Glutathione-S-transferase and tyrosine hydroxylase activity in human adrenal medulla. Differences between fetal and adult tissue

Julio César García, Aida Leiva* y Salvador Viniegra.**

CETEX-CENPALAB, Grupo de Neurotoxicología, Carretera del Cacahual km 3, Bejucal, Apartado Postal 3, La Habana. *Centro de Investigaciones Biomédicas "Victoria de Girón", Avenida 146 esquina a 31, Ciudad de La Habana, Cuba. **Instituto de Neurociencias, Centro Mixto CSIC-Universidad Miguel Hernández, Campus de San Juan. 03550 Alicante, España.

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Palabras clave: catecolaminas, médula adrenal humana, glutatión-S-transferasa, tirosina hidroxilasa, enfermedad de Parkinson.
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RESUMEN. Con el objetivo de caracterizar la glándula adrenal humana en estado fetal (10 a 12 semanas) y adulta (post-morten) se determinaron las actividades específicas de la enzimas glutatión-S-transferasa (GST) y tirosina hidroxilasa (TH), así como las de las catecolaminas (CA): adrenalina (A); noradrenalina (NA) y dopamina (DA), utilizando Cromatografía Líquida de Alta Resolución con detección electroquímica (HPLC-DE). La GST fue determinada utilizando 1-cloro-2,4-dinitrobenzeno como substrato electrofílico y la actividad TH fue determinada por medición de la L-DOPA formada a partir de la L-tirosina utilizando un HPLC-DE. Los resultados indican que la NA fue la principal CA presente en el período fetal estudiado. Una disminución en la actividad de NA acompañado por un ligero incremento en la de A fue detectada con el aumento de la edad fetal. En el tejido adulto la A fue la principal CA y presentó una cantidad de DA (0,046 % del total de CA) 35 veces menor que en el tejido fetal (1,60 %). La actividad específica de la TH presentó diferencias al ser comparada con el tejido fetal y fue significativamente mayor ($p < 0,001$) en el tejido fetal. La actividad de la GST fue también significativamente superior ($p < 0,001$) en el tejido adulto cuando se comparó con el fetal. Los resultados indican un relativamente pobre aporte de la células fetales, en cuanto a su actividad TH. Los cambios detectados en la actividad enzimática específica de la GST entre el tejido adulto y fetal pueden revelar una diferencia en la capacidad entre estos tejidos para metabolizar xenobioticos y toxinas endógenas. Estos resultados pudieran contribuir a la explicación de los resultados del implante de células catecolaminérgicas en la enfermedad de Parkinson.

ABSTRACT. In order to characterize both adult and fetal (10 to 12 weeks) human adrenal gland the tyrosine hydroxylase (TH) and glutathione-S-transferase (GST) specific activities and catecholamine (CA) levels were assayed. Levels of adrenaline (A), noradrenaline (NA) and dopamine (DA) were simultaneously detected using HPLC with electrochemical detection. GSTsa was assayed using 1-chloro-2,4-dinitrobenzene as electrophilic substrate and TH activity was determined by measurement of the L-DOPA formed from L-tyrosine using HPLC with electrochemical detection. These results shows that NA was the main CA present at the fetal ages studied. A decrease in the level of NA accompanied by a slight increase in the levels of A with increasing fetal age was also detected. In adults A was the main CA and the relative amount of DA (0.046 % of the total CA) was 35 times lower than in fetuses (1,60 %). TH specific activity showed differences with the fetal ages and was higher in adult than in fetal adrenal glands ($p < 0.001$). GST_{SA} was also higher ($p < 0.001$) in the adult group and it does not show differences over the fetal ages studied. Our results indicate the relatively poor contribution of fetal cells on TH activity. Changes detected in the GST_{SA} between both adrenal medulla may reflect a different capacity of these tissues to metabolize xenobiotics and endogen toxins. This could explain the performance of catecholaminergic cells in human PD implants.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder in which progressive impairment of movement is caused by the loss of dopaminergic neurons that originate at the substantia nigra and end in the striatum. There is increasing evidence that PD may have more than one cause.[1-3] However, the oxidative stress hypothesis is one of the most commonly accepted to explain both the etiology and progression of PD.

Fifteen years ago, Backlund[4] transplanted autologous adrenal gland into the brain as a therapy for pharmacologically untreated PD, but found little or no clinical improvement. Many other transplants have been subsequently tested. The use of autologous adrenal gland and young or fetal adrenal medullar tissue has been frequently reported. However the efficacy of these techniques in the treatment of PD has not yet been clearly established. The vast majority of studies published on experimental neural transplants have reported significant advantages in using fetal donor adrenal gland tissue because of its high growth potential and functional plasticity.[5]

Studies using human adult and fetal adrenal gland have been reported.[6-8] However, the use of ten to twelve week old human fetal adrenal gland (HFAG) has been scarcely studied.

