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Mitochondrial Na^+/Ca^{2+} exchanger, a new target for neuroprotection in rat hippocampal slices

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ABSTRACT

We tested here the hypothesis that the pharmacological modulation of the mitochondrial Na^+/Ca^{2+} exchanger (mNCX) could be a new neuroprotective strategy to rescue stressed vulnerable neurons from death. We used rat hippocampal slices incubated with veratridine to cause neuronal death through a mechanism involving Na^+ and Ca^{2+} overload. CGP37157 (CGP), an inhibitor of the mNCX, rescued veratridine vulnerable neurons from death, showing an EC_{50} of 5 μM. This neuroprotection was associated to mitigation of veratridine-elicited overproduction of free radicals and to inhibition of the p38 MAPK-linked apoptotic pathway. These results suggest that the mNCX could become a new target to develop compounds with potential therapeutic neuroprotective actions in neurodegenerative diseases.

1. Introduction

Mitochondrial dysfunction and alterations of Ca^{2+} homeostasis has long been implicated in the pathogenesis of neurodegenerative illnesses such as Alzheimer's disease [1], Parkinson's disease [2], or amyotrophic lateral sclerosis [3,4]. The evidence linking mitochondrial dysfunction and neurodegeneration is particularly relevant in Huntington's disease [5–7]. Mitochondrial Ca^{2+} uptake via the uniporter and its ensuing release into the cytosol through the mitochondrial Na^+/Ca^{2+} exchanger (mNCX), so-called mitochondrial Ca^{2+} cycling (mCC), couple neuronal activity to ATP production; this is achieved through activation of Ca^{2+}-dependent dehydrogenases of the Krebs cycle [8,9]. Furthermore, modulation of mCC rate serves as a mechanism that damps the brisk changes of cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_c$) occurring during neuronal activity, thus maintaining the $[Ca^{2+}]_c$ within a physiological critical point [10,11]; beyond this point, chronic elevations of $[Ca^{2+}]_c$ become neurotoxic [12,13]. In this context, it is understandable that alterations of mCC could lead to activation of the apoptotic cascade, particularly in some specific more vulnerable neurons carrying a mutation that affects some of the mechanisms involved in the modulation of Ca^{2+} homeostasis [14–17].

Evidence suggesting that mitochondria can efflux Ca^{2+} ions in exchange for Na^+ ions was reported by Carafoli et al. [18] in isolated heart mitochondria; later on, these authors suggested that this mNCX mechanism could serve to regulate the mCC [19,20]. The discovery of the benzothiazepine derivative CGP37157 (CGP) as a blocker of the mNCX constituted a breakthrough for the functional characterization of the mNCX [21]. CGP causes a sustained elevation of the mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$), thereby causing the augmentation of Ca^{2+}-dependent activity of dehydrogenases, ATP synthesis, and heart contraction [22,23]. Based on this early observation, we raised the supposition that CGP could rescue from death the chromaffin cells subjected to a veratridine stress, a model of neuronal death linked to Na^+ and Ca^{2+} overload [24]. This model was later on adapted in our laboratory to test neuroprotection by drugs targeting Ca^{2+} homeostatic mechanisms in primary cultures of bovine adrenal chromaffin cells [25]. In this model we recently found that CGP causes an efficient cytoprotective action against veratridine stress [26]. Here we report that CGP causes neuroprotection in rat hippocampal slices stressed with veratridine; we attribute such an effect to blockade of the mNCX, that could therefore become a new target for development of neuroprotective drugs with therapeutic potential in neurodegenerative diseases.

2. Materials and methods

Experiments were performed in hippocampal slices prepared from brains of 2-month-old Sprague–Dawley rats (275–325 g

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weight). They were carried out following the European Union Council Directive issued for these purposes and were approved by the Ethics Committee of the Facultad de Medicina, Universidad Autónoma de Madrid, Spain. All efforts were made to minimize the number of animals and their suffering. To prepare the slices we recoursed to the protocol and solutions regularly used in our laboratory [27].

To perform the experiments, we followed the protocols shown on top of the figures and briefly described in their legends. At the end of the different incubation periods, neuronal viability in control or veratridine-treated slices was tested with MTT, a colorimetric method based in the capacity of mitochondria of intact viable neurons to reduce MTT [28]. The production of reactive oxygen species (ROS) was measured with the fluorescent probe CM-H_2DCFDA [29] as previously described [30]. The tissue content of p38 was assayed by Western blot [31].

The following materials and solutions were used: 7-chloro-5-(2-chlorophenyl)-1,5-dihydro-4,1-benzothiazepin-2(3*H*)-one (CGP 37157, CGP) and TTX were purchased from Tocris (Biogen Científica, Spain). Methylthiazolyldiphenyl-tetrazolium bromide, MTT, and veratridine were obtained from Sigma, Madrid, Spain. ω-Conotoxin GVIA (GVIA), ω-agatoxin IVA (IVA), and ω-conotoxin MVIIC (MVIIC) were purchased from Peptide Institute, INC. Japan. Immobilon-P membrane for Western blot was purchased from Millipore Corp., Madrid, Spain. Phospho-p38 MAPK and p38 MAPK antibody were obtained from Cell Signaling, Izasa-Barcelona, Spain. All other chemicals to prepare saline solutions, buffers and so on were reagent grade.

