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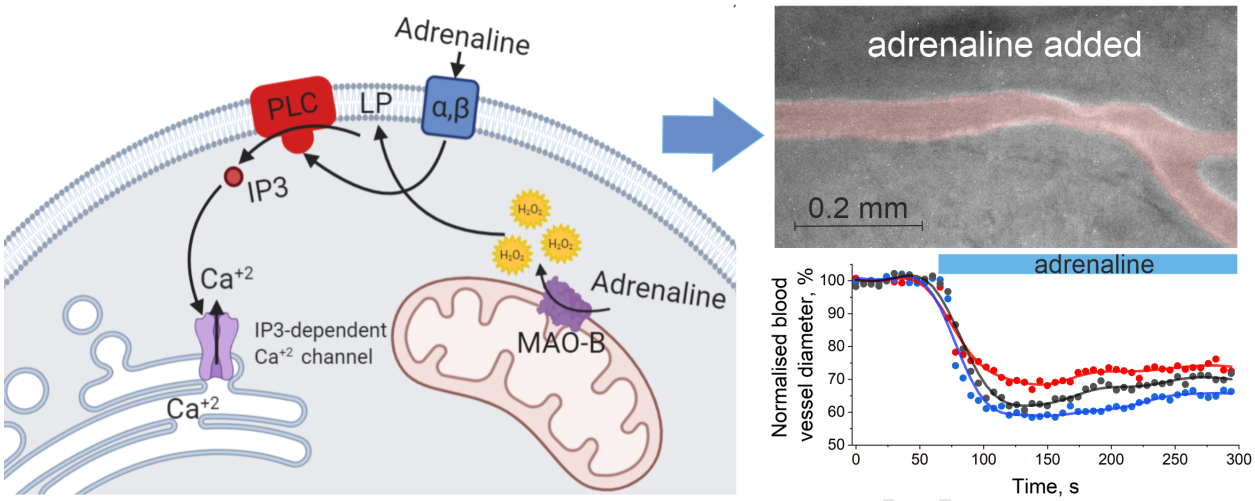

Adrenaline induces calcium signal in astrocytes and vasoconstriction via activation of monoamine oxidase

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Abstract

Adrenaline or epinephrine is a hormone playing an important role in physiology. It is produced de-novo in the brain in very small amounts compared to other catecholamines, including noradrenaline. Although the effects of adrenaline on neurons have been extensively studied, much less is known about the action of this hormone on astrocytes. Here, we studied the effects of adrenaline on astrocytes in primary co-culture of neurons and astrocytes. Application of adrenaline induced calcium signal in both neurons and astrocytes, but only in neurons this effect was dependent on α- and β-receptor antagonists. The effects of adrenaline on astrocytes were less dependent on adrenoreceptors: the antagonist carvedilol had only moderate effect on the calcium signal and the agonist of adrenoreceptors methoxamine induced a signal only in small proportion of the cells. We found that adrenaline in astrocytes activates phospholipase C and subsequent release of calcium from the endoplasmic reticulum. Calcium signal in astrocytes is initiated by the metabolism of adrenaline by the monoamine oxidase (MAO), which activates reactive oxygen species production and induces lipid peroxidation. Inhibitor of MAO selegiline inhibited both adrenaline-induced calcium signal in astrocytes and the vasoconstriction that indicates an important role for monoamine oxidase in adrenaline-induced signalling and function.

Keywords: adrenaline, neurons, astrocytes, monoamine oxidase, calcium, vasoconstriction

Abbreviations: MAO, monoamine oxidase; L-DOPA, l-3,4-dihydroxyphenylalanine; COMT, catechol-methyltransferase; CNS, central nervous system; ROS, reactive oxygen species; HBSS, HEPES-buffered solution; Het, dihydroethidium; ER, endoplasmic reticulum; EGTA, ethyleneglycoltetraacetic acid; IP3, inositol triphosphate;

1. Introduction

Adrenaline (epinephrine) is the first identified hormone produced in adrenal medulla. It plays a number of important functions in the body. Adrenaline is impermeable for the brain blood barrier and in the brain, it is synthetized *de novo*. Adrenaline is one of the catecholamines and the metabolic pathway by which catecholamines are synthesized in

the brain is well established: tyrosine penetrates into the cytoplasm of neurons where it is converted to L-3,4-dihydroxyphenylalanine (L-DOPA) then to dopamine, about half of which is actively transported into storage vesicles where it can be converted into noradrenaline by dopamine-ß-hydroxylase [1]. The final step in L-adrenaline biosynthesis is the transfer of a methyl group from S-adenosyl-L-methionine to the amino group of noradrenaline. The enzyme catalysing this methylation step (phenylethanolamine N-methyltransferase) is the only one unique to adrenaline synthesis.

Catecholamines are degraded by 3-*O*-methylation in catechol-methyltransferase (COMT) or/and oxidative deamination by monoamine oxidase (MAO), a flavoprotein located on the outer membrane of mitochondria. In the brain, one of the isoforms of MAO-A is predominantly neuronal, while MAO-B is mostly located in astrocytes [2]. Both MAO-A and MAO-B catalyse the decomposition of adrenaline in brain tissue at maximal rate [3].

In contrast to dopamine and noradrenaline, adrenaline has neither been mapped in detail nor studied physiologically and pharmacologically with widely varied techniques and regarding numerous physiologic functions. This is mainly due to its low concentration compared to those of the other two catecholamines. Immunohistochemical methods could not discriminate adrenaline and noradrenaline well enough to permit tracking of the relatively sparse adrenaline receptor-containing neurons. Quantitative fluorometric methods distinguished adrenaline receptor from noradrenaline [4]. Central nervous system (CNS) contains very few noradrenergic and even fewer adrenergic neurons compared to glutamatergic and GABAergic neurons. Their cell bodies are in the brain stem within medulla and pons. However, it became more evident that catecholamines, and adrenaline, play important role in astrocytic function. Thus, adrenoceptors (both groups – α- and β-receptors) influence a multitude of parameters in astrocytes, ranging from morphological plasticity through energy metabolism, transport across the cell membrane, gap junction permeability and membrane potential to immunity and scar formation [5].

The role of adrenaline in vasodilation in brain is well established and it mostly regulated by adrenoreceptor mechanism [6,7]. Calcium signal in astrocytes shown to be a trigger for regulation of the regulation of blood vessel diameter in the [8,9] and we suggested that adrenaline can also have effect on $[Ca^{2+}]_c$ of astrocytes in the mechanism of adrenaline induced vasoconstriction.

Previously we found that another catecholamine – dopamine can induce receptor-independent calcium signal in astrocytes via the activation of MAO and the production of ROS followed by activation of phospholipase C [10]. Here we studied the effect of

adrenaline on the calcium signal and the reactive oxygen species (ROS) production in astrocytes and how it regulates blood vessel diameters. We found that adrenaline-induced calcium signal in neurons and astrocytes is completely dependent on receptor antagonist in neurons and only partially in astrocytes. In astrocytes, calcium signal was dependent on the presence of inhibitor of MAO selegiline or an antioxidant. Inhibition of MAO in brain slices significantly reduced the effect of adrenaline on vasoconstriction.

2 Materials and methods

2.1 Cell culture

Primary cell co-cultures of cortical neurons and astrocytes were prepared as described in detail previously [11] with modifications, from the cerebral cortices of Sprague-Dawley P3 rat pups (UCL breeding colony). Experimental procedures were performed in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. After trypsinization of the tissue, the cells were plated on poly-d-lysine-coated coverslips and used on DIV 12-15.

2.2 Live cell imaging

2.2.1 Measurements of $[Ca^{2+}]_c$.

Primary cortical co-culture of neurons and astrocytes were loaded for 30 min with 5 µM fura-2 AM and 0.005% Pluronic in a HEPES-buffered solution (HBSS). $[Ca^{2+}]_c$ was measured in single neurons and astrocytes using excitation light provided by a xenon arc lamp, the beam passing through a monochromator at 340 and 380 nm with bandwidth of 10 nm (Cairn Research, Kent, UK). Emitted fluorescent light passed through a 515-nm long pass filter to a cooled CCD camera (Retiga; QImaging) and was digitized to 12-bit resolution. Ca^{2+}imaging data was acquired at a frame interval of 10 s and analyzed using software from Andor (Belfast, UK). Traces were computed and plotted as fura-2 ratio of excitation acquired at 340 and 380 nm, both with emission at >515 nm. Fura-2 ratio was not calibrated due to inaccuracies arising from different calibration methods.

2.2.2 ROS assessments

Fluorescence measurements were obtained on an epifluorescence inverted microscope equipped with a 20× fluorite objective. For dihydroethidium (HEt) measurements oxidized form of the dye were measured: excitation at 540 nm and emission recorded above 560 nm were used to quantify the oxidized form (ethidium). All data reported in this study were obtained from at least five coverslips and 2-3 different cell and sample preparations. For measurement of cytosolic ROS production, HEt (2 μM) was present in the solution during the experiment. No preincubation ("loading") was used for HEt to limit the intracellular accumulation of oxidized products.

