Neuroscience 301 (2015) 134–143

CATECHOLAMINE SECRETION BY CHEMICAL HYPOXIA IN GUINEA-PIG, BUT NOT RAT, ADRENAL MEDULLARY CELLS: DIFFERENCES IN MITOCHONDRIA

K. HARADA, Y. ENDO, A. WARASHINA AND M. INOUE *

Department of Cell and Systems Physiology, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807-8555, Japan

Abstract—The effects of mitochondrial inhibitors (CN^-, a complex IV inhibitor and CCCP, protonophore) on catecholamine (CA) secretion and mitochondrial function were explored functionally and biochemically in rat and guinea-pig adrenal chromaffin cells. Guinea-pig chromaffin cells conspicuously secreted CA in response to CN^- or CCCP, but rat cells showed a little, if any, secretory response to either of them. The resting metabolic rates in rat adrenal medullae did not differ from those in guinea-pig adrenal medullae. On the other hand, the time course of depolarization of the mitochondrial membrane potential ($\Delta\Psi_m$) in guinea-pig chromaffin cells in response to CN^- was slower than that in rat chromaffin cells, and this difference was abolished by oligomycin, an F_1F_0-ATPase inhibitor. The extent of CCCP-induced decrease in cellular ATP in guinea-pig chromaffin cells, which was indirectly measured using a Mg^{2+} indicator, was smaller than that in rat chromaffin cells. Relative expression levels of F_1F_0-ATPase inhibitor factor in guinea-pig adrenal medullae were smaller than in rat adrenal medullae, and the opposite was true for F_1F_0-ATPase α subunit. The present results indicate that guinea-pig chromaffin cells secrete more CA in response to a mitochondrial inhibitor than rat chromaffin cells and this higher susceptibility in the former is accounted for by a larger extent of reversed operation of F_1F_0-ATPase with the consequent decrease in ATP under conditions where $\Delta\Psi_m$ is depolarized.

Key words: mitochondria, chromaffin cell, oxidative phosphorylation, chemical hypoxia, secretion.

INTRODUCTION

Utilization of O_2 has markedly enhanced efficiency for energy production in the cell with the consequent sophistication of living things (Costa et al., 2014). This use, however, has made the organism depend upon O_2 for survival. Thus, organisms have been forced to develop various defense mechanisms against hypoxia (Fujishiro et al., 2004; Semenza, 2011). How hypoxia is detected in the organism has been explored for more than fifty years (Anichkov and Belen'kii, 1963) and is still a hot topic (López-Barneo et al., 2008; Salman et al., 2014). The cells that rapidly respond to a decrease in blood O_2 pressure are carotid body (CB) type I cells, adrenal medullary chromaffin cells, and smooth muscle cells in the pulmonary artery (Fujishiro et al., 2004). All of these cells exhibit an increase in intracellular Ca^{2+} concentration in response to hypoxia, resulting in secretion or contraction. Various cellular mechanisms for these responses have been proposed, but are still a matter of controversy (Fujishiro et al., 2004; López-Barneo et al., 2008; Salman et al., 2014).

Carotid body type I cells and adrenal chromaffin cells originate from the neural crest (Donoghue et al., 2008), and both synthesize and secrete catecholamine (CA) in response to a depolarization (Rigual et al., 1991). It has been repeatedly reported that mitochondria play a major role in hypoxic detection in CB type I cells (Duchen and Biscoe, 1992a,b; Williams and Buckler, 2004; Wyatt and Buckler, 2004; Wyatt et al., 2007) and chromaffin cells (Inoue et al., 2002). The strong evidence for the notion is that the effect of hypoxia on membrane excitability is reproduced by various mitochondrial inhibitors (Anichkov and Belen'kii, 1963; Inoue et al., 2002; Wyatt and Buckler, 2004). Several mechanisms for such an involvement of mitochondria have been proposed: a decrease in cellular ATP (Inoue et al., 2002; Williams and Buckler, 2004) and a decrease in ROS production (Thompson et al., 2007). In adult guinea-pig chromaffin cells, CA secretion is evoked by various mitochondrial inhibitors (Inoue et al., 2002) and depolarization is induced by the application of CN^- or chemical hypoxia (Inoue et al., 1998). Furthermore, severe hypoxia produces CA secretion and an inward current at potentials near the resting membrane potential (Inoue et al., 1999). In rat chromaffin cells, on the other hand, the sensitivity to hypoxia disappears about one week after birth (Mojet et al., 1997; Thompson et al., 1997; Garcia-Fernandez et al., 2007). What mechanism is involved in this disappearance remains an open question. If the mitochondrial hypothesis is correct, the properties of mitochondria in rat adrenal chromaffin cells may change during the development. It,

*Corresponding author. Tel: +81-93-691-7235; fax: +81-93-602-9883.
E-mail address: minoue@med.uoeh-u.ac.jp (M. Inoue).
Abbreviations: Ab, antibody; CA, catecholamine; CB, carotid body; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; FWHM, full width at half-maximal intensity; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; IF_1, inhibitor factor; MgG, Magnesium Green; NAD(P)H, reduced nicotinamide adenine dinucleotide (phosphate); PVDF, polyvinylidene fluoride; $\Delta\Psi_m$, mitochondrial membrane potential.

http://dx.doi.org/10.1016/j.neuroscience.2015.05.080
0306-4522/

however, would be difficult to investigate biochemically and functionally how mitochondria change during the development, because neonatal adrenal medullae are very tiny. If rat adult chromaffin cells exhibit little secretion in response to mitochondrial inhibitors, the elucidation of how mitochondria in rat chromaffin cells differ from those in guinea pig would provide an important cue on the mechanism involved in hypoxia detection. The first aim in the present experiment is to examine quantitatively the effects of mitochondrial inhibitors on CA secretion in rat and guinea-pig chromaffin cells. If there is a difference in efficiency for CA secretion between them, the mechanism responsible for the difference will be explored by functional and biochemical analyses of the mitochondrial function.