To prepare stock solutions of the various reagents, veratridine, CGP, and nimodipine were dissolved in dimethylsulfoxide (DMSO) at the concentration of 10^{-2} M. GVIA, IVA, and MVIIC were prepared in Millipore water at the concentration of 10^{-4} M. All solutions were stored in aliquots at −20 °C. Once defrosted for a given experiment, the aliquot was discarded. The final concentrations of DMSO used (always <0.1%) did not cause neuronal toxicity.

Data on cell viability were expressed as percentage of MTT reduction, taking the maximum control tissue capability in each individual experiment as 100%. In some figures, data underwent a transformation to determine the percentage of protection afforded by a given treatment; for instance, a 50% decrease of MTT reduction means 50% cell death; a decrease of 25% cell death by a given treatment means 50% neuroprotection. Statistical differences between groups of data were assayed using one-way ANOVA followed by a Newman–Keuls test. The level of significance was established at $p \leqslant 0.05$.

3. Results and discussion

We first performed an experiment to delineate the neurotoxic effects of veratridine in rat hippocampal slices [24]. Fig. 1A shows the protocol followed to estimate the neuronal damage elicited by slice incubation during 4 h with veratridine that caused 15–45% loss of MTT reduction at 1–100 μM. The threshold toxic concentration was at 1 μM and the EC_{50} to cause neuronal death was 2 μM (Fig. 1C). To have an adequate window of opportunity to test drug elicited neuroprotection, we selected 30 μM veratridine that reliably produced about 40% of neuronal damage during a 3.5-h incubation period.

To test the action of CGP on veratridine neurotoxicity, slices were preincubated first with increasing concentrations of the compound for a 30-min period, to allow its equilibration with the neuronal biophase; thereafter, slices continued in the presence of CGP plus veratridine, for an additional 3.5-h period (Fig. 2A). As expected from the results of Fig. 1B, in this series of experiments veratridine caused 39% diminution of MTT reduction; this neuronal

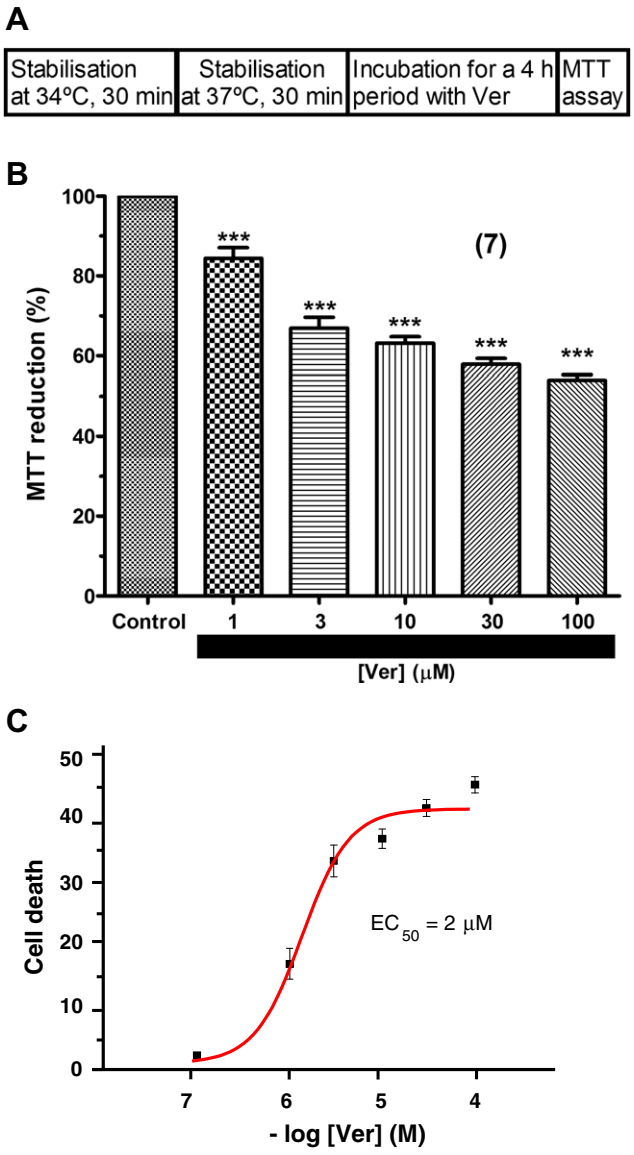

Fig. 1. Neurotoxic effects of veratridine (Ver) in rat hippocampal slices. (A) Experimental protocol consisting of two sequential incubation periods to equilibrate the tissue with oxygenated Krebs-bicarbonate solutions (see Section 2) and to study the effects of Ver on neuronal viability and death. (B) MTT reduction by the slices, in the absence (control) and the presence of increasing concentrations of Ver, after 4 h incubation, an indicator of neuronal viability, normalized as % of control. (C) Transformation of MTT reduction data into neuronal death by subtraction of values obtained in slices treated with Ver from those obtained in control slices. Data are means ± S.E. of seven quadruplicate experiments performed in seven different animals. $^{***}p < 0.001$ with respect to control (ANOVA).

lesion was gradually counteracted by increasing concentrations of CGP (Fig. 2B) that caused neuroprotection with an EC_{50} of 5 μM (Fig. 2C).