2.2.3 Lipid peroxidation

The rate of lipid peroxidation was measured using confocal microscopy. Confocal images were obtained with a Zeiss 710 LSM with an integrated META detection system. To assess lipid peroxidation C11-BODIPY (581/591, 2 μM, Molecular probes) was excited using the 488 and 543 nm laser line and fluorescence measured using a band-pass filter from 505 to 550 nm and 560 nm long-pass filter (40x objective). Illumination intensity was kept to a minimum (at 0.1-0.2% of laser output) to avoid phototoxicity and the pinhole set to give an optical slice of ~2 μm. Addition of a bright field image allowed separation between neurons and glia, that are visibly different and are situated on different focal planes. Data were acquired and analysed using ZEN2009 software.

2.3 Vessel diameter measurements in brain slices

Brains from the 6-8-week-old male Wistar rats were dissected and prepared for the measurements. The studies were conducted in cortical slices which contained vessels not damaged by the incision. For isolation and preparation of brain slices all animal work was approved by Institutional ethical committee in compliance of Russian Federation legislation.

All slices were divided into two groups. In the first group the measurement of the fluorescence level and the blood vessels diameter were conducted immediately after loading and washing. In the second group, after washing, the slices were kept in HBSS with 20 μM selegiline for 8 minutes.

The imaging setup for brain slice imaging included a Mitutoyo M Plan APO 5X planar apochromatic lens (Thorlabs, Inc. USA) and a LB1945-A biconvex lens with a focal length of 200 mm (Thorlabs, Inc. USA). The excitation channel included 455 nm M455F1 Fiber-Coupled LED (Thorlabs, Inc. USA). The radiation from the source was directed at the sample through MD480 dichroic filter (Thorlabs, Inc. USA) and MF445-45 bandpass filter (Thorlabs, Inc. USA).

The images were recorded by 340M-USB fast frame rate monochrome scientific CCD camera (Thorlabs, Inc. USA) with the FGL495 (Thorlabs, Inc. USA) glass filter placed before detector. The filter blocked wavelengths shorter than 495 nm. The imaging linear field of view for the system was 1.04 x 0.78 mm (height x width) at working distance 34 mm.

During the measurements the slices were fixed on a glass slide contained 150 µl HBSS. After adjusting focus the images of the blood vessels were registered for 1 minute. Then 2 µM adrenaline was applied. The imaging of the blood vessels has been conducted for 4 minutes after adding adrenaline. The image processing was done in ImageJ software using Vessel Diameter plugin (version 1.0)[12].

2.4 Data analysis and statistics

Data and statistical analysis were performed using OriginPro (OriginLab Corp., Northampton, USA) and GraphPad Prism (GraphPad software, Inc. San Diego, USA) software. Data are presented as means expressed ± standard error of the mean (SEM).

3 Results

3.1 Adrenaline induce $[Ca^{2+}]_c$ elevation in neurons and astrocytes via different mechanisms

Application of the adrenaline (10 nM-1 µM) dose-dependently induced peak-like calcium signal in neurons (Fig.1a, g, h; in 58±9% of cells; n=321) and astrocytes (Fig. 1c, g, h; in 94±5% of cells; n=367). Carvedilol, a nonselective beta blocker/alpha-1 blocker (20 µM) inhibited adrenaline-induced calcium signal in most of neurons (in 146/154 neurons; Fig. 1b, h) and reduced the number of the astrocytes with elevated $[Ca^{2+}]_c$ but not the amplitude of the signal (Fig. 1d, h; in 177/210 astrocytes with Ca^{2+}signal). Thus,

calcium effect of adrenaline in neurons is receptor dependent while in astrocytes effect of adrenalin is only partially dependent on adrenoreceptors.

In order to prove receptor-independent effects of adrenaline on astrocytes we used agonist of the adrenoreceptors methoxamine (500 µM). Application of methoxamine induced calcium signal in small percentage of astrocytes (Fig. 1e, h; in 14/142 cells). Importantly, preincubation of the primary astrocytes with 20 µM carvedilol completely blocked effect of adrenoreceptor agonist on $[Ca^{2+}]_c$ of astrocytes (Fig. 2f, h; n=89). Thus, only small proportion of the astrocytes activates adrenaline-induced calcium signal through receptor-dependent mechanism.

3.2 Adrenaline induce release of Ca^{2+} from endoplasmic reticulum (ER) via activation of phospholipase C

In order to unravel the source of Ca^{2+} and mechanism of adrenaline-induced calcium signal in astrocytes we used Ca^{2+}-free medium (plus 0.5 mM ethyleneglycoltetraacetic acid (EGTA)) that exclude extracellular calcium influx. Addition of the adrenaline (500 nM) in these conditions after a short delay (1-2 min) induced elevation of $[Ca^{2+}]_c$ of cortical astrocytes (Fig. 2a; n=102). Inhibition of the SERCA with 1 µM Thapsigargin irreversibly release Ca^{2+} from ER to cytosol. Addition of the 100 µM ATP induced no further increase that confirmed empty Ca^{2+}-pool of ER that completely block adrenaline induced calcium signal (Fig. 2b; n=114). This confirmed that the astrocytic adrenaline induced Ca^{2+} signals are dependent on intracellular stores. One of the most common triggers of release of calcium from intracellular stores in astrocytes is activation of phospholipase C. Application of the inhibitor of phospholipase C – 20 µM U73122 completely prevented the astrocytic adrenaline-induced Ca^{2+} signal (Fig. 2c; n=148). Taken together, these experiments suggest that in astrocytes adrenaline activates phospholipase C, inducing inositol triphosphate (IP3)-dependent Ca^{2+} release from ER, resulting in a rise of $[Ca^{2+}]_c$.

3.3 Adrenalin-induced calcium signal in astrocytes dependent on MAO and antioxidants

Previously we have shown that other catecholamines – tyrosine and dopamine can induce receptor-independent calcium signal and that this signal can be suppressed by inhibition of monoaminoxidase [10,13]. Here we found that pre-incubation of the co-culture of neurons and astrocytes with 20 µM selegiline (20 min; in this concentration selegiline

non-selectively inhibits both MAO-A and MAO-B) reduces the amplitude of adrenaline-induced calcium signal in neurons (Fig. 3a, e; n=198; from 0.89±11 to 0.62±0.06 fura-2 ratio; $p<0.05$) and blocks the $[Ca^{2+}]_c$ rise in the majority of astrocytes (Fig. 3b; in 312/336 astrocytes). It should be noted that in astrocytes with calcium response the amplitude of the $[Ca^{2+}]_c$ elevation was significantly lower (0.11±0.01 compared to the fura-2 ratio in control, 0.7±0.1; $p<0.001$; Fig. 3e).

MAO produces aldehydes and hydrogen peroxide. H_2O_2 can both- induce calcium signal in astrocytes and increase receptor-activated signal [14]. Incubation of the primary co-culture of cortical neurons and astrocytes with water-soluble analogue of vitamin E – Trolox (20 min, 100 µM) reduced the amplitude of adrenaline-induced $[Ca^{2+}]_c$ rise in neurons (from 0.89±11 to 0.61±0.07 fura-2 ratio; n=68, $p<0.05$; Fig. 3b, e) and reduced the overall number of cells with response and the amplitude of calcium signal in astrocytes (Fig. 3c, e; n=46/121 astrocytes; from 0.7±0.1 fura-2 ratio to 0.32±03; $p<0.001$). Thus, adrenaline-induced calcium signal in astrocytes and partially in neurons is dependent on the activity of MAO.

3.4 Adrenaline induces ROS production and lipid peroxidation in astrocytes

Application of 10 nM adrenaline induced immediate profound elevation in the rate of ROS production (to 156±11% of the basal rate; n=220; $p<0.001$; Fig. 4a, c). Increase of the concentration to 500 nM had a higher effect on the rate of Het fluorescence (to 205±11% of basal rate; $p<0.001$; Fig.4b, c). Importantly, 10 min pre-incubation of the cells with 20 µM selegiline had no effect on the basal rate of Het fluorescence, but almost completely reduced the effect of both – 10 nM and 500 nM adrenaline (n=72 and n=111, respectively; Fig. 4b, c). Thus, adrenaline activates ROS production even in small concentrations.

Previously we showed that activation of phospholipase C in astrocytes can be associated with lipid peroxidation [10,14]. Application of 10 nM or 500 nM to primary co-culture induced a dose-dependent increase in the rate of lipid peroxidation of astrocytes (to 154±10.7% and 238±15 of basal rate, respectively; (Fig. 4e-g) and in neurons (to 263±13% of basal rate; Fig. 4g-j).

Pre-incubation of primary cortical co-culture with 20 µM selegiline significantly reduced the basal rate of lipid peroxidation in both – neurons and astrocytes (Fig. 4e, f, h, j). Importantly, inhibition of MAO in neurons completely prevented adrenaline-induced

elevation in the rate of lipid peroxidation (Fig. 4e, f, h, j). Thus, adrenaline-induced lipid peroxidation acts via activation of monoamine oxidase.

