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Preservation of Alpha-3 Neuronal Nicotinic Acetylcholine Receptor Expression in Sympathetic Ganglia After Brain Death

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Abstract The goal of this study was to evaluate if the immunohistochemical expression of alpha-3 neuronal nicotinic acetylcholine receptor subunit in sympathetic ganglia remains stable after brain death, determining the possible use of sympathetic thoracic ganglia from subjects after brain death as study group. The third left sympathetic ganglion was resected from patients divided in two groups: BD—organ donors after brain death and CON—patients submitted to sympathectomy for hyperhidrosis (control group). Immunohistochemical staining for alpha-3 neuronal nicotinic acetylcholine receptor subunit was performed; strong and weak expression areas were quantified in both groups. The BD group showed strong alpha-3 neuronal nicotinic acetylcholine receptor expression in 6.55% of the total area, whereas the CON group showed strong expression in 5.91% (p=0.78). Weak expression was found in 6.47% of brain-dead subjects and in 7.23% of control subjects (p=0.31). Brain death did not affect the results of the immunohistochemical analysis of sympathetic ganglia, and its use as study group is feasible.

Keywords Brain death · Ganglia, sympathetic · Receptors, nicotinic · Nicotinic receptor subunit alpha3 · Immunohistochemistry · Human

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Introduction

The autonomic nervous system is responsible for homeostasis [1]. The preganglionic fibers of the sympathetic nervous system form synapses with sympathetic ganglia that are mediated by acetylcholine and its neuronal nicotinic receptor (nAChR [2]). This receptor is a pentamer formed by two alpha and three beta subunits (except the monomer formed only by alpha-7 subunits [1, 2]). In sympathetic ganglia, alpha-3 is the predominant alpha subunit and, in association to beta-4 subunit, forms the "ganglia-type nAChR" [1–5].

To assess sympathetic function, indirect methods such as the sympathetic skin response have been used, but the direct analysis of human sympathetic ganglion function has not been achieved to date due, at least in part, to the lack of adequate control subjects [6]. The goal of this study was to evaluate if the immunohistochemical expression of alpha-3 neuronal nicotinic acetylcholine receptor subunit in sympathetic ganglia remains stable after brain death, determining the possible use of sympathetic thoracic ganglia from subjects after brain death as study group.

Methods

This cross-sectional study was approved by the Ethics Committee for Analysis of Research Projects. All participants (or their legal guardians) provided written consent. Ganglia were resected from individuals divided in two groups: BD, organ donors after brain death, and CON, patients submitted to sympathectomy for hyperhidrosis (control group). The exclusion criteria were the presence of any known or suspected neurological, endocrine, or metabolic disease; a BMI higher than 25 kg/m^2; and donor family or patient refusal to participate in this study.

In the search for potential organ donors, the study was explained, and family consent was obtained by the Organ Procurement Organization of our institution. The ganglia were resected while taking care not to delay or interfere with organ donation for transplantation. Control specimens were derived from the Thoracic Surgery Division database.

Sample Size Calculation In agreement with previous studies [7], the excitability recovery of sympathetic skin response that was observed was 40% faster in patients with hyperhidrosis. The sample size was calculated with sufficient power to detect such a difference, assuming that α=0.05 and β=0.10, two tailed. These calculations demonstrated that a sample of 20 individuals per group was necessary.

Specimen Obtainment The third left sympathetic ganglion was resected, fixed in formalin (less than 48 h), and embedded in paraffin.

Immunohistochemical Technique Histological sections (3 μm) from specimens were deparaffinized, hydrated, and placed in 10 mM citrate solution (pH 6.0) for 1 min at 125° C for high-temperature antigen recovery. Endogenous peroxidases and nonspecific IgG were then blocked. The primary antibody against the alpha-3 neuronal nicotinic acetylcholine receptor (Abcam, Inc., Cambridge, MA; ab55773, dilution 1:100) was dissolved in bovine serum albumin. The slides were dried around the specimens, and the antibody was pipetted. The slides were then incubated overnight in a humid chamber, washed in PBS, placed in chromogenic medium, counterstained with Harris hematoxylin, and mounted with synthetic resin and coverslips.

Image Analysis Images were taken from ten noncoincident, representative fields of each slide at ×400 magnification, and the areas expressing the alpha-3 neuronal nicotinic acetylcholine receptor (strong expression, weak expression and total area—excluding artifact areas) were then quantified using Image Pro Plus software (©Media Cybernetics), and the percentages of strong and weak expression were calculated in relation to the total area. The researcher was blinded to which group each slide belonged.

Statistical Analysis The Shapiro–Wilk test was performed, demonstrating that the data were normally distributed for weak expression, but not to strong expression. A comparison of the median values was then performed using a nonparametric two-tailed t test (Mann–Whitney test). Statistical significance was defined as a p value of less than 0.05.

Results

From December 2008 to March 2010, 46 subjects were enrolled. Four were excluded due to BMI >25 kg/m^2 and two due to diabetes; all of these patients were in the BD group. Demographic data are summarized in Table 1. No significant difference in alpha-3 nicotinic acetylcholine receptor expression was observed between the groups, as illustrated in Fig. 1.

The BD group showed strong alpha-3 neuronal nicotinic acetylcholine receptor expression in 6.55% (2.77–7.55) of the total area, whereas the CON group showed strong expression in 5.91% (3.29–9.14%), as shown in Fig. 2 (p=0.78). Weak expression was found in 6.47% (3.65–7.52) of brain-dead subjects and in 7.23% (4.98–9.5%) of control subjects (p=0.31; see Fig. 3).