CGP is being widely used to selectively inhibit the mNCX, at concentrations of 10 μM in neuronal cultures [32,33] and at 15 μM in brain slices [34]. In chromaffin cells transfected with aequorins, 20 μM CGP slows down the mitochondrial Ca^{2+} efflux into the cytosol without inhibiting the K^+-evoked $[Ca^{2+}]_c$ transient [35]. In contrast, in rat dorsal root ganglion cells, CGP blocks the K^+-elicited $[Ca^{2+}]_c$ transients with an IC_{50} of 4 μM [36]. Furthermore, 1 μM CGP does not affect I_{Ca} in isolated neonatal rat ventricular myocytes, while it causes mNCX inhibition in isolated heart mitochondria with an IC_{50} of 0.36 μM [22]. However, in rat atrial myocytes the compound inhibits I_{Ca} with an IC_{50} of 0.27 μM [37]. In bovine

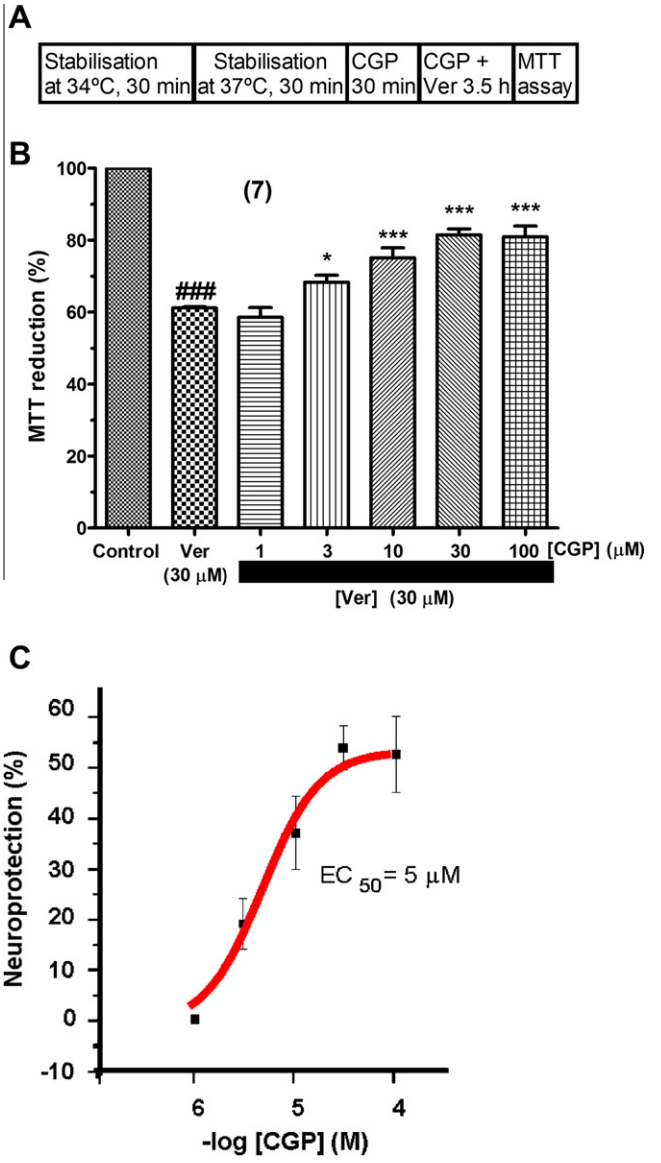

Fig. 2. CGP37157 (CGP) protected hippocampal slices against the neurotoxic effects of veratridine (Ver). (A) Experimental protocol consisting of two 30-min equilibration periods at 34 °C and 37 °C, followed by a 30-min preincubation period with CGP at the concentrations indicated in the abscissa, and by a 3.5-h co-incubation period with CGP and Ver. (B) MTT reduction by slices subjected to the different CGP concentrations, as an indicator of neuronal survival, expressed in the ordinate as % of control. (C) Transformation of MTT reduction data into neuronal protection by normalizing to 100% the damage caused by Ver in the absence of CGP (i.e., 40% inhibition of MTT reduction) as a window of opportunity for neuroprotection afforded by CGP. A calculated EC_{50} value of 5 μM to induce neuroprotection was found. Data are means ± S.E. of seven quadruplicate experiments performed in seven different rats. $^{###}p < 0.05$ with respect to control; $^{*}p < 0.05$, $^{***}p < 0.001$ with respect to Ver-elicited neuronal damage.