3.5 Adrenaline induced MAO-dependent vasoconstriction in acute cortical brain slices

In order to identify if adrenaline-induced calcium signal in astrocytes has a physiological relevance, we studied effect of adrenaline on vascular diameter in cortical slices from adult (6-8 weeks) rats. Application of 1 μM adrenaline, expectably induced reduction in blood vessel diameters (N=3; n=9 slices; Fig. 5a). Incubation of the slices with 20 μM selegiline (8-10 min) significantly reduced the effect of adrenaline on vasoconstriction (N=3 rats; n=7 slices; Fig. 5b, c). Thus, metabolism of adrenaline in MAO has an effect of astrocytic calcium signal and vasoconstriction in cortex.

4 Discussion

Here we demonstrated that adrenaline induces calcium signal in cortical neurons and astrocytes. While in neurons it stimulates Ca^{2+} signal via activation of receptors, in astrocytes the signal in only several cells is dependent on receptor stimulation. This could be confirmed by experiments with adrenoreceptor agonists and antagonists (Fig. 1d, e, f). Although expression and functional activity of the adrenoreceptors in astrocytes was reported [15,16] our data confirm only moderate activation of astrocytic calcium signal by methoxamine [17]. Adrenaline is shown to be present in brain in much lower concentrations, compared to noradrenaline [4] and in a small number of adrenergic neurons [18]. However, in our experiments adrenaline effectively induced calcium signal in small concentrations, and importantly, most of this activation was induced in receptor-independent way, confirming that adrenaline can play important physiological role in very small concentrations.

The inhibitor of MAO, selegiline successfully inhibited adrenaline-induced ROS production (Fig. 4c). These data cannot exclude that adrenaline first activates the calcium signal, which in turn stimulates calcium-dependent activation of NADPH oxidase that produces these ROS [19,20]. But ability of selegiline to inhibit not only adrenaline-induced lipid peroxidation, but also to significantly reduce basal rate of oxidation of lipids strongly suggests the importance of monoamine oxidase in lipid redox homeostasis but it also suggests that adrenaline-induced ROS are initially generated in MAO.

ROS are now widely accepted to be not only a trigger for various pathologies, but also to be an essential player in the mechanism of cell signalling [21,22]. Activation of MAO can stimulate calcium uptake directly via hydrogen peroxide production [23,24], but the absence of adrenaline-induced calcium signal in Ca^{2+}-free medium in our experiments can exclude an effect of hydrogen peroxide on the plasmalemmal ion channels (Fig. 3a). Considering the presented results, the pathway through which adrenaline-induced production of hydrogen peroxide leads to lipid peroxidation via activation of phospholipase C and IP3-dependent calcium signal in astrocytes seems more plausible. The same pathway we showed previously for activation of calcium signal in astrocytes by dopamine [10]. Importantly, although adrenaline induced lipid peroxidation in neurons, the effect of selegiline on the calcium signal in these cells was much smaller that confirmed for receptor-activated mechanism of stimulation of neurons by adrenaline.

More pronounced effect of inhibitor of MAO in astrocytes compare to neurons could be explained by higher expression of adrenoreceptors in neurons and, possibly, by different level of MAO-A and MAO-B expression in these cells. The level of these enzymes in the cells depending on several factors and varies on the brain region, stage of development and pathology [25–27].

Inhibitors of monoamine oxidase are widely used for inhibition of catecholamine degradation and maintenance of the active monoamine levels with expectations for increased or prolonged action of these compounds [28]. However, inhibition of MAO by selegiline in our experiments significantly reduced the effect of adrenaline on the vasoconstriction that suggests other mechanism takes place (Fig. 5). Considering the effect of calcium signal on vasoconstriction [8,29], suppression of the calcium signal in astrocytes by selegiline could be the most feasible mechanism for inhibition of vasoconstriction. In agreement to our findings, it has been reported that MAO-produced H_2O_2 enhances the effect of serotonin on rat basilar artery [30].

Thus, we propose here the importance of monoamine oxidase and redox signalling in the mechanism of adrenaline-induced signal in astrocytes and cerebral vasoconstriction.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] R. Kvetnansky, E.L. Sabban, M. Palkovits, Catecholaminergic systems in stress: structural and molecular genetic approaches., Physiol. Rev. 89 (2009) 535–606. https://doi.org/10.1152/physrev.00042.2006.

[2] P. Riederer, C. Konradi, V. Schay, E. Kienzl, G. Birkmayer, W. Danielczyk, E. Sofic, M.B. Youdim, Localization of MAO-A and MAO-B in human brain: a step in understanding the therapeutic action of L-deprenyl., Adv. Neurol. 45 (1987) 111–118.

[3] G. Oanca, J. Stare, J. Mavri, How fast monoamine oxidases decompose adrenaline? Kinetics of isoenzymes A and B evaluated by empirical valence bond simulation., Proteins. 85 (2017) 2170–2178. https://doi.org/10.1002/prot.25374.

[4] S.-F. Li, H.-L. Wu, A.-L. Xia, S.-H. Zhu, J.-F. Nie, Y.-J. Yu, R.-Q. Yu, Quantitative analysis of epinephrine in human plasma samples using kinetic fluorometric method combined with second-order calibration., Anal. Sci. 25 (2009) 1231–1236. https://doi.org/10.2116/analsci.25.1231.

[5] L. Hertz, Y. Chen, M.E. Gibbs, P. Zang, L. Peng, Astrocytic adrenoceptors: a major drug target in neurological and psychiatric disorders?, Curr. Drug Targets. CNS Neurol. Disord. 3 (2004) 239–267. https://doi.org/10.2174/1568007043337535.

[6] L. Cao, M.H. Weil, S. Sun, W. Tang, Vasopressor agents for cardiopulmonary resuscitation., J. Cardiovasc. Pharmacol. Ther. 8 (2003) 115–121. https://doi.org/10.1177/107424840300800204.

[7] Z. Chen, K.P. Minneman, Recent progress in alpha1-adrenergic receptor research., Acta Pharmacol. Sin. 26 (2005) 1281–1287. https://doi.org/10.1111/j.1745-7254.2005.00224.x.

[8] S.J. Mulligan, B.A. MacVicar, Calcium transients in astrocyte endfeet cause cerebrovascular constrictions., Nature. 431 (2004) 195–199. https://doi.org/10.1038/nature02827.

[9] A. Beiersdorfer, A. Scheller, F. Kirchhoff, C. Lohr, Panglial gap junctions between astrocytes and olfactory ensheathing cells mediate transmission of Ca(2+) transients and neurovascular coupling., Glia. 67 (2019) 1385–1400. https://doi.org/10.1002/glia.23613.

[10] A. Vaarmann, S. Gandhi, A.Y. Abramov, Dopamine induces Ca2+ signaling in astrocytes through reactive oxygen species generated by monoamine oxidase., J. Biol. Chem. 285 (2010) 25018–25023. https://doi.org/10.1074/jbc.M110.111450.

[11] P.R. Angelova, K.Z. Iversen, A.G. Teschemacher, S. Kasparov, A. V Gourine, A.Y. Abramov, Signal transduction in astrocytes: Localization and release of inorganic polyphosphate., Glia. 66 (2018) 2126–2136. https://doi.org/10.1002/glia.23466.

[12] M.J.M. Fischer, S. Uchida, K. Messlinger, Measurement of meningeal blood vessel diameter in vivo with a plug-in for ImageJ., Microvasc. Res. 80 (2010) 258–266. https://doi.org/10.1016/j.mvr.2010.04.004.

[13] S. Gandhi, A. Vaarmann, Z. Yao, M.R. Duchen, N.W. Wood, A.Y. Abramov, Dopamine induced neurodegeneration in a PINK1 model of Parkinson's disease., PLoS One. 7 (2012) e37564. https://doi.org/10.1371/journal.pone.0037564.

[14] A.-M. Domijan, S. Kovac, A.Y. Abramov, Lipid peroxidation is essential for phospholipase C activity and the inositol-trisphosphate-related Ca(2)(+) signal., J. Cell Sci. 127 (2014) 21–26. https://doi.org/10.1242/jcs.138370.

[15] P. Marin, J.C. Delumeau, J. Cordier, J. Glowinski, J. Premont, Both Astrocytes and Neurons Contribute to the Potentiation Mediated by alpha1-Adrenoceptors of the beta-Adrenergic-Stimulated Cyclic AMP Production in Brain., Eur. J. Neurosci. 2 (1990) 1110–1117. https://doi.org/10.1111/j.1460-9568.1990.tb00022.x.

[16] C. Giaume, P. Marin, J. Cordier, J. Glowinski, J. Premont, Adrenergic regulation of intercellular communications between cultured striatal astrocytes from the mouse., Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 5577–5581. https://doi.org/10.1073/pnas.88.13.5577.

[17] L. Venance, J. Prémont, J. Glowinski, C. Giaume, Gap junctional communication and pharmacological heterogeneity in astrocytes cultured from the rat striatum., J. Physiol. 510 (Pt 2) (1998) 429–440. https://doi.org/10.1111/j.1469-7793.1998.429bk.x.