The primary function of the adrenal medulla is to synthesize and secrete catecholamines (CA). The CA is a dihydroxylated phenolic, highly unstable compound, which are synthesized in the brain, adrenal medulla and sympathetic nerve endings. Tyrosine hydroxylase (TH), (EC 1.14.16.2) the rate limiting enzyme in the production of CA is present in both adult and fetal adrenal gland. TH catalyzes the conversion of L-tyrosine to L-dopa.[9]

Chromaffin cells have been widely explored as a potential source of CA in animal models and human cases of striatal dopamine deficiency.[10] In general, the recovery of motor function following chromaffin cell implants has been quite modest, which may be related to the poor survival rate of implanted chromaffin cells.[10,11] Furthermore, susceptibility, which is associated with the altered expression of enzymes regulating the metabolism of endogenous and exogenous neurotoxins, is implicated in the pathophysiology of PD. There are a vast number of enzyme systems such as monoamine oxidase, superoxide dismutase and glutathione-S-transferase (GST) that are involved in the metabolism of xenobiotic, with most attention having been focused on GST.

A recent group of communications has indicated the neuroprotective role of GST and glutathione (GSH) systems in the detoxification of CA oxidized metabolites in the Central Nervous System (CNS).[12-17]

The purpose of the present study was to characterize both adult and fetal (10 to 12 weeks) human adrenal gland, studying the TH and GST specific activity and CA levels.

MATERIAL AND METHODS

Tissue sampling

Human fetal adrenal glands (HFAG) were obtained with the aid of a stereomicroscope, from therapeutically aborted fetuses, at the Eusebio Hernandez Hospital in Havana City. The fetal adrenal glands can be clearly distinguished at 6 week's gestation.[18] This allowed the easy obtainment of the tissue in the age range studied.

Human adult adrenal gland (HAAG) was obtained (4.0 ± 2.1) h post mortem, (mean ± SD) from six male patients 6(2.4 ± 3.4) years of age (means ± SD) with no known history of neuropsychiatry or neurodegenerative disorders. (Table 1). HFAG and HAAG were immediately frozen (–70 °C) until assayed.

The Eusebio Hernández Hospital Review Board gave approval for the use of human tissue specimens.

Catecholamine assay

Catecholamine assay was performed as previously described[18] for the simultaneous detection of adrenaline (A), noradrenaline (NA) and dopamine (DA) in both HFAG and HAAG, using a HPLC procedure with electrochemical detection. The adrenal tissue was homogenized (1 : 5 wet weight : volume) in glass potters using 0.1 mol/L $HCLO_4$ containing $NaHSO_3$ (1.9 mmol/L) as an antioxidant.

The homogenate was immediately centrifuged for 30 min at 10 000 g, 4 °C , and the protein free supernatants subsequently passed through a 0.22 mm membrane filter. Dihydroxybenzylamine was used as an internal standard. A 5-20 µL sample volume was injected into a Unicam isocratic HPLC System consisting of a PU4100 pump and PU4022 electrochemical detector set at 70 mV with a sensitivity of 30 nA .

Separation was carried out on a Hypersil H5 ODS column (100 X 4.6 mm i.d.). The electrochemical detector's signal was recorded on a PM 8252 A two channel recorder. Composition of the mobile phase was 13.8 g of NaH_2PO_4, 60 mg of Na_2EDTA, 20 mg of 1-octanesulfonic acid and 2 % ethanol per liter. The pH was adjusted to 3.70 with H_3PO_4 before addition of ethanol. The flow-rate was 1.0 mL/min .

Assay tyrosine hydroxylase (TH) specific activity (TH_{SA})

TH activity was determined by measurement of the L-DOPA formed from L-tyrosine using HPLC with electrochemical detection, as originally described by Nagatsu[9] with minor modifications.[20]

Briefly, 50 µL of homogenate was analyzed at pH 6.0 in the presence of 1 mol/L sodium acetate, 10 mmol/L DL-6-methyl-5,6,7,8-tetrahydropterin, 10 mmol/L $Fe(NH_4)_2SO_4 \cdot 6H_2O$; 1.5 mmol/L L-tyr in a total volume of 120 µL for 15 min at 37 °C . The reaction was stopped by the addition of 600 µL, 0,5 mol/L perchloric acid. The HPLC assay was carried out under the same conditions as the CA assay, apart from the phase mobile pH, which was changed to 3.35.