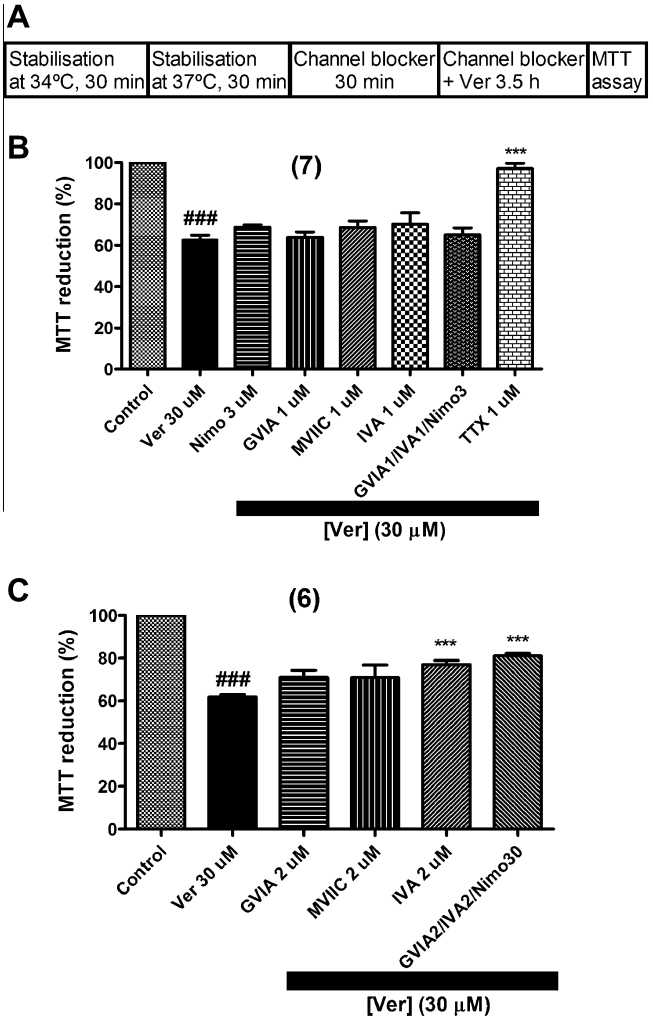

Fig. 3. Effects of ion channel blockers on veratridine (Ver)-elicited neuronal damage. (A) Protocol consisted in four successive periods of incubation of hippocampal slices in Krebs-bicarbonate solution that was bubbled continuously with carbogen; the first two periods allowed tissue equilibration; during the third period tissues were incubated with blockers of VDCCs of L-(nimodipine), *N*-(ω-conotoxin GVIA (GVIA)), P/Q (ω-agatoxin IVA (IVA)), a mixture of the three blockers, or tetrodotoxin (TTX), at the concentrations indicated at the abscissa of panel B; during the fourth period, media were exchanged with fresh solution containing the blockers plus 30 μM Ver, and slices were further incubated for a 3.5-h period. Control slices had no blockers nor Ver; an additional group of slices were treated with Ver alone in order to determine the reference neuronal lesion in each individual experiment. (B) MTT reduction capability (ordinate) of slices subjected to the different treatments, expressed as % of control. (C) This experiment was made following the same protocol but with higher concentrations of the blockers, as indicated in the abscissa. Data are means ± S.E. of quadruplicate experiments performed in different animals, as indicated in parentheses. $^{###}p < 0.001$ compared with control; $^{***}p < 0.001$ compared with reference Ver.

chromaffin cells CGP causes 18% inhibition of I_{Ca} at 10 μM and 60% blockade at 30 μM. In the light of these variable data we tested whether supramaximal concentrations of selective blockers of VDCCs were mimicking the neuroprotective effects of CGP.

In the experiments of Fig. 3A, slices run in parallel were preincubated during 30 min with concentrations known to cause a maximal blockade of specific VDCC subtypes, i.e., 3 μM nimodipine (L-type, Cav1.3), 1 μM GVIA (N-type, Cav 2.2), 1 μM MVIIC (N- and PQ-type, Cav2.1), or 1 μM IVA (PQ-type). Veratridine produced 37.2% decrease of MTT reduction, a figure similar to that of previous experiments; none of the VDCC blockers either given alone or in combination, mitigated this neuronal damage. As a positive control we included a variable with tetrodotoxin (TTX, 1 μM) that by neutralizing the veratridine action on Na^+ channels, afforded full protection (Fig. 3B). In another experiment we doubled the blocker concentrations, but only IVA reduced the veratridine damage by 39.5%. Slice incubation with 2 μM each of GVIA and IVA plus 30 μM nimodipine elicited 50% neuroprotection (Fig. 3C). This nimodipine concentration is at least 10-fold higher that required to block L channels [38–40]. At these high concentrations, other 1,4-dihydropyridine (DHP) derivatives such as nitrendipine and isradipine blocks the mNCX [41,42]. It is therefore plausible that at 30 μM, the DHP derivative nimodipine is also inhibiting the mNCX to afford such neuroprotective action.