[18] I. Armando, M. Jezova, C. Bregonzio, G. Baiardi, J.M. Saavedra, Angiotensin II AT1 and AT2 receptor types regulate basal and stress-induced adrenomedullary catecholamine production through transcriptional regulation of tyrosine hydroxylase.,

Ann. N. Y. Acad. Sci. 1018 (2004) 302–309. https://doi.org/10.1196/annals.1296.036.

[19] A.Y. Abramov, J. Jacobson, F. Wientjes, J. Hothersall, L. Canevari, M.R. Duchen, Expression and modulation of an NADPH oxidase in mammalian astrocytes., J. Neurosci. 25 (2005) 9176–9184. https://doi.org/10.1523/JNEUROSCI.1632-05.2005.

[20] S. Kovac, A.-M. Domijan, M.C. Walker, A.Y. Abramov, Seizure activity results in calcium- and mitochondria-independent ROS production via NADPH and xanthine oxidase activation., Cell Death Dis. 5 (2014) e1442. https://doi.org/10.1038/cddis.2014.390.

[21] P.R. Angelova, A.Y. Abramov, Functional role of mitochondrial reactive oxygen species in physiology., Free Radic. Biol. Med. 100 (2016) 81–85. https://doi.org/10.1016/j.freeradbiomed.2016.06.005.

[22] P.R. Angelova, V. Kasymov, I. Christie, S. Sheikhbahaei, E. Turovsky, N. Marina, A. Korsak, J. Zwicker, A.G. Teschemacher, G.L. Ackland, G.D. Funk, S. Kasparov, A.Y. Abramov, A. V Gourine, Functional Oxygen Sensitivity of Astrocytes., J. Neurosci. 35 (2015) 10460–10473. https://doi.org/10.1523/JNEUROSCI.0045-15.2015.

[23] P. Angelova, W. Muller, Oxidative modulation of the transient potassium current IA by intracellular arachidonic acid in rat CA1 pyramidal neurons., Eur. J. Neurosci. 23 (2006) 2375–2384. https://doi.org/10.1111/j.1460-9568.2006.04767.x.

[24] G. Sandri, E. Panfili, L. Ernster, Hydrogen peroxide production by monoamine oxidase in isolated rat-brain mitochondria: its effect on glutathione levels and Ca2+ efflux., Biochim. Biophys. Acta. 1035 (1990) 300–305. https://doi.org/10.1016/0304-4165(90)90092-b.

[25] J.M. Luque, S.W. Kwan, C.W. Abell, M. Da Prada, J.G. Richards, Cellular expression of mRNAs encoding monoamine oxidases A and B in the rat central nervous system., J. Comp. Neurol. 363 (1995) 665–680. https://doi.org/10.1002/cne.903630410.

[26] J. Tong, G. Rathitharan, J.H. Meyer, Y. Furukawa, L.-C. Ang, I. Boileau, M. Guttman, O. Hornykiewicz, S.J. Kish, Brain monoamine oxidase B and A in human parkinsonian dopamine deficiency disorders., Brain. 140 (2017) 2460–2474. https://doi.org/10.1093/brain/awx172.

[27] A. Nicotra, F. Pierucci, H. Parvez, O. Senatori, Monoamine oxidase expression during development and aging., Neurotoxicology. 25 (2004) 155–165. https://doi.org/10.1016/S0161-813X(03)00095-0.

[28] G.B. Baker, R.T. Coutts, K.F. McKenna, R.L. Sherry-McKenna, Insights into the mechanisms of action of the MAO inhibitors phenelzine and tranylcypromine: a review., J. Psychiatry Neurosci. 17 (1992) 206–214.

[29] G.R.J. Gordon, C. Howarth, B.A. MacVicar, Bidirectional Control of Blood Flow by Astrocytes: A Role for Tissue Oxygen and Other Metabolic Factors., Adv. Exp. Med. Biol. 903 (2016) 209–219. https://doi.org/10.1007/978-1-4899-7678-9_15.

[30] C.C.W. Poon, S.W. Seto, A.L.S. Au, Q. Zhang, R.W.S. Li, W.Y.W. Lee, G.P.H. Leung, S.K. Kong, J.H.K. Yeung, S.M. Ngai, H.P. Ho, S.M.Y. Lee, S.W. Chan, Y.W. Kwan, Mitochondrial monoamine oxidase-A-mediated hydrogen peroxide generation enhances 5-hydroxytryptamine-induced contraction of rat basilar artery., Br. J. Pharmacol. 161 (2010) 1086–1098. https://doi.org/10.1111/j.1476-5381.2010.00941.x.

Figure legends

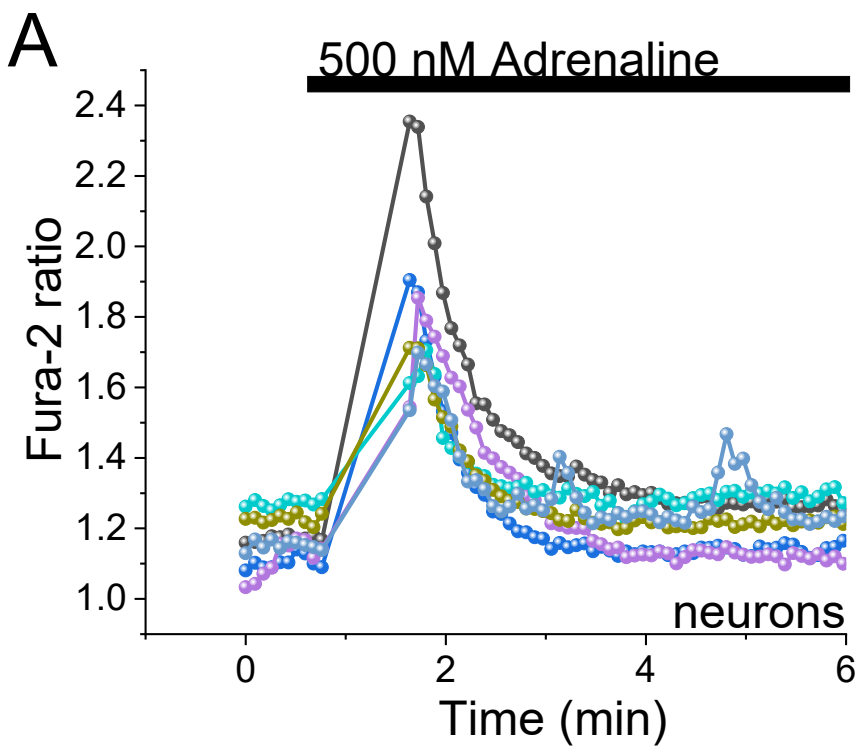
Fig. 1. Adrenaline induces elevation of $[Ca^{2+}]_c$ of astrocytes and neurons. Adrenaline induces changes in $[Ca^{2+}]_c$ of cortical neurons (a) and astrocytes (c). Antagonist of adrenoreceptors carvedilol (20 µM) completely prevent adrenaline-induced calcium signal in neurons (b) but not in astrocytes (d). Agonist of adrenoreceptors methoxamine (500 µM) induced $[Ca^{2+}]_c$ elevation in astrocytes (e) which can be completely prevented by 20 µM carvedilol (f). (g) Dose-dependent characteristics of the values of fura-2 ratio increase in astrocytes and neurons in response to different concentrations of adrenaline are represented in panel. (h) Percentage of cells with calcium response; 100 µM of ATP or 10 µM glutamate were added in some experiments for astrocytic or neuronal identification.

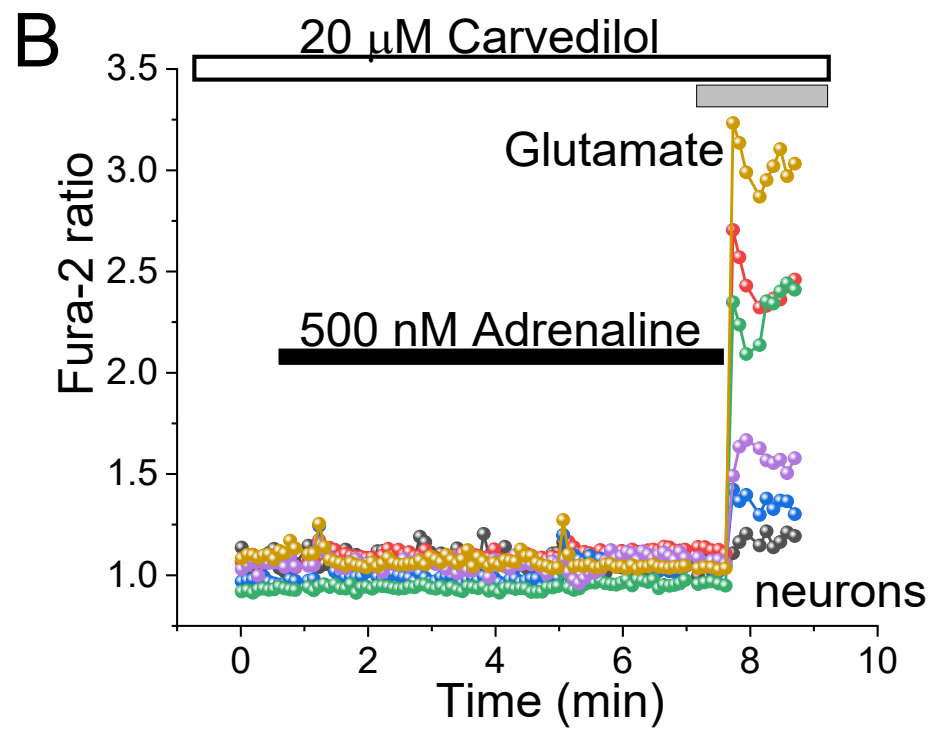
Fig. 2. $[Ca^{2+}]_c$ responses to adrenaline in astrocytes are dependent on intracellular Ca^{2+} stores. (a) The removal of external Ca^{2+} (Ca^{2+} free media with 0.5 mM EGTA) delays the adrenaline (500 nM)-induced Ca^{2+} responses in astrocytes. (b) Depletion of intracellular calcium pool with inhibitor of ER Ca^{2+} pump, thapsigargin (0.5 µM) abolishes the adrenaline-induced calcium signal in astrocytes. Astrocytic changes in $[Ca^{2+}]_c$ in response to adrenaline was dependent on presence of inhibitor of phospholipase C U73122 (10 µM).

