Extra-adrenal chromaffin cells of the Zuckerkandl´s paraganglion: morphological and electrophysiological study.

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Cell Biology of the Chromaffin Cell
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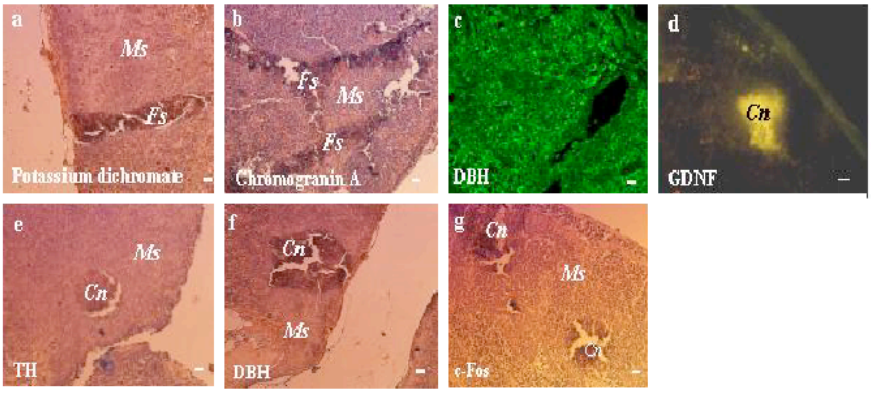
Parkinson´s disease is one of the most important neurodegenerative disorders that affects to one out of a hundred of the world population elder than 65. It has been observed in our laboratory, for the first time, that intrabrain transplantation of chromaffin cell aggregates from the Zuckerkandl´s organ, an extraadrenal paraganglion located adjacent to the lower abdominal aorta, induced gradual improvement of functional deficits in animal models of Parkinson´s disease[1]. This functional regeneration was likely caused by long-survival of grafted cells and chronic trophic action of dopaminotrophic factors, glial cell line-derived factor (GDNF)[2,3] and transforming growth factor beta1 (TGF-β_1)[4,5], which are expressed and delivered by long-surviving grafted chromaffin cells. The objective of this study is to discern the morphological and cytological characteristics of extra-adrenal cells of the Zuckerkandl's organ. On the other hand, long survival of extra-adrenal chromaffin cells could be related to resistance to hypoxia, since it is certainly know that hypoxia is a primary factor involved in cell death after intrabrain grafting. Thus, resistance to hypoxia seems to be a critical factor involved in survival fate after grafting because, among cells of the chromaffin lineage, adrenal medulla cells (non responsive to hypoxia) die shortly after transplantation, but carotid body cells (hypoxia responsive) present a sustained and long-term survival after grafting[6,7]. For this reason, we are currently studying the electrophysiological characteristics as well as sensitivity to hypoxia of these extra-adrenal chromaffin cells.

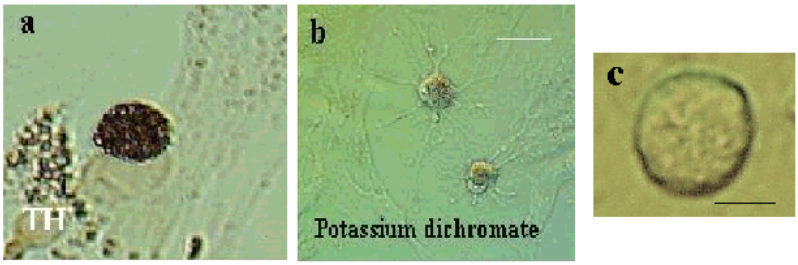
RESULTS

Paraganglia tissue was observed to be composed of chromaffin cells (size around 15 μm) and mesenchyma. Chromaffin tissue (around 22% of the organ) was found to be distributed making up longitudinal fascicles with the appearance of rounded "cell nests" on coronal sections. As typical chromaffin cells, they react with potassium dichromate (classical Orth's reaction), and was also detected the presence of chromogranin A, a chromaffin cell lineage protein that participates on exocytosis.

