this concentration U73122 blocks receptor-activated PLC in astrocytes (Venance et al., 1997). Activation of phospholipase C generates IP_3 and diacylglycerol, which liberate Ca^{2+} from intracellular calcium stores and activate protein kinase C, respectively. Protein kinase C activation leads to transmitter release from neurones and cultured cells (Browning and Dudek, 1992; Dekker et al., 1991; Goodall et al., 1997; Murphy et al., 1992; Zimmermann, 1990). However, since staurosporine and calphostin C, at concentrations known to block protein kinase C (Fukao et al., 1997; Zhang and Weight, 1995), had no effect on the glutamate response to 5-HT, it is unlikely that protein kinase C plays a role in this effect of 5-HT.

4.2. Role of intracellular calcium stores in the 5-HT-induced release of glutamate from C6 glioma cells

We have previously shown that there is a clear association between raised intracellular calcium and glutamate release from C6 glioma cells (Meller et al., 2002). In support of these findings, in the current study, the Ca^{2+} ionophores ionomycin and calcimycin increased glutamate efflux from C6 glioma cells in a Ca^{2+}-dependent manner. The glutamate response to 5-HT in the C6 glioma cells was also inhibited following pre-treatment with thapsigargin (Fig. 3). Previous studies have shown that at the concentration used (1 μM), thapsigargin depletes intracellular calcium stores in endothelial cells, astrocytes and C6 glioma cells (Sabala et al., 1997; Thastrup et al., 1990; Venance et al., 1997). Therefore, our data would suggest that 5-HT-induced Ca^{2+} mobilisation from intracellular stores is involved in the glutamate response to 5-HT.

There are at least two separate intracellular calcium stores, one sensitive to ryanodine and the other to IP_3 (Berridge, 1993; Tanaka and Tashjian, 1993; Verma et al., 1992). The ryanodine-sensitive calcium store is activated by cyclic ADP ribose, ryanodine, caffeine and Ca^{2+} (Ehrlich et al., 1994; Galione, 1992; Galione et al., 1991; Sitsapesan et al., 1995). Ryanodine has biphasic effects, at low concentrations (<1 μM), ryanodine promotes the opening of the ryanodine receptor to cause Ca^{2+} release, however, at higher concentrations (10 μM) ryanodine inhibits the opening of the ryanodine receptor thereby inhibiting Ca^{2+} release. Since 5-HT-evoked glutamate release from C6 glioma cells was unaffected by ryanodine (10 μM), this suggests that ryanodine-sensitive calcium stores do not mediate the effect of 5-HT.

Caffeine mobilises ryanodine-sensitive calcium stores (Ehrlich et al., 1994; Parker and Ivorra, 1991), however, at the concentration tested (20 mM), caffeine did not stimulate the efflux of glutamate from C6 glioma cells, providing further support that ryanodine-sensitive calcium stores are not involved. Caffeine blocked the effect of 5-HT, which could be due to its ability to block IP_3 receptors (Ehrlich et al., 1994; Parker and Ivorra, 1991). In addition to its effects on intracellular stores, caffeine can inhibit phosphodiesterase (Scotini et al., 1983), however, 5-HT has no effect on cyclic AMP formation in C6 glioma cells (Carson et al., 1996). Therefore, the inhibition of the effect of 5-HT by caffeine could be due to blockade of the IP_3 receptor. However, taking together the effects of U73122, thapsigargin, caffeine and ryanodine, our data supports the view that the glutamate response to 5-HT is mediated through the release of Ca^{2+} from the IP_3-sensitive intracellular calcium store.

4.3. Role of extracellular Ca^{2+} in the glutamate response to 5-HT

The L-type calcium channel blockers nifedipine and verapamil did not inhibit the glutamate response to 5-HT in C6 glioma cells. Our observations agree with the findings of Cholewinski and Leslie (1996) who found that verapamil and nifedipine (both 50 μM) had no effect on the 5-HT-induced increase in intracellular Ca^{2+} in C6 glioma cells. The glutamate response to 5-HT was not inhibited by the N-type voltage-gated calcium channel blocker ω-conotoxin GVIA (Williams et al., 1992; Zhang et al., 1993), although it was by Ni^{2+} (Fig. 4). There are many other types of voltage-gated calcium channels which are inhibited by Ni^{2+} ions such as P, R and X-type Ca^{2+} channels (Zhang et al., 1993); however, other groups have reported that voltage-gated calcium channels are not expressed by C6 glioma cells (Imoto et al., 1996), and these were not investigated further.