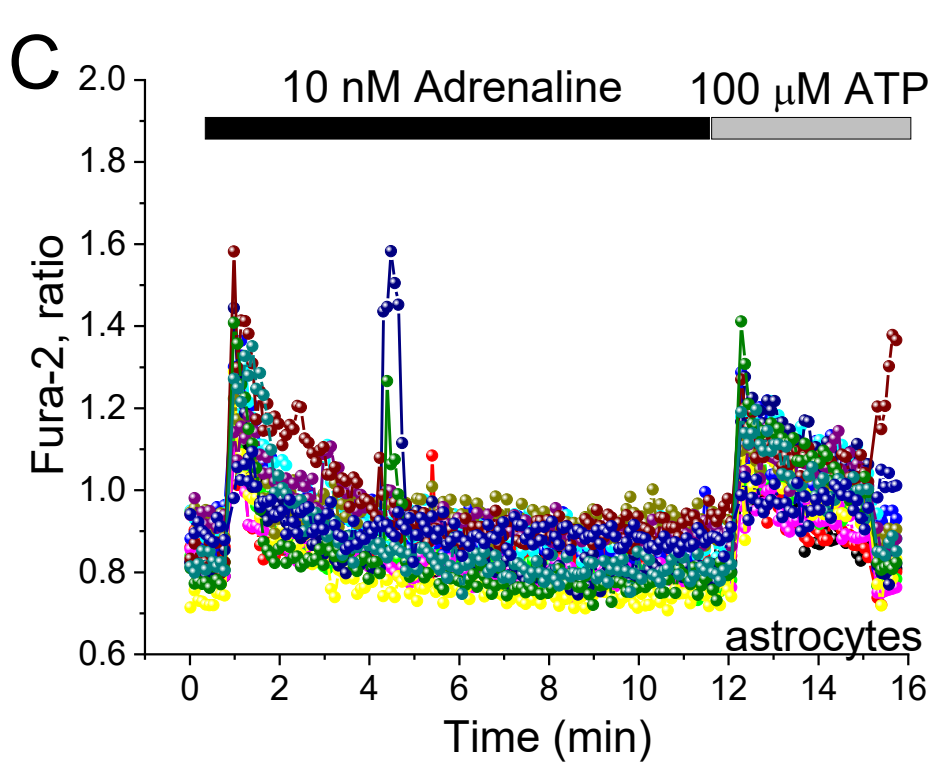
Fig. 3. Adrenaline-induced Ca^{2+} signal in astrocytes is activates by production of ROS from MAO. The MAO inhibitor, selegiline (20 µM) blocks the Ca^{2+} signal in astrocytes (a), but not in neurons (b). (c) Adrenaline-induced astrocytic Ca^{2+} signal can be reduced by preincubation with antioxidant Trolox (100 µM). Effect of Trolox is smaller in neurons (d). (e) Summary of fura-2 ratio increases in astrocytes and neurons in the presence of 20 µM selegeline or 100 µM Trolox. Error bars indicate SEM; *p< 0.05; **p < 0.01; ***p < 0.001.

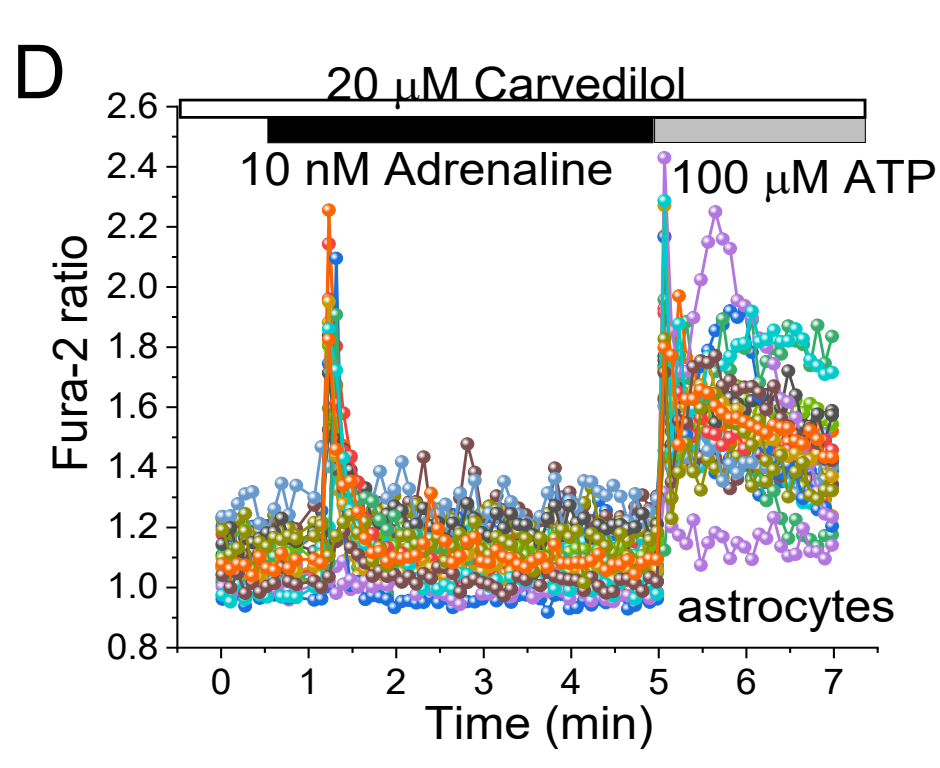
Fig. 4. Effect of adrenaline on ROS production and lipid peroxidation in neurons and astrocytes. Adrenaline induce dose dependent increase in the rate of ROS production (a-b), which can be blocked by preincubation with 20 µM selegiline. (c) Quantification of ROS production in astrocytes under 10 nM or 500 nM adrenaline exposure. (d) Adrenaline induce activation of lipid peroxidation in astrocytes, which can be completely inhibited by incubation of the cells with 20 µM selegiline (e). Summary of the effects of adrenaline on lipid peroxidation in astrocytes (f). The effect of adrenaline on lipid peroxidation in neurons with or without incubation with 20 µM selegiline (g-h). Quantification of the effects of adrenaline rate of lipid peroxidation in neurons and astrocytes. Error bars indicate SEM; *<0.05; **p < 0.01; ***p < 0.001.

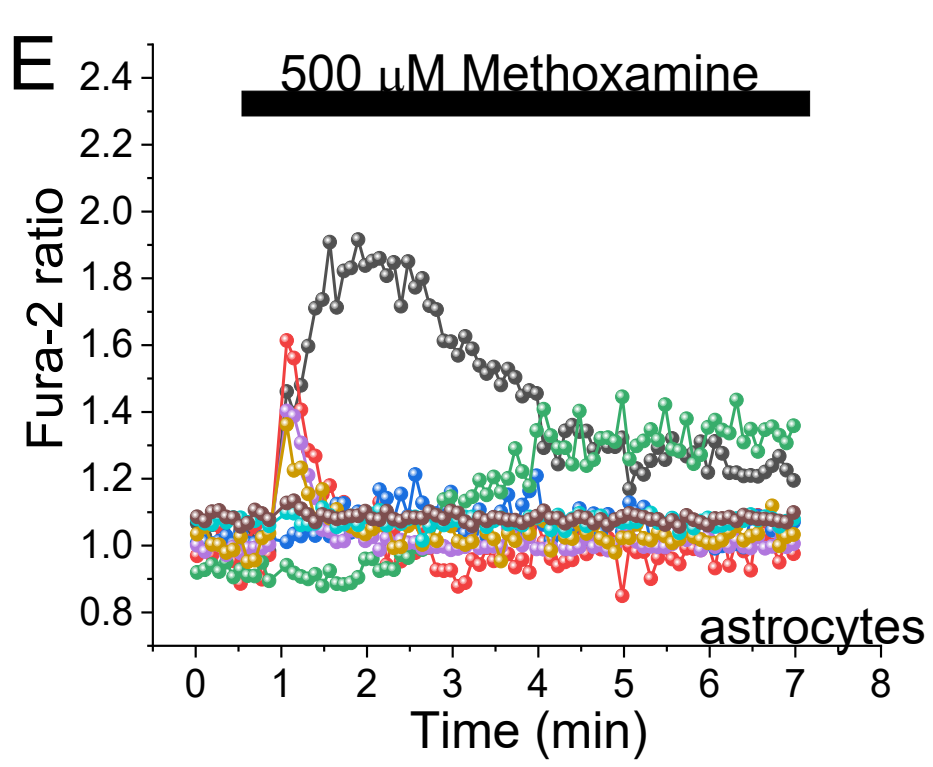
Fig. 5. Inhibition of MAO decreases effect of adrenaline on vasoconstriction. Left, representative acute brain slices not pre-treated (a) and pre-treated (b) for 8 min with 20 µM selegiline before and after adding adrenaline; right, corresponding traces of the normalised blood vessel diameters. (c) Relative decrease of the blood vessel diameters with (N = 3 mice, n = 9 slices, 3 ROI per slice) and without (N = 3 mice, n = 7 slices, 3 ROI per slice) selegiline pre-treatment. The error bars depict mean ± SEM, P = 0.000054. The P value was calculated by ANOVA with the Tukey post hoc test.