A


a
Ms
Fs
Potassium dichromate
b
Fs
Ms
Fs
Chromogranin A
c
DBH
d
Cn
GDNF
e
Ms
Cn
TH
f
Cn
Ms
DBH
g
Cn
Ms
Cn
c-Fos

B


a
TH
b
Potassium dichromate
c

C

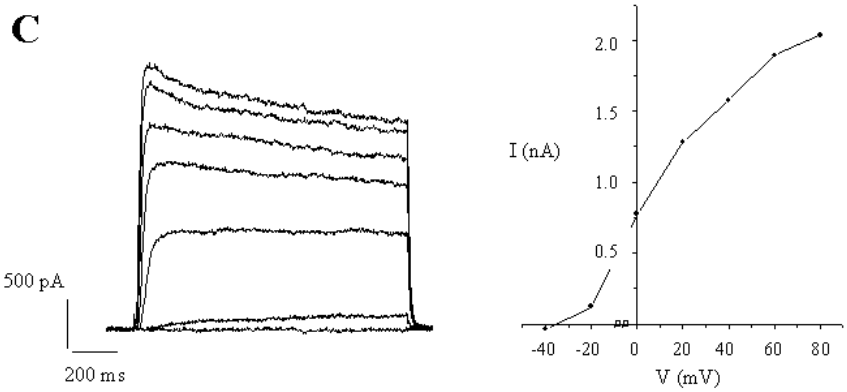

500 pA
200 ms
I (nA)
2.0
1.5
1.0
0.5
-40
-20
0
20
40
60
80
V (mV)

Figure 1. A) Morphological features of the Zuckerkandl's organ**.** Orth´s reaction showing that chromaffin cells clustered making up a fascicle (Fc), reacted with potassium dichromate (**a)**. Immunohystochemistry and immunofluorescence staining revealed that chromaffin cells, forming fascicles (Fc) or cell nests (Cn), are positive to chromogranin A **(b)**, dopamine-beta-hydroxylase (**c) and (f)**; glial cell line-derived neurotrophic factor (GDNF) **(d)**; tyrosine-hydroxylase (TH) **(e)**, and that they are transcriptionally active in vivo because they express c-fos **(g)**. Phenylethanolamine-N-methyl-transferase (PNMT, adrenaline synthesizing enzyme) is not expressed. Scale bars: 100 µm, except in c, 10µm. **B) (a, b)** Positive reaction to TH and potassium dichromate. **(c)** Isolated chromaffin cell of the Zuckerkandl´s organ. Bar, 10µm. **C**). **(a)** Ionic currents in extra-adrenal chromaffin cells. The currents traces are recorded every 8 s for 125 ms voltage steps in 20 mV increments from –40 to +80 mV from a holding potential of –70 mV. In all cells explored outward currents exhibiting sigmoid activation kinetics were observed with amplitude ranging from 0.5 to 2.5 nA. The outwards currents show activation threshold at approximately -40 to –35 mV. **(b)** Current-voltage relationship obtained by measuring the peak amplitude of traces shown in figure **C (a)**. These results suggest that these cells could possess different potassium channels.

Immunostaining (following standard procedures) also indicated that extra-adrenal chromaffin cells were noradrenergic (TH and DBH positive), and expressed the neurotrophic factor GDNF, that is known to be dopaminotrophic. Thus GDNF protects dopaminergic neurons from degeneration in vitro and in animal models of PD, when delivered by intraventricular injections or via transplanted cells or viruses[2,8].

Regarding isolated cell experiments (cytochemical and electrophysiological studies) chromaffin cells of the Zuckerkandl´s organ were cultured following a similar protocol to that used for isolating adrenal chromaffin cells, with few modifications[9]. Cultured extra-adrenal chromaffin cells were found to express TH and to react with potassium dichromate. An isolated typical chromaffin cell two days after cultured can be observed in Figure 1B (c).

Ionic currents from isolated cells were recorded using the whole cell variant of the patch-clamp technique[11] with an EPC-7 patch clamp amplifier. Data adquisition was performed by an ITC-16 computer interface and (Pulse + Pulsefit) software. Linear leak currents, through membrane capacitance, were cancelled on-line using P/4 procedure[12]. The

preliminary electrophysiological results are summarized in legend of figure 1C.

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