The neurotransmitter-mediated release of Ca^{2+} from intracellular stores is usually accompanied by an influx of Ca^{2+} from extracellular sources that may be mediated by the extent of store depletion (Berridge, 1993). Whether the influx is controlled by receptor activation, G-proteins (Fasolato et al., 1994), a second messenger such as inositol-1,3,4,5-tetrakisphosphate (IP_4) (Luckhoff and Clapham, 1992) or another factor released from the store, such as calcium influx factor (Randriamampita and Tsien, 1993), is the subject of much debate. Ca^{2+} influx due to store depletion is inhibited by Ni^{2+} ions (5 mM) and depolarisation of the cell with high K^+ (Glowinski et al., 1994; Yagodin et al., 1995). Interestingly, the 5-HT-induced rise in intracellular Ca^{2+} in C6 glioma cells is inhibited by depolarising the cell with high K^+ (Manor et al., 1994). Given that both Ni^{2+} ions and high K^+ inhibit the effect of 5-HT on glutamate release, this would suggest that the Ca^{2+} influx, which is necessary for glutamate release, may involve a pathway which is similar in nature to that described following Ca^{2+} store depletion.

In summary, a number of agents were used to determine the source of calcium involved in the 5-HT-evoked release

of glutamate from C6 glioma cells. It is proposed that there are two salient features of the mechanism by which 5-HT receptor activation leads to glutamate release. First, the release of Ca^{2+} from intracellular IP_3-sensitive calcium stores and, secondly, the influx of calcium from external sources, the pathway of which is unknown.

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References

Araque, A., Sanzgiri, R.P., Parpura, V., Haydon, P.G., 1999. Astrocyte-induced modulation of synaptic transmission. Can. J. Physiol. Pharmacol. 77, 699–706.

Barres, B.A., 1991. New roles for glia. J. Neurosci. 11, 3685–3694.

Berridge, M.J., 1993. Inositol trisphosphate and calcium signalling. Nature 361, 315–325.

Bezzi, P., Carmignoto, G., Pasti, L., Vesce, S., Rossi, D., Rizzini, B.L., Pozzan, T., Volterra, A., 1998. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 391, 281–285.

Borst, J.G., Sakmann, B., 1996. Calcium influx and transmitter release in a fast CNS synapse. Nature 383, 431–434.

Browning, M.D., Dudek, E.M., 1992. Activators of protein kinase C increase the phosphorylation of the synapsins at sites phosphorylated by cAMP-dependent and Ca^{2+}/calmodulin-dependent protein kinase in the rat hippocampal slice. Synapse 10, 62–70.

Carson, M.J., Thomas, E.A., Danielson, P.E., Sutcliffe, J.G., 1996. The 5HT5A serotonin receptor is expressed predominantly by astrocytes in which it inhibits cAMP accumulation: a mechanism for neuronal suppression of reactive astrocytes. Glia 17, 317–326.

Cholewinski, A.J., Leslie, R.A., 1996. 5-Hydroxytryptamine-evoked [Ca^{2+}]i oscillations in rat C6 glioma cells. Neurochem. Int. 28, 583–589.

Dekker, L.V., De Graan, P.N., Gispen, W.H., 1991. Transmitter release: target of regulation by protein kinase C? Prog. Brain Res. 89, 209–233.

Ehrlich, B.E., Kaftan, E., Bezprozvannaya, S., Bezprozvanny, I., 1994. The pharmacology of intracellular Ca^{2+}-release channels. Trends Pharmacol. Sci. 15, 145–149.

Elliott, J.M., Newberry, N.R., Cholewinski, A.J., Bartrup, J.T., Briddon, S.J., Carey, J.E., Flanigan, T.P., Newton, R.A., Phipps, S.L., Reavley, A.C., et al., 1995. Characterization of the 5-hydroxytryptamine$_{2A}$ receptor-activated cascade in rat C6 glioma cells. Neuroscience 69, 1119–1131.