Table 1. Fetuses and adults employed according to age.

N	Age
5	10 weeks
5	11 weeks
5	12 weeks
6	(62 ± 5.6) years

Glutathione-S-transferase specific activity (GSTsa)

GSTsa was assayed with 1-chloro-2,4-dinitrobenzene (CDNB) as the electrophilic substrate according to Habig's method.[21] Protein concentration was measured by the Bradford method,[22] using BSA as the standard protein.

On the day of analysis the samples (HFAG and HAAG) were homogenized in a glass-Teflon potter and centrifuged at 10 000 X g for 30 min, 4 °C .

Aliquots of the supernatant were used to measure total protein content and GST activity by spectrophotometric methods as previously described.[13] Briefly, GST activity was measured by determining the rate of formation of dinitrophenylglutathione from CDNB and GSH. One unit of GST activity was the quantity of enzyme in 1 mL required to catalyze the conjugation of 1 µmol/min of CDNB.

Statistical analysis

A one-way analysis of variance was used to determine the overall statistical significance of the data. In the case of significant difference ($p < 0.05$) among groups, the LSD parametric test was performed post-hoc. Values are expressed as mean ± SEM.

RESULTS

Fetal CA are synthesized mainly in the adrenal medulla from the amino acids phenylalanine and tyrosine.

It was observed that NA was the main CA present at all the fetal ages studied (Table 2). A decrease in the level of NA with increasing fetal age was also detected. This decrease was accompanied by a slight increase in the levels of A. In adults A was the main CA and DA the least, as was the case in fetal tissue.

NA is the main CA in prenatal life

The highest relative amount of DA was detected in the10-12 week old fetuses, (8.31 % of the total CA),

being 35 times higher than in adults (0.046 %).

To determine the effect of age on the capacity for CA biosynthesis, TH activity was assessed in adult and fetal adrenal medulla homogenates. TH activity was significantly greater in the adult tissue (Table 3). There were differences in TH_{SA} at different fetal ages. TH_{SA} was significantly higher in 11 to 12 week fetuses ($p < 0.01$) when compared with the 10-week-old fetuses. In general, TH_{SA} was higher in the adults than in fetuses ($p < 0.001$).

To determine the effect of age on the protective capacity in the detoxification of electrophilic compounds, GST specific activity (GST_{SA}) was assessed in adult and fetal adrenal medulla homogenates (Table 3).

GST_{SA} was shown to be significantly higher ($p < 0.001$) in the adult group. Unlike the results for TH_{SA}, GST_{SA} was not seen to vary significantly over the fetal ages studied.

Table 3. Tyrosine hydroxylase specific activity (TH_{SA}) and glutathione-S-transferase specific activity (GST_{SA}) in fetal and adult human adrenal medulla.

Source	Age	N	TH_{SA}	GST_{SA}
Fetus	10 weeks	5	2.61 ± 0.37*°	0.0281 ± 0.0011
Fetus	11 weeks	5	9.95 ± 1.76	0.0303 ± 0.0016
Fetus	12 weeks	5	11. 36 ± 0.70	0.0290 ± 0.0012
Adult	(62.4 ± 3.4) years	6	39.66 ± 3. 76	0.3134 ± 0.0151°

TH_{SA} is expressed in pmol L-DOPA · min^{-1} · mg of $protein^{-1}$. GST_{SA} is expressed in micromoles GST · min^{-1} · mg of $protein^{-1}$. Values shown represent the means ± SEM of triplicate determinations for individual samples in each group.

DISCUSSION

The adrenal glands are believed to fulfill vital functions in human fetal life as well as after birth. Their greater fetal size and proportion relative to other organs when compared to the postnatal condition, reflects their relative importance to the fetus. Fetal adrenal glands can be clearly distinguished at 6 week's gestation, and for this reason were relatively easy to obtained (Table 1).

Fetal CA is produced primarily in the adrenal medulla. Additional synthesis occurs in adrenergic nerve endings and within masses of extramedullary paraaortic chromaffin tissue, the largest of which are known as the organs of Zuckerklandl.

It was observed that the main CA for all the fetal ages studied was NA (Table 2). A decrease in the level of NA with increasing fetal age was observed. This decrease is accompanied by a slight increase in the levels of A. This is in agreement with previously published results.[6,19]

This modification with the age of the HFAG CA could be related to the morphogenesis of the adrenal medulla, which originates in the neural crest, and develops along with the rest of the sympathetic nervous system. In the process of maturation, neuroblastic cells migrate from the neural crest.