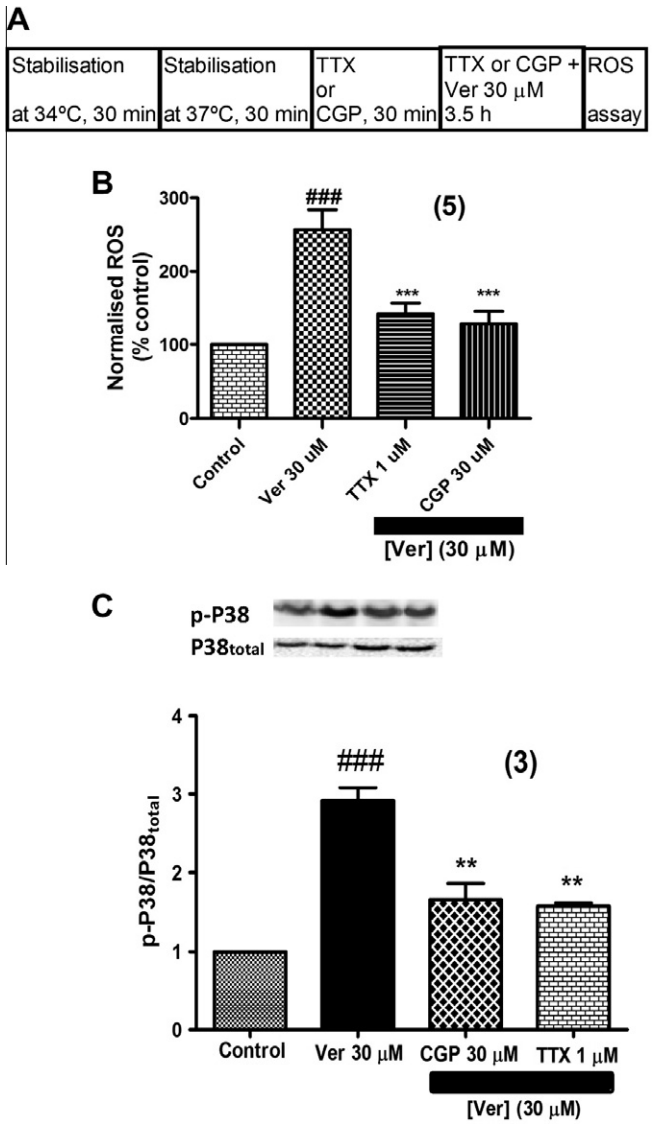

Fig. 4. Inhibition by CGP37157 (CGP) of veratridine (Ver)-elicited production of reactive oxygen species (ROS) in hippocampal slices. (A) Experimental protocol consisting in four sequential incubation periods of slices in Krebs-bicarbonate solutions continuously bubbled with carbogen. After stabilisation, slices were incubated with TTX or CGP during 30 min at the indicated concentrations and subsequently with the same concentrations of the compounds plus 30 μM Ver during a further 3.5 h period. Control slices were incubated in solutions containing no drugs. (B) ROS production by slices at the end of each treatment, measured with the fluorescent probe CM-H_2DCFDA (Arbitrary Fluorescence Units – AFU – ordinate). ROS production by control slices incubated for 3.5 h in the absence of drugs, and expressed in AFU was normalized to 100% and the values of drugs treated slices were expressed as % of control. Data are means ± S.E. of five quadruplicate experiments done in five different animals. $^{###}p < 0.001$ with respect to control; $^{***}p < 0.001$ with respect to Ver. (C) CGP blocks the phosphorylation of p38 induced by veratridine in rat hippocampal slices. Western blot analysis for p38 obtained from protein extracts of rat hippocampal slices maintained in control solution or subjected to 3.5 h veratridine in the absence or presence of 30 μM CGP or 1 μM TTX. The top part shows representative blots and the bottom part represents the means ± S.E. of three experiments. $^{###}p < 0.001$ with respect to basal and $^{**}p < 0.01$ with respect to veratridine-stressed slices.

How CGP produces neuroprotection could be explained in the context of the ionic pathway leading to neuronal death in veratridine-treated slices. This pathway sequence commences with slowing down of Na^+ channel inactivation elicited by veratridine [43] that causes Na^+ accumulation in the cytosol [44,45]. This produces cell depolarization [46], leading to VDCC opening and increased $[Ca^{2+}]_c$ [25,26,46]. This will cause mitochondrial Ca^{2+} overload and activation by enhanced $[Na^+]_c$ of the mNCX, thereby producing a futile mCC with a concomitant excessive production of ROS; this could be the signal to initiate the process of neuronal death and neurodegeneration [47]. By slowing down such futile mCC, CGP could contribute to mitigate the excessive ROS generation. This was tested in the following experiment. Slices were subjected to the protocol shown in Fig. 4A. At the end of the 3.5-h incubation period with veratridine, the tissues contained 2.5-fold higher ROS concentration, compared with the control tissue. TTX reduced veratridine-elicited ROS production by 73% while CGP caused a 77.1% reduction.

To further inquire into the intracellular death pathway involved in veratridine neurotoxicity and in the neuroprotective effects of CGP we measured p38 MAPK activation, a pathway linked to cell death [48,49]. In fact, inhibition of p38 MAPK has been investigated as a potential target for neuroprotection in different human diseases [50,51]. As Fig. 4C shows, veratridine elicited near 3-fold increase of p38 phosphorylation, compared to basal hippocampal slices. CGP significantly reduced veratridine-elicited p38 phosphorylation by 70%; this reduction was similar to that obtained with TTX.