Fasolato, C., Innocenti, B., Pozzan, T., 1994. Receptor-activated Ca^{2+} influx: how many mechanisms for how many channels? Trends Pharmacol. Sci. 15, 77–83.

Fukao, M., Hattori, Y., Kanno, M., Sakuma, I., Kitabatake, A., 1997. Sources of Ca^{2+} in relation to generation of acetylcholine-induced endothelium-dependent hyperpolarization in rat mesenteric artery. Br. J. Pharmacol. 120, 1328–1334.

Furuichi, T., Mikoshiba, K., 1995. Inositol 1, 4, 5-trisphosphate receptor-mediated Ca^{2+} signaling in the brain. J. Neurochem. 64, 953–960.

Galione, A., 1992. Ca^{2+}-induced Ca^{2+} release and its modulation by cyclic ADP-ribose. Trends Pharmacol. Sci. 13, 304–306.

Galione, A., Lee, H.C., Busa, W.B., 1991. Ca^{2+}-induced Ca^{2+} release in sea urchin egg homogenates: modulation by cyclic ADP-ribose. Science 253, 1143–1146.

Glowinski, J., Marin, P., Tence, M., Stella, N., Giaume, C., Premont, J., 1994. Glial receptors and their intervention in astrocyto–astrocytic and astrocyto–neuronal interactions. Glia 11, 201–208.

Goodall, A.R., Turner, N.A., Walker, J.H., Ball, S.G., Vaughan, P.F., 1997. Activation of protein kinase C-alpha and translocation of the myristoylated alanine-rich C-kinase substrate correlate with phorbol ester-enhanced noradrenaline release from SH-SY5Y human neuroblastoma cells. J. Neurochem. 68, 392–401.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P., 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–203.

Imoto, K., Takemura, H., Kaneko, M., Ohshika, H., 1996. Lack of voltage-operated calcium channel in rat glioma C6 cells. Res. Commun. Mol. Pathol. Pharmacol. 93, 249–256.

Luckhoff, A., Clapham, D.E., 1992. Inositol 1,3,4,5-tetrakisphosphate activates an endothelial Ca^{2+}-permeable channel. Nature 355, 356–358.

Manor, D., Moran, N., Segal, M., 1994. Interactions among calcium compartments in C6 rat glioma cells: involvement of potassium channels. J. Physiol. 478, 251–263.

Meller, R., Harrison, P.J., Elliott, J.M., Sharp, T., 2002. In vitro evidence that 5-hydroxytryptamine increases efflux of glial glutamate via 5-HT$_{2A}$ receptor activation. J. Neurosci. Res. 67, 399–405.

Murphy, S., Pearce, B., 1987. Functional receptors for neurotransmitters on astroglial cells. Neuroscience 22, 381–394.

Murphy, N.P., McCormack, J.G., Ball, S.G., Vaughan, P.F., 1992. The effect of protein kinase C activation on muscarinic-M3- and K(+)-evoked release of [^{3}H]noradrenaline and increases in intracellular Ca^{2+} in human neuroblastoma SH-SY5Y cells. Biochem. J. 282, 645–650.

Parker, I., Ivorra, I., 1991. Caffeine inhibits inositol trisphosphate-mediated liberation of intracellular calcium in Xenopus oocytes. J. Physiol. 433, 229–240.

Parpura, V., Basarsky, T.A., Liu, F., Jeftinija, K., Jeftinija, S., Haydon, P.G., 1994. Glutamate-mediated astrocyte-neuron signalling. Nature 369, 744–747.

Randriamampita, C., Tsien, R.Y., 1993. Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. Nature 364, 809–814.

Reiser, G., Donie, F., Binmoller, F.J., 1989. Serotonin regulates cytosolic Ca^{2+} activity and membrane potential in a neuronal and in a glial cell line via 5-HT$_3$ and 5-HT$_2$ receptors by different mechanisms. J. Cell Sci. 93, 545–555.

Sabala, P., Amler, E., Baranska, J., 1997. Intracellular Ca^{2+} signals induced by ATP and thapsigargin in glioma C6 cells. Calcium pools sensitive to inositol 1,4,5-trisphosphate and thapsigargin. Neurochem. Int. 31, 55–64.