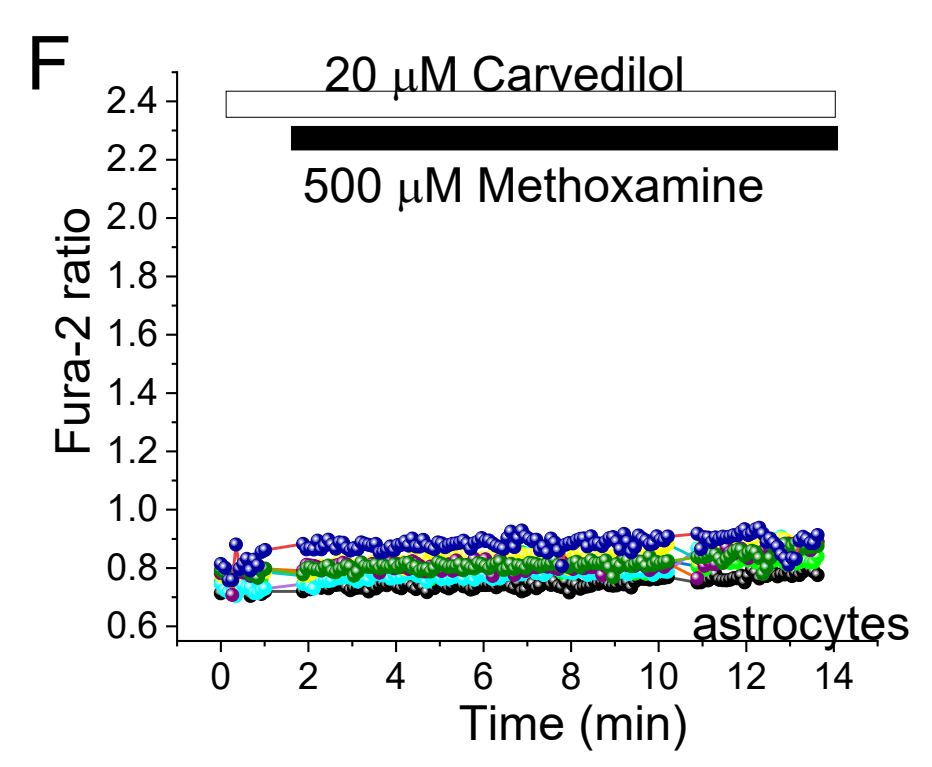

A
500 nM Adrenaline
Fura-2 ratio
Time (min)
neurons

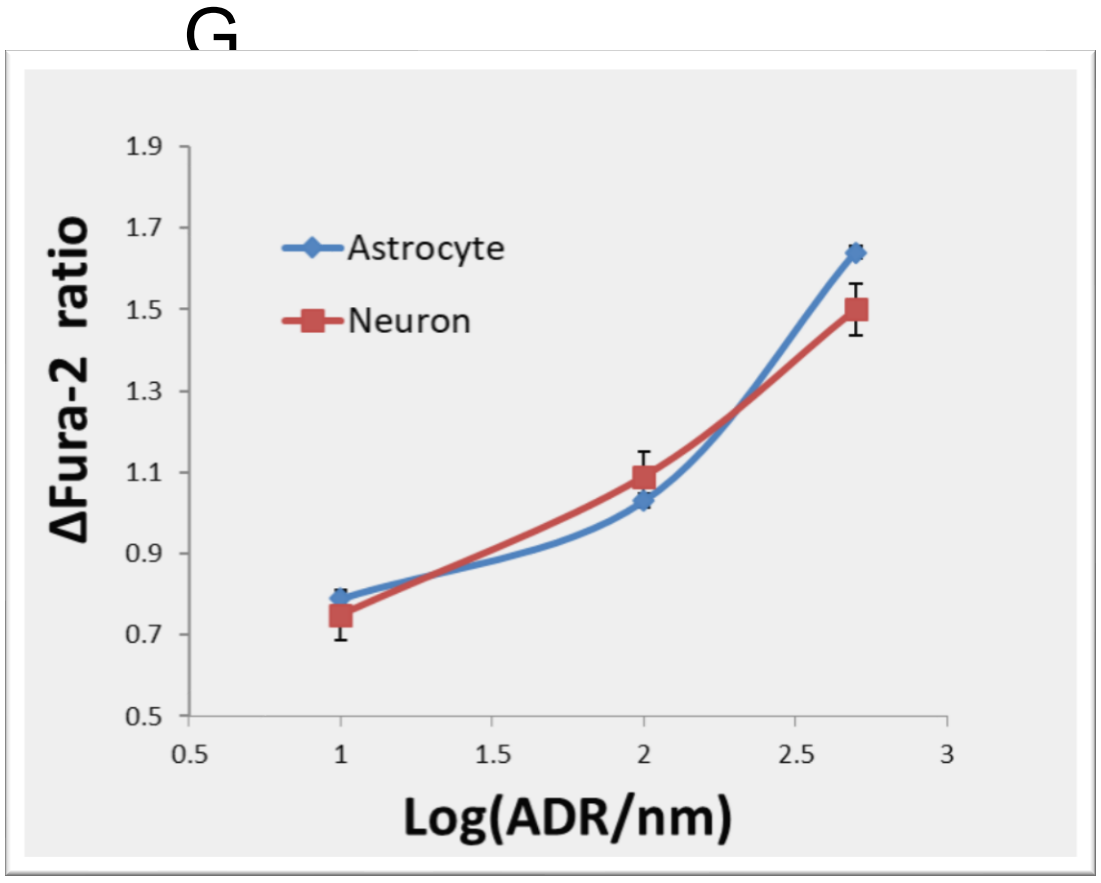

B
20 μM Carvedilol
Glutamate
500 nM Adrenaline
Fura-2 ratio
Time (min)
neurons


C
10 nM Adrenaline
100 μM ATP
Fura-2, ratio
Time (min)
astrocytes


D
20 μM Carvedilol
10 nM Adrenaline
100 μM ATP
Fura-2 ratio
Time (min)
astrocytes


E
500 μM Methoxamine
Fura-2 ratio
Time (min)
astrocytes


F
20 μM Carvedilol
500 μM Methoxamine
Fura-2 ratio
Time (min)
astrocytes


G
ΔFura-2 ratio
Astrocyte
Neuron
Log(ADR/nm)