At first sight it seems paradoxical that CGP-elicited mNCX blockade could lead to neuroprotection. In fact, a recent elegant experiment from Duchen's laboratory suggests the opposite: a mutation of the mitochondrial protein PINK1, encoded by a gene believed to be involved in Parkinson's disease, caused a decrease of mNCX activity; this results in mitochondrial Ca^{2+} overload that sensitizes the mitochondria permeability transition pore (mPTP) opening, thereby impairing respiration and rendering neurons vulnerable to Ca^{2+}-dependent cell death [17]. However, there is an opposite view that considers the high Ca^{2+} buffering capacity of mitochondria as a mechanism to remove excess Ca^{2+} from the cytosol during a stressful stimulus, leading to neuroprotection [52,53]. This fits well in the hypothesis that, beyond a critical physiological range, the $[Ca^{2+}]_c$ elevations become neurotoxic [12]. In this context, a mild inhibition of the mNCX by CGP could slow down the rate of delivery of Ca^{2+} into the cytosol to maintain the $[Ca^{2+}]_c$ within a physiological range. Furthermore, mitigation of mNCX activity by CGP enhances dehydrogenase activity and ATP synthesis [22] and augments histamine-elicited ATP production in Hela cells [54]. This is in the line with the concept that the high-capacity mitochondrial Ca^{2+} uptake pathway provides a mechanism that couples energy demand to increased ATP production through the Ca^{2+}-dependent up-regulation of mitochondrial enzyme activity [55]. This pharmacological strategy may be particularly useful to rescue from death the vulnerable neurons of neurodegenerative diseases that have an alteration of Ca^{2+} homeostatic mechanisms [2,3,14,15].

In conclusion, we have shown here for the first time that CGP causes neuroprotection in rat hippocampal slices stressed with veratridine. Although an action of CGP on VDCCs cannot be completely discarded, neuroprotection is likely linked to blockade of the mNCX. Data suggest that the pharmacological modulation of the mNCX may become a novel and efficacious strategy to mitigate the Ca^{2+}-dependent death of vulnerable neurons occurring in neurodegenerative diseases.

