Taken together, these results suggest a clear involvement of PKC in the signalling pathways through which NPY modulates glutamate release.

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P19. Role of the NCX in neurotoxicity mediated by AMPA receptor activation in cultured hippocampal neurons

Bruno P. Carreira[1], Inês M. Araújo[1], António F. Ambrósio[2], Caetana M. Carvalho[1]
1- Center for Neuroscience and Cell Biology, Department of Zoology, University of Coimbra, Coimbra, Portugal; 2- Center of Ophthalmology of Coimbra, IBILI, Faculty of Medicine, University of Coimbra, 3000-354 Coimbra, Portugal.

The Na^+/Ca^{2+} exchanger (NCX) is a plasma membrane ion transporter protein that exchanges Na^+ for Ca^{2+} in a bi-directional mode. Physiologically, NCX extrudes Ca^{2+} in exchange for Na^+, whereas upon neuronal depolarization Na^+ is pumped out by NCX while Ca^{2+} is pumped in, working in the reverse mode. In pathophysiological conditions, overactivation of glutamate receptors can cause the reversal of NCX to occur leading to Ca^{2+} entry into the cell. In this work, we investigated the consequences of the reversal of NCX in the neurotoxicity induced by the activation of AMPA receptors, in cultured hippocampal neurons.

We have previously shown that activation of AMPA receptors by short-term exposure (5 min) to kainate (KA; 100 μM), in non-desensitizing conditions (cyclothiazide present; CTZ, 30 μM) causes a neurotoxic effect and leads to an increase in calpain activity that is involved in cell death (Araújo et al., 2004, J. Neurochem., **91**(6): 1322-31). We now found that the Na^+/Ca^{2+} exchanger inhibitor (KB-R7943; 20 μM; present during the 5 min stimulus), a selective inhibitor of NCX reverse mode, prevented the neurotoxic effect of KA, in non-desensitizing conditions, as evaluated by the MTT reduction assay ($p<0.01$). KB-R7943 also prevented significantly the appearance of condensed nucleae following exposure to KA ($p<0.05$). Furthermore, Western blot analysis of spectrin, which is used as a marker for calpain activation, showed that inhibition of NCX prevented proteolysis of spectrin, as well as of other endogenous calpain substrates, like the GluR1 subunit of AMPA receptors and the neuronal nitric oxide synthase (nNOS).

Taken together, these data suggest that reversal of NCX is an early phenomenon in the neurotoxicity induced by activation of AMPA receptors, and it is likely that Ca^{2+} entry due to activation of AMPA receptors occurs via reversal of NCX and is directly involved in the activation of calpains, thus causing neuronal demise.

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P20. Involvement of the adenosine A_1 receptor in the recovery of hippocampal metabolism after hypoxia

J. M. N. Duarte, R. A. Cunha, R. A. Carvalho
Center for Neuroscience and Cellular Biology, University of Coimbra, Coimbra.

Hypoxia compromises neuronal function in brain tissue (Trends Neurosci 17, 251). During a hypoxic insult there is not only the release of excitatory neurotransmitters that may cause excytotoxicity, but also neuromodulators such as adenosine, which may have a protective role. Adenosine is a ubiquitous neuromodulator causing predominantly an inhibition of neurotransmitter release through activation of adenosine A_1 receptors (Pharmacol Toxicol 77, 299). During hypoxia, there is a complete inhibition of synaptic transmission, which is fully reversible by re-oxygenation, but blockade of adenosine A_1 receptors attenuates the recovery of synaptic transmission after the insult (J Neurosci 21, 8564; Neurosci 132, 575). Acting on A_1 receptor, adenosine has also a homeostatic role modulating neuronal and astrocytic intermediary metabolism (J Neurochem 74, 327). Thus, we now investigated the involvement of the adenosine A_1 receptor in the recovery of the hippocampal intermediary metabolism from hypoxia, using hippocampal slices superfused with the ^{13}C enriched substrates.

Hippocampal slices (400μm thick) were prepared from male Wistar rats (8 weeks old). After a 45min resting period in a Krebs solution gassed with $95\%O_2/5\%CO_2$, at room temperature, the slices were superfused (3mL/min) with the same solution during 60min, at 37ºC, to stabilize, followed by a 90min superfusion either in normoxic or hypoxic conditions (perfusate bubbled with $95\%N_2/5\%CO_2$), in the presence of 50μM 4-amino-pyridine (4AP) to allow slice stimulation. Then, a group of slices was superfused during 3hours in normoxic conditions in the presence of either unlabelled glucose (5.5mM) and sodium acetate (2mM) or [U-^{13}C]glucose and [2-^{13}C]acetate, with 4AP present. The superfusions were repeated in the presence of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), a selective A_1 antagonist, during the hypoxia and re-oxygenation and the respective control conditions. Water-soluble metabolites were extracted from the slices with perchloric acid. The concentration of metabolites and the incorporation of 13C atoms into different carbon positions of metabolic intermediates were determined by ^1H- and ^{13}C-NMR spectroscopy (Varian Unity-500 spectrometer using a 5mm broadband NMR probe), respectively. Adenine nucleotide concentrations were measured by HPLC analysis and used to calculate energy charge (EC) of the tissue.