NA is the main CA in prenatal life. The proportion of NA relative to other CA's begins to decrease only after birth, at which time A significantly increases, consequently becoming the principle CA in adult life. The striking differences between fetal and human adrenal gland A levels may be explained by the increase in TH_{SA} which was 3,5 times higher in the adult tissue (Table 2).

The DA : A and DA : NA ratios found in the HFAG between 10 and 12 weeks were 0,18 and 0,04 respectively. That is 360 and 12,5 times higher than the ratios detected for human adult adrenal gland. The DA : A ratio observed in this work is similar to that reported in author's previous study on HFAG (13 to 18 week fetuses).[19]

The TH_{SA} in HAAG was at a higher level than that of HFAG. This difference was most apparent when comparing 10-week fetuses with adults (TH_{SA} 15 times higher in the adult tissue).

TH, the first enzyme in CA biosynthesis, is the product of a single, multiple-exonic gene.[23]

TH activity in most catecholaminergic cells and tissue is regulated by several factors. In adrenal gland cells, activity is regulated by compounds such as adrenal cortical hormone, adrenaline and neurotrofic factors.[5,24,25] However, such differences in TH_{SA} levels are assumed to reflect changes in the amount of TH per cell.

These results indicate the relatively poor contribution of fetal cells on TH activity, and this may be important in explaining the performance of catecholaminergic cells in human PD implants.[11]

Oxidative stress has been implicated in PD. The GST supergene family encodes isoenzymes that appear to be critical in protection against both toxic and oxidative stress.[12-15,26]

The GST are believed to play an important role in the protection of cellular macromolecules from attack by reactive electrophiles. GST functions as an intra and intercellular detoxification system acting on mutagens, carcinogens, and other toxic compounds. In addition their GSH-dependent peroxidase activity, it may play an important action in protecting tissue from endogenous organic hydroperoxides produced during oxidative stress. A marked increase in GST activity has been observed in HAAG, and has raised the question of a possible role for GST in protection against both toxic and oxidative stress during CA synthesis, which is also increased in HAAG. The changes detected in the GST_{SA} in both fetal and adult medullar tissue may reflect a metabolic coupling between free radical generating and scavenging systems.

Table 2. Levels of noradrenaline (NA), adrenaline (A) and dopamine (DA) in human fetal and adult adrenal gland.

Age	N	NA	A	DA
10 weeks	5	27.18 ± 3.01	3.06 ± 1.46*°	0.56 ± 0.14
11 weeks	5	20.8 ± 2.16	5.41 ± 0.36	0.40 ± 0.10
12 weeks	4	15.06 ± 1.06&°	8.01 ± 0.03	2.09 ± 0.06&
62 years	6	58.2 ± 11.26	352.3 ± 114.9	0.19 ± 0.07°

The values are the means ± SD, expressed in nanograme/milligram of wet weight. * $p < 0,01$ for differences from 10 week to 11 or 12 weeks. ° $p < 0,001$ for difference from adult and fetal ages (10-12 weeks). & $p < 0,01$ for differences from 12 week to 10 or 11 weeks.

In conclusion this study clearly demonstrates differences between the TH_{SA} of HFAG and HAAG ($p < 0.001$) that are associated with CA levels in the adrenal gland. Moreover increased GST activity was observed in HAAG compared to HFAG ($p < 0.001$). The physiological consequences of lower GST_{SA} in the HFAG are less clear, since the precise functional role of these enzymes, at this stage of fetal development remains unknown. The present results show a direct relationship between TH and GST specific activities. The high HFAG TH_{SA}/ GST_{SA} ratio in compared to HAAG could explain the performance of catecholaminergic cells in human PD implants. Indeed changes detected in the TH_{SA} and GST_{SA} between both fetal and adult adrenal medulla may reflect a different capacity of these tissues to metabolize xenobiotics and endogen toxins.

Finally it is well known that GST M2-2 catalyze the detoxification of catecholamines acting as neuroprotective antioxidant system.[26,27] On the other hand recently was demonstrated that GST pi was a dopamine-inducible suppressor of dopamine induced apoptosis in PC 12 cells through inhibition of Jun-N-terminal Kinase (JNK).[28] These well-documented results are example of the relationship between catecholamine pathway and detoxifying enzymes like GST. It is also demonstrate that the degree of DA metabolites -induced damage depends not only of the TH regulation but also of the activity of different GST isoforms. Further studies on the contribution of GST isoforms and other detoxification enzymes in catecholamine pathway are in progress.

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