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References

[1] M.P. Mattson, Calcium and neurodegeneration, Aging Cell 6 (2007) 337–350.
[2] Z. Yao, N.W. Wood, Cell death pathways in Parkinson's disease: role of mitochondria, Antioxid. Redox Signal. 11 (2009) 2135–2149.
[3] F. von Lewinski, B.U. Keller, Ca^{2+}, mitochondria and selective motoneuron vulnerability: implications for ALS, Trends Neurosci. 28 (2005) 494–500.
[4] J. Grosskreutz, L. Van Den Bosch, B.U. Keller, Calcium dysregulation in amyotrophic lateral sclerosis, Cell Calcium 47 (2010) 165–174.
[5] M. Damiano, L. Galvan, N. Déglon, E. Bronillet, Mitochondria in Huntington's disease, Biochim. Biophys. Acta 1802 (2010) 52–61.
[6] J.M.A. Oliveira, Nature and cause of mitochondrial dysfunction in Huntington's disease: focusing on huntingtin and the striatum, J. Neurochem. 114 (2010) 1–12.
[7] R.A. Quintanilla, G.W.W. Johnson, Role of mitochondrial dysfunction in the pathogenesis of Huntington disease, Brain Res. Bull. 80 (2009) 242–247.
[8] J.G. McCormack, R.M. Denton, Mitochondrial Ca^{2+} transport and the role of intramitochondrial Ca^{2+} in the regulation of energy metabolism, Dev. Neurosci. 15 (1993) 165–173.
[9] A.D. Arco, J. Satrustegui, New mitochondrial carriers: an overview, Cell. Mol. Life Sci. 62 (2005) 2204–2227.
[10] T.E. Gunter, D.R. Pfeiffer, Mechanisms by which mitochondria transport calcium, Am. J. Physiol. 258 (1990) C755–C786.
[11] R. Rizzuto, C. Bastianutto, M. Brini, M. Murgia, T. Pozzan, Mitochondrial Ca^{2+} homeostasis in intact cells, J. Cell Biol. 126 (1994) 1183–1194.
[12] T. Koike, D.P. Martin, E.M. Johnson Jr., Role of Ca^{2+} channels in the ability of membrane depolarization to prevent neuronal death induced by trophic-factor deprivation: evidence that levels of internal Ca^{2+} determine nerve growth factor dependence of sympathetic ganglion cells, Proc. Natl. Acad. Sci. USA 86 (1989) 6421–6425.
[13] J.L. Franklin, E.M. Johnson Jr., Suppression of programmed neuronal death by sustained elevation of cytoplasmic calcium, Trends Neurosci. 15 (1992) 501–508.
[14] M.J. Berridge, M.D. Bootman, P. Lipp, Calcium—a life and death signal, Nature 395 (1998) 645–648.
[15] M.R. Duchen, Mitochondria and calcium: from cell signalling to cell death, J. Physiol. 529 (Pt 1) (2000) 57–68.
[16] M. Brini, P. Pinton, M.P. King, M. Davidson, E.A. Schon, R. Rizzuto, A calcium signaling defect in the pathogenesis of a mitochondrial DNA inherited oxidative phosphorylation deficiency, Nat. Med. 5 (1999) 951–954.
[17] S. Gandhi, A. Wood-Kaczmar, Z. Yao, H. Plun-Favreau, E. Deas, K. Klupsch, J. Downward, D.S. Latchman, S.J. Tabrizi, N.W. Wood, M.R. Duchen, A.Y. Abramov, PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death, Mol. Cell 33 (2009) 627–638.
[18] E. Carafoli, R. Tiozzo, G. Luggli, F. Crovetti, C. Kratzing, The release of calcium from heart mitochondria by sodium, J. Mol. Cell. Cardiol. 6 (1974) 361–371.
[19] M. Crompton, M. Capano, E. Carafoli, The sodium-induced efflux of calcium from heart mitochondria. A possible mechanism for the regulation of mitochondrial calcium, Eur. J. Biochem. 69 (1976) 453–462.
[20] E. Carafoli, Historical review: mitochondria and calcium: ups and down on an unusual relationship, Trends Biochem. Sci. 28 (2003) 175–181.
[21] M. Chiesi, R. Schwaller, E. Eichenberger, Structural dependency of the inhibitory action of benzothiazepines and related compounds on the mitochondrial Na^+–Ca^{2+} exchanger, Biochem. Pharmacol. 37 (1988) 4399–4403.
[22] D.A. Cox, L. Conforti, N. Sperelakis, M.A. Matlib, Selectivity of inhibition of Na^+–Ca^{2+} exchange of heart mitochondria by benzothiazepine CGP-37157, J. Cardiovasc. Pharmacol. 21 (1993) 595–599.
[23] D.A. Cox, M.A. Matlib, Modulation of intramitochondrial free Ca^{2+} concentration by antagonists of Na^+–Ca^{2+} exchange, Trends Pharmacol. Sci. 14 (1993) 408–413.
[24] D. Ashton, R. Willems, R. Marrannes, P.A. Janssen, Extracellular ions during veratridine-induced neurotoxicity in hippocampal slices: neuroprotective effects of flunarizine and tetrodotoxin, Brain Res. 528 (1990) 212–222.
[25] R. Maroto, M.T. De la Fuente, A.R. Artalejo, F. Abad, M.G. Lopez, J. Garcia-Sancho, A.G. Garcia, Effects of Ca^{2+} channel antagonists on chromaffin cell death and cytosolic Ca^{2+} oscillations induced by veratridine, Eur. J. Pharmacol. 270 (1994) 331–339.
[26] S.M. Nicolau, A.M. de Diego, L. Cortes, J. Egea, J.C. Gonzalez, M. Mosquera, M.G. Lopez, J.M. Hernandez-Guijo, A.G. Garcia, Mitochondrial Na^+/Ca^{2+}-exchanger blocker CGP37157 protects against chromaffin cell death elicited by veratridine, J. Pharmacol. Exp. Ther. 330 (2009) 844–854.
[27] J. Egea, A.O. Rosa, M. Sobrado, L. Gandia, M.G. Lopez, A.G. Garcia, Neuroprotection afforded by nicotine against oxygen and glucose deprivation in hippocampal slices is lost in alpha7 nicotinic receptor knockout mice, Neuroscience 145 (2007) 866–872.
[28] F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, J. Immunol. Methods 89 (1986) 271–277.
[29] H.C. Ha, P.M. Woster, J.D. Yager, R.A. Casero Jr., The role of polyamine catabolism in polyamine analogue-induced programmed cell death, Proc. Natl. Acad. Sci. USA 94 (1997) 11557–11562.