Hypoxia altered metabolic pools and EC, either in the presence or absence of DPCPX. Metabolic pools after hypoxia plus re-oxygenation are not different from the control. However, the presence of DPCPX caused a significant decrease of EC and the modification of some metabolite concentrations. Hypoxia decreased glutamine content, which was exacerbated by blockade of A_1 receptors, suggesting a modification of the glutamine-glutamate cycle flux. The incorporation of ^{13}C atoms in glutamate and GABA carbon positions was used to evaluate the TCA cycle fluxes. The TCA cycle of the compartment where glucose is oxidized (neurons plus glia) is not modified in the hypoxia plus re-oxygenation, but the presence of DPCPX increases this flux in the period after hypoxia. Acetate is metabolized exclusively in glial cells and the TCA cycle flux in this compartment seems not to be affected by the blockade of adenosine A_1 receptor. Thus, we conclude that the metabolic modifications that occur in hippocampal slices during hypoxia recover upon reperfusion, and this recovery is prevented by blockade of the adenosine A_1 receptor.

P21. Differential cytotoxic responses of PC12 cells chronically exposed to psychostimulants or to hydrogen peroxide

Teresa Oliveira[1,2], Ana Cristina Rego[1,2], Maria Teresa Morgadinho[3], Ana Bela Sarmento[1,2], Tice Macedo[3] and Catarina Resende Oliveira[1,2]
1- Center for Neuroscience and Cell Biology and 2- Institute of Biochemistry, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 3- Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.

Stimulant drugs of abuse directly induce extraneuronal dopamine accumulation in specific brain areas. Dopamine, in high concentrations, may be cytotoxic, in part due to the

generation of H_2O_2 and other ROS through oxidative metabolism. In this work we compared the responses of cells chronically or acutely exposed to H_2O_2 or to the stimulant drugs of abuse, cocaine and amphetamine, using PC12 cells as a dopaminergic neuronal model. Furthermore, chronic incubation with H_2O_2 was used as a model of cellular adaptation to oxidative stress. We assessed the viability of cells chronically incubated with the drugs of abuse, or H_2O_2, after acute exposure to H_2O_2 (50-75 µM) or to the stimulant drugs (1-3 mM). We further analyzed dopamine and DOPAC levels, ATP levels, cell morphology and the expression of Bcl-2 and Bax. We showed that chronic cocaine induces sensitization to acute cocaine toxicity, and increased cocaine-evoked accumulation of extracellular dopamine. Moreover we showed that chronic amphetamine induced dopamine depletion. Nevertheless, acute amphetamine toxicity was maintained in cells lacking dopamine, indicating that dopamine is not involved in acute amphetamine cytotoxicity. We also showed that chronic cocaine treatment protected the cells against H_2O_2 challenge. Although exposure to H_2O_2 decreased intracellular ATP in cells chronically treated with cocaine, these cells were less injured by H2O2 challenge. In addition PC12 cells adapted to chronic H_2O_2 were shown to be resistant to acute H_2O_2 exposure, as determined by MTT, cell morphology and ATP levels. Despite differences in susceptibility of PC12 cells chronically exposed to the stimulant drugs, cocaine and amphetamine, and H_2O_2, the changes could not be attributed to the alteration of Bcl-2/Bax levels.

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P22. The Role of the Caudal Ventrolateral Medulla in the Development of Hyperension by Adenosine Receptor Blockade in Rat

D. Pinho[1], J. Marques-Lopes[1], M. Morato[1,3], T. Sousa[1], I. Tavares[2], A. Albino-Teixeira[1]
1- Institute of Pharmacology and Therapeutics; 2- Institute of Histology and Embryology, Faculty of Medicine of Porto and IBMC; 3- Pharmacology Department, Faculty of Pharmacy of Porto, Portugal.

The continuous infusion for 7 days of the adenosine receptor antagonist 1,3-dipropyl-8-sulfophenylxanthine (DPSPX) causes in rats a sustained hypertensive state associated with hypoalgesia. It has been shown that lesion of the lateralmost part of CVLM (VLMlat) causes transient increases in blood pressure and heart rate in normotensive rats and reverts the hypoalgesia in DPSPX-hypertensive rats, indicating that this region participates both in the descending inhibition pathway of pain and in the vasodepressor actions ascribed to the CVLM. Therefore, we decided to investigate the long-term effects of lesioning VLMlat in the induction process of hypertension by adenosine receptor blockade. We also lesioned the adjacent medullary region spinal trigeminal nucleus, *pars caudalis* (Sp5C) in order to define more accurately the boundaries of the effector area.