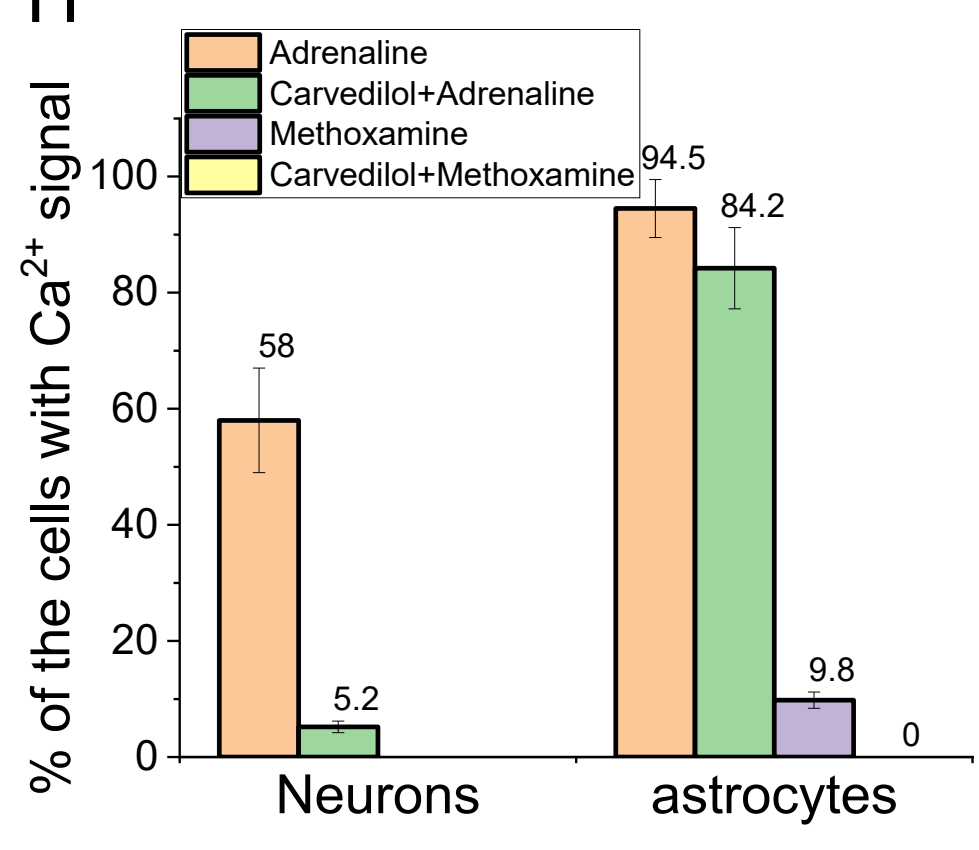

H
Adrenaline
Carvedilol+Adrenaline
Methoxamine
Carvedilol+Methoxamine
% of the cells with Ca2+ signal
58
5.2
94.5
84.2
9.8
0
Neurons
astrocytes

Figure 1

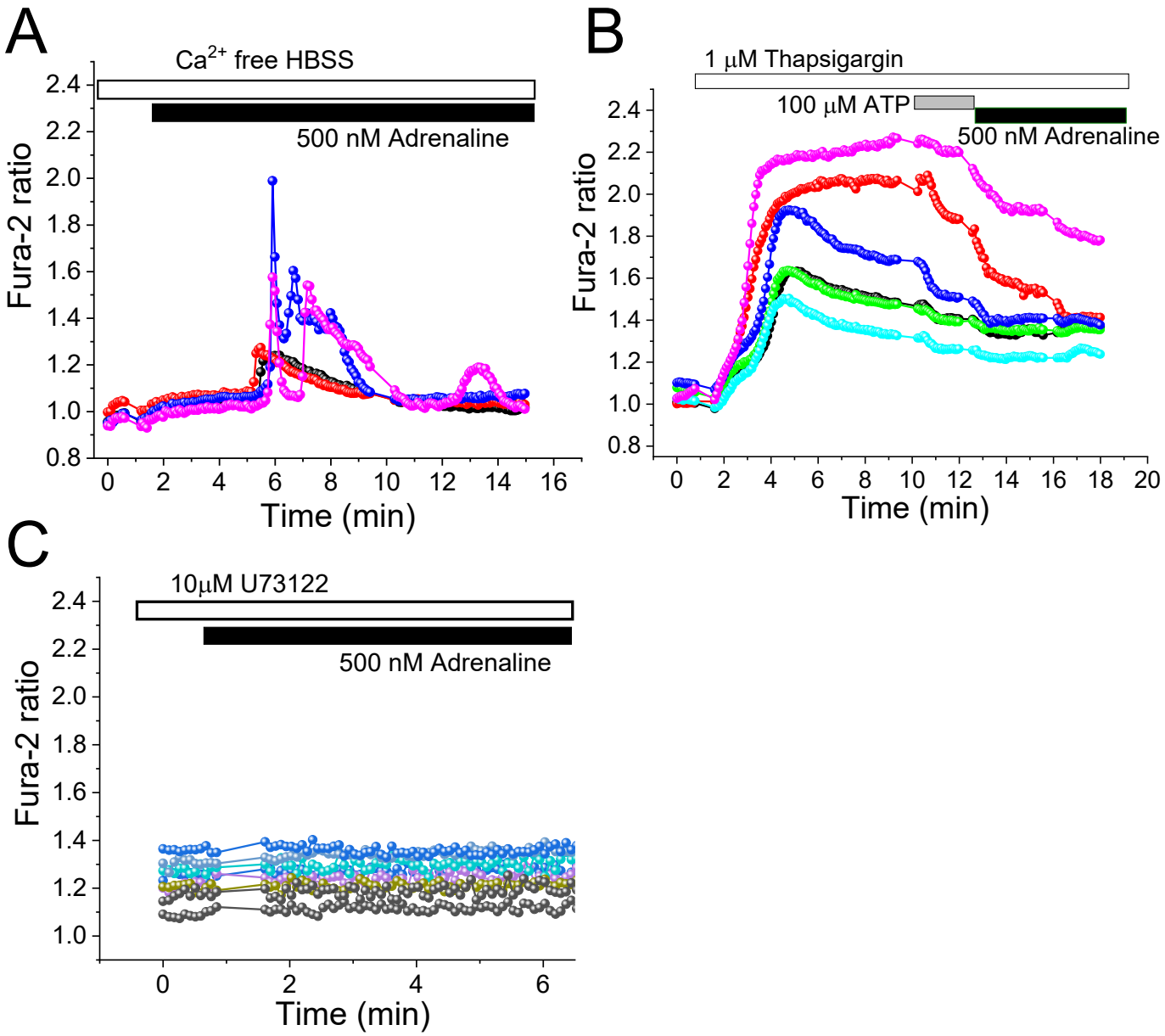

A
Ca2+ free HBSS
500 nM Adrenaline
Fura-2 ratio
Time (min)
B
1 μM Thapsigargin
100 μM ATP
500 nM Adrenaline
Fura-2 ratio
Time (min)
C
10μM U73122
500 nM Adrenaline
Fura-2 ratio
Time (min)

Figure 2

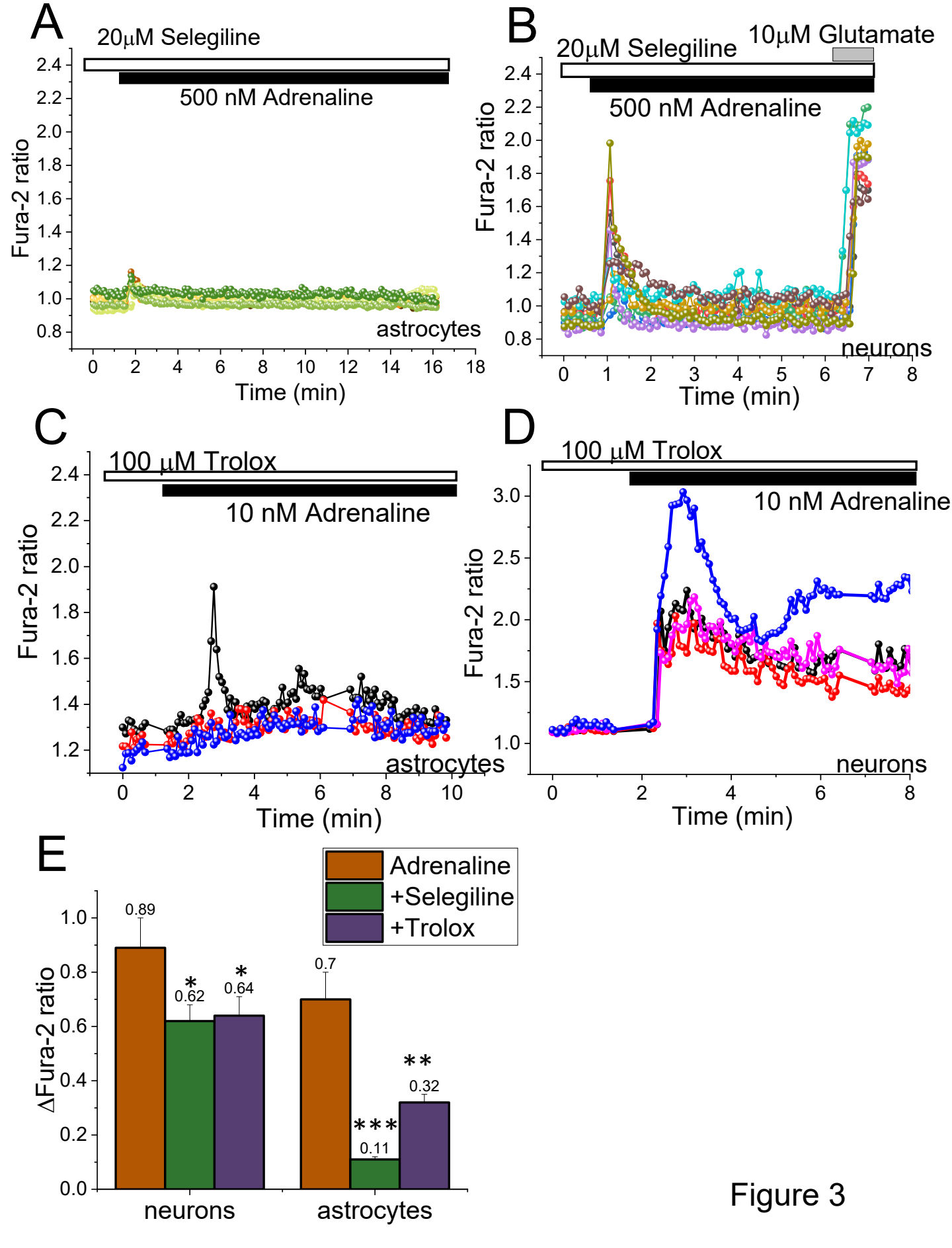

A
20μM Selegiline
500 nM Adrenaline
Fura-2 ratio
astrocytes
Time (min)
B
20μM Selegiline
10μM Glutamate
500 nM Adrenaline
Fura-2 ratio
neurons
Time (min)
C
100 μM Trolox
10 nM Adrenaline
Fura-2 ratio
astrocytes
Time (min)
D
100 μM Trolox
10 nM Adrenaline
Fura-2 ratio
neurons
Time (min)
E
Adrenaline
+Selegiline
+Trolox
ΔFura-2 ratio
0.89
0.62
0.64
0.7
0.11
0.32
*
*
**
***
neurons
astrocytes