[30] A.I. Rojo, P. Rada, J. Egea, A.O. Rosa, M.G. Lopez, A. Cuadrado, Functional interference between glycogen synthase kinase-3 beta and the transcription factor Nrf2 in protection against kainate-induced hippocampal cell death, Mol. Cell. Neurosci. 39 (2008) 125–132.
[31] A.P. Rigon, F.M. Cordova, C.S. Oliveira, T. Posser, A.P. Costa, I.G. Silva, D.A. Santos, F.M. Rossi, J.B. Rocha, R.B. Leal, Neurotoxicity of cadmium on immature hippocampus and a neuroprotective role for p38 MAPK, Neurotoxicology 29 (2008) 727–734.
[32] Y.V. Medvedeva, M.S. Kim, Y.M. Usachev, Mechanisms of prolonged presynaptic Ca^{2+} signaling and glutamate release induced by TRPV1 activation in rat sensory neurons, J. Neurosci. 28 (2008) 5295–5311.
[33] D. Ryan, A.J. Drysdale, C. Lafourcade, R.G. Pertwee, B. Platt, Cannabidiol targets mitochondria to regulate intracellular Ca^{2+} levels, J. Neurosci. 29 (2009) 2053–2063.
[34] R. Kovacs, J. Kardos, U. Heinemann, O. Kann, Mitochondrial calcium ion and membrane potential transients follow the pattern of epileptiform discharges in hippocampal slice cultures, J. Neurosci. 25 (2005) 4260–4269.
[35] M. Montero, M.T. Alonso, E. Carnicero, I. Cuchillo-Ibanez, A. Albillos, A.G. Garcia, J. Garcia-Sancho, J. Alvarez, Chromaffin-cell stimulation triggers fast millimolar mitochondrial Ca^{2+} transients that modulate secretion, Nat. Cell Biol. 2 (2000) 57–61.
[36] K.T. Baron, S.A. Thayer, CGP37157 modulates mitochondrial Ca^{2+} homeostasis in cultured rat dorsal root ganglion neurons, Eur. J. Pharmacol. 340 (1997) 295–300.
[37] T. Thu le, J.R. Ahn, S.H. Woo, Inhibition of L-type Ca^{2+} channel by mitochondrial Na^+–Ca^{2+} exchange inhibitor CGP-37157 in rat atrial myocytes, Eur. J. Pharmacol. 552 (2006) 15–19.
[38] D. Ashton, R. Willems, J. Wynants, J. Van Reempts, R. Marrannes, G. Clincke, Altered Na^+-channel function as an in vitro model of the ischemic penumbra: action of lubeluzole and other neuroprotective drugs, Brain Res. 745 (1997) 210–221.
[39] Y. Arakawa, C. Nishijima, N. Shimizu, T. Urushidani, Survival-promoting activity of nimodipine and nifedipine in rat motoneurons: implications of an intrinsic calcium toxicity in motoneurons, J. Neurochem. 83 (2002) 150–156.
[40] J.C. Fernandez-Morales, L. Cortes-Gil, A.G. Garcia, A.M. De Diego, Differences in the quantal release of catecholamines in chromaffin cells of rat embryos and their mothers, Am. J. Physiol. Cell Physiol. 297 (2009) C407–C418.
[41] J.J. Ballesta, A.G. Garcia, L.M. Gutierrez, M.J. Hidalgo, M. Palmero, J.A. Reig, S. Viniegra, Separate [^{3}H]-nitrendipine binding sites in mitochondria and plasma membranes of bovine adrenal medulla, Br. J. Pharmacol. 101 (1990) 21–26.
[42] G. Uceda, A.G. Garcia, J.M. Guantes, P. Michelena, C. Montiel, Effects of Ca^{2+} channel antagonist subtypes on mitochondrial Ca^{2+} transport, Eur. J. Pharmacol. 289 (1995) 73–80.
[43] M. Ota, T. Narahashi, R.F. Keeler, Effects of veratrum alkaloids on membrane potential and conductance of squid and crayfish giant axons, J. Pharmacol. Exp. Ther. 184 (1973) 143–154.
[44] Z. Deri, V. Adam-Vizi, Detection of intracellular free Na^+ concentration of synaptosomes by a fluorescent indicator, Na^+-binding benzofuran isophthalate: the effect of veratridine, ouabain, and alpha-latrotoxin, J. Neurochem. 61 (1993) 818–825.
[45] C.R. Rose, B.R. Ransom, Regulation of intracellular sodium in cultured rat hippocampal neurones, J. Physiol. 499 (Pt 3) (1997) 573–587.
[46] M.G. Lopez, A.R. Artalejo, A.G. Garcia, E. Neher, J. Garcia-Sancho, Veratridine-induced oscillations of cytosolic calcium and membrane potential in bovine chromaffin cells, J. Physiol. 482 (Pt 1) (1995) 15–27.
[47] J.A. Dykens, Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca^{2+} and Na^+: implications for neurodegeneration, J. Neurochem. 63 (1994) 584–591.
[48] Z. Xia, M. Dickens, J. Raingeaud, R.J. Davis, M.E. Greenberg, Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis, Science 270 (1995) 1326–1331.
[49] M. Hetman, K. Kanning, J.E. Cavanaugh, Z. Xia, Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase, J. Biol. Chem. 274 (1999) 22569–22580.
[50] F.C. Barone, E.A. Irving, A.M. Ray, J.C. Lee, S. Kassis, S. Kumar, A.M. Badger, J.J. Legos, J.A. Erhardt, E.H. Ohlstein, A.J. Hunter, D.C. Harrison, K. Philpott, B.R. Smith, J.L. Adams, A.A. Parsons, Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia, Med. Res. Rev. 21 (2001) 129–145.
[51] K.W. Ward, J.W. Proksch, L.M. Azzaranot, J.A. Mumawa, T.J. Roethke, G.J. Stelman, M.J. Walsh, K.S. Zeigler, J.E. McSurdy-Freed, J.R. Kehlert, J. Chokshi, M.A. Levy, B.R. Smith, Preclinical pharmacokinetics of SB-203580, a potent inhibitor of p38 mitogen-activated protein kinase, Xenobiotica 31 (2001) 783–797.
[52] D.G. Nicholls, A role for the mitochondrion in the protection of cells against calcium overload?, Prog Brain Res. 63 (1985) 97–106.
[53] R.J. White, I.J. Reynolds, Mitochondria and Na^+/Ca^{2+} exchange buffer glutamate-induced calcium loads in cultured cortical neurons, J. Neurosci. 15 (1995) 1318–1328.
[54] E. Hernandez-SanMiguel, L. Vay, J. Santo-Domingo, C.D. Lobaton, A. Moreno, M. Montero, J. Alvarez, The mitochondrial Na^+/Ca^{2+} exchanger plays a key role in the control of cytosolic Ca^{2+} oscillations, Cell Calcium 40 (2006) 53–61.
[55] L.S. Jouaville, P. Pinton, C. Bastianutto, G.A. Rutter, R. Rizzuto, Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming, Proc. Natl. Acad. Sci. USA 96 (1999) 13807–13812.