EXPERIMENTAL PROCEDURES

Animals

All the experimental animal procedures were performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments, and were approved by the Institutional Animal Care and Use Committee of the University of Occupational and Environmental Health. All efforts were made to minimize the number of animals used and their suffering. Wister rats (n = 30) and Hartley guinea pigs (n = 26) weighing 250–400 g were used.

Amperometry

Catecholamine secretion from single or clustered adrenal chromaffin cells was measured by using amperometry, as described elsewhere (Inoue et al., 2002). Briefly, adrenal medullae, which were cut into two or three pieces, were incubated for 30 min at 35 °C with 0.25% collagenase dissolved in Ca^{2+}-deficient saline, where Ca^{2+} was removed from a standard saline containing 137 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.53 mM NaH_2PO_4, 5.5 mM D-glucose, 5 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), and 4 mM NaOH (pH 7.4). Digested preparations were kept at 5 °C in Ca^{2+}-deficient saline until the commencement of the experiments. One or two pieces of tissue were placed in the bath apparatus on an inverted microscope, and chromaffin cells were dissociated mechanically using fine needles 20 min before the bath was continuously perfused in the standard saline at 24–26 °C. A carbon fiber was carefully approached to the chromaffin cell, and 600 mV was applied to the tip of the electrode under voltage clamp conditions. The current due to oxidation of CA at the tip of the electrode was recorded using a patch-clamp amplifier. Data were fed into a thermal recorder after low-pass filtering at 15 Hz and into a digital audio tape recorder. Chemicals were bath-applied. For quantitative analysis, the total charge of evoked currents was measured.

Perfused adrenal gland

Perfused adrenal glands of rats or guinea pigs were used to measure changes in reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) autofluorescence in adrenal chromaffin cells *in situ* in response to a mitochondrial inhibitor. Microfluometric methods used were similar to those described elsewhere (Warashina, 2001; Warashina and Inoue, 2012). Briefly, adrenal glands were removed under pentobarbital (i.p. 60 μg Kg^{-1}) anesthesia, and then perfused retrogradely via the adrenal vein with standard saline at a rate of 0.15 ml min^{-1}. Part of the adrenal cortex covering the medulla was carefully removed using microscissors. The gland was transferred to a chamber with the naked medulla down to the glass bottom, and then the chamber was mounted on the stage of an inverted fluorescence microscope. For the measurement of NAD(P)H fluorescence, the preparation was illuminated through a 20X objective lens by the light from a xenon lamp (100 W), which was filtered at 340 nm, and the emission was transmitted through a 420-nm dichroic mirror and a 440-nm barrier filter. NAD(P)H fluorescence diminished with each illumination. This decrease in autofluorescence was approximated by a polynomial function ($at^2 + bt + c$, where a, b, and c are constants and t is time), and then intensity in autofluorescence was corrected for photobleaching. The adrenal gland was continuously perfused at a rate of 0.15 ml min^{-1} via the adrenal vein at 25–28 °C.

Magnesium Green and rhodamine 123 fluorescence

To measure a change in intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$), dissociated cells were loaded at 25–28 °C with the acetoxymethyl (AM) ester form of Magnesium Green (MgG) at 5 mM in the presence of 0.05% Pluronic F-127 for 40 min and then kept for 20 min in standard saline without the dye (Leyssens et al., 1996; Inoue et al., 2002), whereas for the measurement of a change in mitochondrial membrane potential ($\Delta\Psi_m$), the cells were incubated in standard saline containing 26 μM rhodamine 123 for 10 min (Nicholls and Ward, 2000). The dish in which the loaded cells had settled was placed on a Zeiss Axiovert microscope (63X objective lens, NA 1.4) attached to an LSM5 Pascal confocal laser microscope (Carl Zeiss, Tokyo, Japan). Illumination at 488 nm was provided by an argon laser and emission was monitored between 505 and 530 nm. Fluorescence images were acquired every 2 or 2.5 s at 25–28 °C. Magnesium Green fluorescence was obtained with a full width at half-maximal intensity (FWHM) of 7.59 because of the low intensity. FWHM under our experimental conditions corresponds to optical slice thickness. The rhodamine 123 fluorescence was acquired with a FWHM of 0.92 or 1.49. To investigate the effects of mitochondrial inhibitors, half of the 2-ml solution in the dish was replaced with a test solution containing the inhibitor, and the administration was completed within 20 s. After testing with an inhibitor, the solution in the dish was exchanged at least five times with saline. The intensity of MgG usually decreased with each illumination. Thus, 15–20 frames of MgG fluorescence were obtained prior to the application of chemicals and the extent of photobleaching was estimated by a curve fitting of the intensities in the frames with a linear function ($at + b$, where a and b are constants and t is