Sabria, J., Pastor, C., Clos, M.V., Garcia, A., Badia, A., 1995. Involvement of different types of voltage-sensitive calcium channels in the presynaptic regulation of noradrenaline release in rat brain cortex and hippocampus. J. Neurochem. 64, 2567–2571.

Scotini, E., Carpenedo, F., Fassina, G., 1983. New derivatives of methylxanthines: effect of thiocaffeine thiotheophylline and 8-phenyltheophylline on lipolysis and on phosphodiesterase activities. Pharmacol. Res. Commun. 15, 131–143.

Segovia, J., Lawless, G.M., Tillakaratne, N.J., Brenner, M., Tobin, A.J., 1994. Cyclic AMP decreases the expression of a neuronal marker (GAD67) and increases the expression of an astroglial marker (GFAP) in C6 cells. J. Neurochem. 63, 1218–1225.

Sheppard, C.A., Simpson, P.B., Sharp, A.H., Nucifora, F.C., Ross, C.A., Lange, G.D., Russell, J.T., 1997. Comparison of type 2 inositol 1,4,5-trisphosphate receptor distribution and subcellular Ca^{2+} release sites that support Ca^{2+} waves in cultured astrocytes. J. Neurochem. 68, 2317–2327.

Sitsapesan, R., McGarry, S.J., Williams, A.J., 1995. Cyclic ADP-ribose, the ryanodine receptor and Ca^{2+} release. Trends Pharmacol. Sci. 16, 386–391.

Sudhof, T.C., 1995. The synaptic vesicle cycle: a cascade of protein–protein interactions. Nature 375, 645–653.

Tanaka, Y., Tashjian Jr., A.H. 1993. Functional identification and quantitation of three intracellular calcium pools in GH4C1 cells: evidence that the caffeine-responsive pool is coupled to a thapsigargin-resistant, ATP-dependent process. Biochemistry 32, 12062–12073.

Thastrup, O., Cullen, P.J., Drobak, B.K., Hanley, M.R., Dawson, A.P., 1990. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. Proc. Natl. Acad. Sci. U. S. A. 87, 2466–2470.

Ullmer, C., Boddeke, H.G., Schmuck, K., Lubbert, H., 1996. 5-HT_{2B} receptor-mediated calcium release from ryanodine-sensitive intracellular stores in human pulmonary artery endothelial cells. Br. J. Pharmacol. 117, 1081–1088.

Venance, L., Stella, N., Glowinski, J., Giaume, C., 1997. Mechanism involved in initiation and propagation of receptor-induced intercellular calcium signaling in cultured rat astrocytes. J. Neurosci. 17, 1981–1992.

Verma, A., Hirsch, D.J., Snyder, S.H., 1992. Calcium pools mobilized by calcium or inositol 1,4,5-trisphosphate are differentially localized in rat heart and brain. Mol. Biol. Cell 3, 621–631.

Williams, M.E., Brust, P.F., Feldman, D.H., Patthi, S., Simerson, S., Maroufi, A., McCue, A.F., Velicelebi, G., Ellis, S.B., Harpold, M.M., 1992. Structure and functional expression of an omega-conotoxin-sensitive human N-type calcium channel. Science 257, 389–395.

Yagodin, S., Holtzclaw, L.A., Russell, J.T., 1995. Subcellular calcium oscillators and calcium influx support agonist-induced calcium waves in cultured astrocytes. Mol. Cell. Biochem. 149–150, 137–144.

Zhang, J.F., Randall, A.D., Ellinor, P.T., Horne, W.A., Sather, W.A., Tanabe, T., Schwarz, T.L., Tsien, R.W., 1993. Distinctive pharmacology and kinetics of cloned neuronal Ca^{2+} channels and their possible counterparts in mammalian CNS neurons. Neuropharmacology 32, 1075–1088.

Zhang, L., Oz, M., Weight, F.F., 1995. Potentiation of 5-HT_3 receptor-mediated responses by protein kinase C activation. NeuroReport 6, 1464–1468.

Zimmermann, H., 1990. Neurotransmitter release. FEBS Lett. 268, 394–399.