On day 0, saline (S) or quinolinic acid (QA; 54 nmol in 0.3 µL) were stereotaxically injected in the left VLMlat or in Sp5C of male Wistar rats (≈300 g) and osmotic minipumps were intraperitoneally implanted, filled either with saline or DPSPX (Px; 90 µg/kg/h). Systolic BP (SBP) was measured by the tail-cuff technique on the 8 groups of animals: QA/Px, QA/S, S/Px and S/S, injected either in VLMlat or Sp5C (n=6 for all groups). Statistical analysis was performed by 1-way ANOVA followed by Tukey test.

VLMlat lesion prevented DPSPX-induced hypertension (*e.g.* on day 10, SBP values (mean±SEM) were QA/Px: 128.0±2.3 *vs* S/Px: 144.2±2.5 mmHg, P<0.05). On the other hand, sham lesions in VLMlat or QA lesions in the adjacent Sp5C did not prevent the onset of hypertension (*e.g.* on day 6: QA/Px: 134.7±3.3 *vs* S/Px: 140.8±1.7 mmHg). Rather, lesion of Sp5C caused by itself moderate increases in blood pressure (on day 6: QA/S: 141.0±3.2 *vs* S/S: 122.3±1.6 mmHg, P<0.05).

We conclude that the VLMlat is an important medullary region in the induction process of hypertension by adenosine receptor blockade in rats.

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P23. Contractile responses and metabolism of purines in detrusor muscle from patients with hyperactive urinary bladder

M. Faria[1], M. Silva-Ramos[2], T. Magalhães-Cardoso[1], J.-M. la Fuente-de-Carvalho[2] & P. Correia-de-Sá[1]
1- Laboratório de Farmacologia, Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP); 2- Serviço de Urologia, Hospital Geral de Santo António, SA (HGSA-SA), Portugal.

Contractile responses of the detrusor muscle from normal human bladders are mediated exclusively by cholinergic activity. However, under some pathological conditions (e.g. interstitial cystitis, neurogenic or hyperactive bladders), contractile activity of the detrusor is resistant to atropine. Contractions resistant to atropine can be prevented by co-application of the stable ATP analogue, alfa-beta-methylene ATP, which induces desensitization of P2X receptors when applied in high concentrations. In most tissues, ATP is rapidly metabolized in the extracellular milieu by the ecto-nucleotidase pathway. In the present work, we aimed at studying the kinetics of the extracellular metabolism of adenine nucleotides and in order to probe their role on contractile activity of the detrusor from patients with hyperactive urinary bladder.

Fragments from human detrusor muscle were collected from patients that underwent transvesical prostatic surgery. Some experiments were performed with fragments of porcine detrusor muscle obtained from the local slaughterhouse shortly after sacrifice. These proceedings had the approval of the Ethics Committees of HGSA-SA and ICBAS-UP. For transportation, the fragments were immediately placed in a M-400 solution (composition g/100 ml: manitol 4.19, KH2PO4 0.205, K2HPO4•3H2O 0.97, KCl 0.112, NaHCO3 0.084) at 4-6ºC. Muscle stripes with 1 mm width were mounted in 12-ml organ bath superfused with carbogen-gassed (95% O2 + 5% CO2) Tyrode's solution at 37ºC. The isometric tension generated by the tissues was measured with a isometric force transducer and registered in a oscilograph (Hugo-Sachs, Germany). The resting load was 10 mN and the preparations were allowed to equilibrate for a 90-min period. To test the contractile responses induced by ATP, this compound was cumulatively applied to the organ bath (0,01-3 mM). In some experiments, the relaxing effect of adenosine was tested in preparations pre-contracted with acetylcholine (10 µM). To study the kinetics of ATP catabolism in detrusor muscle, samples (75 µl) were collected from the organ bath during a 45-min period. The disappearance of ATP and extracellular formation of ATP metabolites were quantified by HPLC analysis.

In keeping with previously released data, the detrusor muscle from patients with hyperactive urinary bladder respond to ATP (0,01-3 mM) by increasing the isometric tension in a concentration-dependent manner. At the maximum concentration tested, the ATP (3 mM) effect was about 40% of that produced by acetylcholine (10 µM). ATP (0,01-3 mM)-induced contractions were abolished by the P2X receptor antagonist, pyridoxal phosphate-6-azo(benzene-2,4-disulfonic) acid (PPADS, 10 µM). The ATP