Discussion

Significance of sympathetic system disorders are increasing, but finding an adequate comparison parameter for sympathetic ganglia studies is a challenge. The resection of normal ganglia from healthy subjects is not feasible for ethical reasons. One alternative is the resection of ganglia from cadavers; however, cellular and humoral degeneration after death and unequal times between death and ganglion resection make such analysis unreliable.

In the eighteenth century, Marie François Xavier Bichat (1771–1802) proposed a division of life (and, consequently, the nervous system): the "organic life" would be characterized by continuity, asymmetry, disharmony, and indepen-

Table 1 Demographic data

	BD	CON
Age (years)[a]	46.5 (28.25–53)	26 (24–30.25)
Sex (M/F)	9/11	3/17

[a] Age expressed as the median (interquartile range)

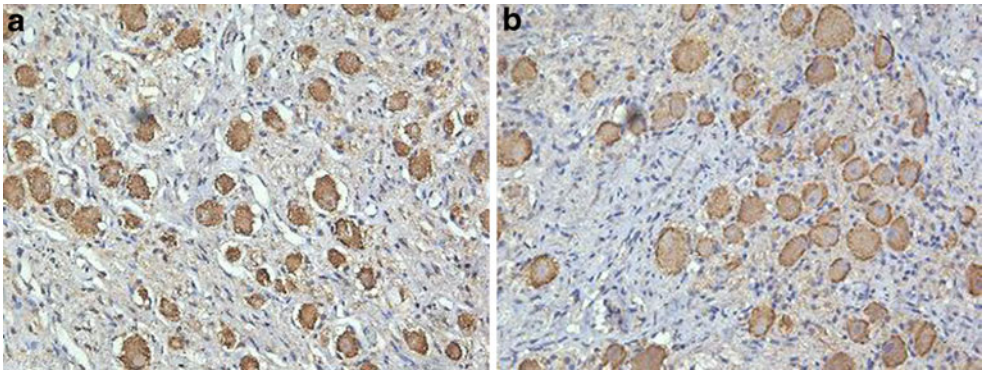

Fig. 1 Alpha-3 neuronal nicotinic acetylcholine receptor expression. **a** Brain-dead subject. **b** Control

dence of habit and education, and its nervous system should be commanded by ganglia and would end at the death of the heart; in contrast, the "animal life" would be discontinuous, symmetric, harmonic, and depend on the environment, and the center of its nervous system would be the brain, and its death could occur before the death of many other organs [8]. Thus, sympathetic ganglia from organ donors after brain death could be an alternative for such studies. Our results have proven Bichat's theory, demonstrating a level of alpha-3 neuronal nicotinic acetylcholine receptor subunit expression in the sympathetic ganglia of subjects after brain death that is similar to that in controls, demonstrating the stability of these structures after cerebral death and the feasibility of the immunohistochemical analysis of these ganglia. The use of organs from subjects after brain death as controls is warranted in upcoming studies.

One limitation of the present study is the differences between groups with respect to age and sex. This difference is the result of the greater demand for sympathectomy among younger women [9], despite the fact that hyperhidrosis affects all age groups and both sexes equally [10–13]. In contrast, the profile of our institution's organ donors shows a predominance of subjects in the 41- to 60-year-old group and sex equivalence [14]. Given the small number of organ donors, the group of organ donors used in this study was considered the best control group available. Furthermore, the ganglia were resected immediately after cardiac arrest, but with different times after brain death.

Conclusions

Brain death did not affect the results of the immunohistochemical analysis of sympathetic ganglia, and its use as study group is feasible.

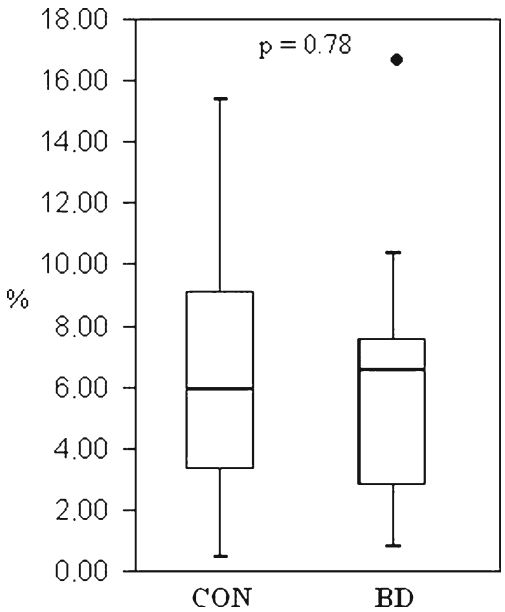

Fig. 2 Quantification of nAChR alpha-3 subunit strong expression by group

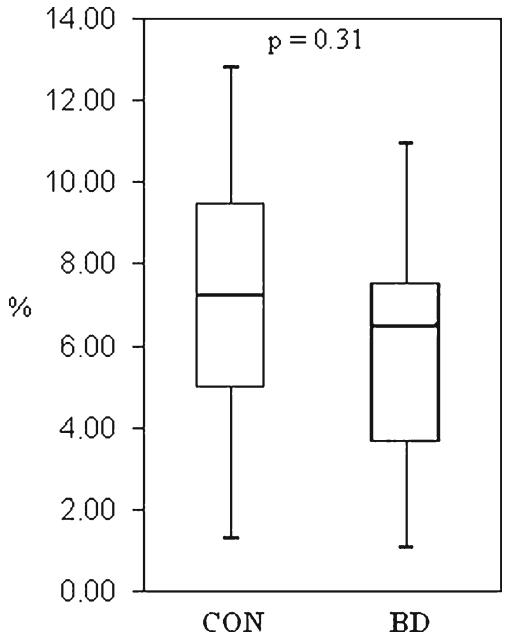

Fig. 3 Quantification of nAChR alpha-3 subunit weak expression by group

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