Figure 3

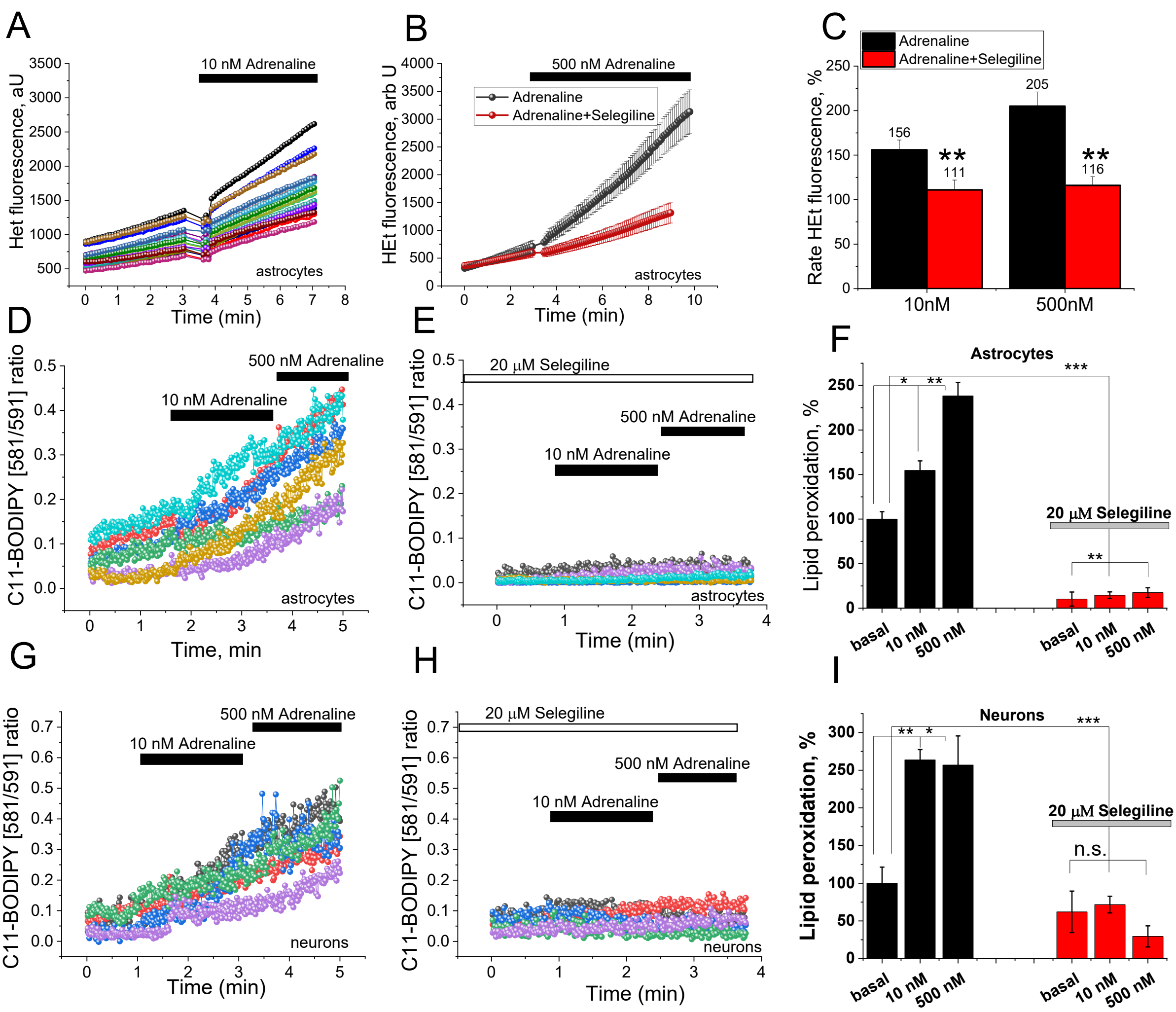
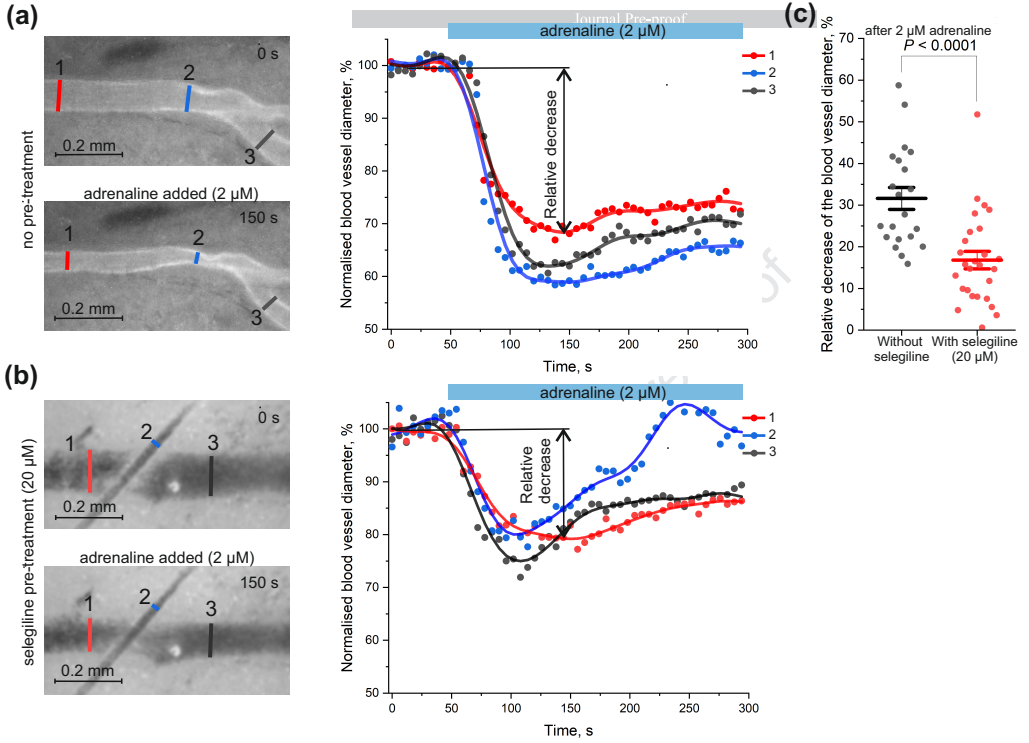

A
10 nM Adrenaline
Het fluorescence, aU
astrocytes
Time (min)
B
500 nM Adrenaline
Adrenaline
Adrenaline+Selegiline
HEt fluorescence, arb U
astrocytes
Time (min)
C
Adrenaline
Adrenaline+Selegiline
Rate HEt fluorescence, %
156
111
205
116
**
**
10nM
500nM
D
500 nM Adrenaline
10 nM Adrenaline
C11-BODIPY [581/591] ratio
astrocytes
Time, min
E
20 μM Selegiline
500 nM Adrenaline
10 nM Adrenaline
C11-BODIPY [581/591] ratio
astrocytes
Time (min)
F
Astrocytes
Lipid peroxidation, %
20 μM Selegiline
basal
10 nM
500 nM
G
500 nM Adrenaline
10 nM Adrenaline
C11-BODIPY [581/591] ratio
neurons
Time (min)
H
20 μM Selegiline
500 nM Adrenaline
10 nM Adrenaline
C11-BODIPY [581/591] ratio
neurons
Time (min)
I
Neurons
Lipid peroxidation, %
20 μM Selegiline
n.s.
basal
10 nM
500 nM

Figure 4



Highlights

* Adrenaline induce calcium signal in cortical astrocytes and neurons
* α- and β-adrenoreceptors antagonist had only minor effect on calcium signal in astrocytes
* Adrenaline induce production of ROS and lipid peroxidation in MAO of astrocytes
* Inhibitor of MAO selegiline and antioxidants suppress adrenaline induced Ca^{2+} signal in astrocytes
* Selegiline inhibit adrenaline-induced vasoconstriction in acute brain slices